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FORMULATION AND EVALUATION OF ANTI INFLAMMATORYTRANSDERMAL PATCHES USING LEAF EXTRACTS OF ARISTOLOCHIA INDICA AND BOERHAVIA DIFFUSA

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ABSTRACT

Indian birthwort or *Aristolochia indica,* is one of the most significant plants found throughout India and has a lot of therapeutic uses. The main components of *Aristolochia indica* are alkaloids, essential oils, bitter principles (aristolochin), fixed oil, tannic acid, resin, gum, and sugar. It works well to treat ulcers, bronchitis, asthma, and bites from insects and reptiles. *Boerhavia diffusa* is a well-known ethnomedical plant, a perennial creeping weed that grows in tropical and subtropical regions. The entire plant as well as leaves, roots, stems and plant extracts, has extensively employed in numerous traditional and folkloric medical systems for the treatment of a wide range of illnesses. The study mainly aims at the anti-inflammatory action of both *Aristolochia indica* and *Boerhavia diffusa* against the poisonous insect bites and stings. A transdermal patch is prepared by combining the extracts of *Aristolochia indica* and *Boerhavia diffusa* which seems to be another goal of this study. The Anti-inflammatory activity of the extracts are determined by Protein Denaturation Inhibition Test.

KEYWORDS: Protein denaturation assay, *Aristolochia indica*, *Boerhavia diffusa* transdermal patches.

INTRODUCTION

Arthropoda is regarded as the largest phylum with around nine lakh species worldwide species (cockroaches, crabs, beetles, centipedes, scorpions, shrimp, spiders, lobsters, and bees). Bites and stings of these species have the potential to be toxic causing swelling, redness, itching, and other reactions at the location. It is possible to lessen the inflammation of these stings by using anti-inflammatory medications. There are many medicinal plants which possess anti-inflammatory action, *Aristolochia indica* and *Boerhavia diffusa* are one of them. An adhesive patch that has been medicated and applied to the skin to administer a predetermined dosage of medication is called a transdermal patch. *Aristolochia indica*, is a perennial herb withtwining leaves, belonging to the Aristolochiaceae family. It is used to treat worm infections, fever, acne, and snake and scorpion venom. It benefits those with arthritis and inflammation. *Boerhavia diffusa* is a well-known medicinal plant belonging to the family Nyctaginaceae. It possess antiaging, rejuvenating, enhancing life and mental capacity, and disease prevention qualities. This herb increases the body's resistance to any attack by offering hepatoprotection and immunomodulation. The study mainly aims at the anti-inflammatory action of both *Aristolochia indica* and *Boerhavia diffusa* against the poisonous insect bites and stings. A transdermal patch is prepared by combining the extracts of *Aristolochia indica* and *Boerhavia diffusa* which seems to be a another goal of this study. The leaves are collected and authenticated. The leaves are dried under the shade and powdered for carrying out the further steps. The Anti-inflammatory activity of the extracts are determined by Protein Denaturation Inhibition Test. The extract showed potent anti-inflamamtory activity. The Ethosomal formulation of the extract was prepared. Using the nanoformulation, a transdermal patch of these extracts was prepared and evaluated.

Collection of plant material

The leaves of *Aristolochia indica* and *Boerhavia diffusa* were collected in the month of April from Thrissur district. The leaves were authenticated by M.Bheemalingappa, scientist B, Forest Botany Department, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India. The fresh leaves were used for study of macroscopic and microscopic characters. Collected Leaves were shade dried and coarsely powdered.

Extraction

Using soxhelet apparatus, various constituents was extracted from leaves of *Aristolochia indica* and *Boerhavia diffusa*. Leaves were dried at room temperature in the shade:

- Dried leaves were ground into powder using a mill
- Powder was kept at room temperature in a bottle with tight lid
- Leaf powder from *Aristolochia indica* and *Boerhavia diffusa*, 30 gm was packed in soxhelet apparatus separately with 250 ml of 99% ethanol to make ethanolic extract.
- Extraction was carried out at room temperature for 12 hours with 2-3 cycles per hour until the extract become colourless
- To prevent contamination and the destruction of bioactive substance by light and temperature, sample was collected in an amber bottle after extraction.[1]

In vitro anti-inflammatory activity test

Inhibition of albumin denaturation test

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation:

Control Solution (50 ml)

2 ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

Standard Drug (50 ml)

2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of standard drug (aspirin) concentration of 100, 200, 400, 800 and 1000 µg/ml.

Test Solution (50 ml)

2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of plant extract (*Aristolochia indica and Boerhavia diffusa* aqueous leaf extract) concentration of 100, 200, 400, 800, 1000µg/ml. All of the above solutions were adjusted to ph. using a small amount of 1 N HCl. The samples were incubated at 37^{o}C for 15 minutes and heated at 70^{o}C for 5 minutes. After cooling, the absorbance (at 230 nm) of the above solutions percentage inhibition of protein denaturation was calculated using the following formula.[2]

Calculation

Percentage Inhibition = [Vt / Vc – 1] × 100

Where,

Vt = Absorbance of Test Sample

Vc = Absorbance of Control

Formulation of trandermal patches

Preparation of Ethosomes (Nanoparticles) by Hot Method

- Add leaf extract of *Aristolochia indica* (0.5g) and *Boerhavia diffusa* (0.5 g) to the aqueous solution of Phospholipids (1g).
- Heat in a waterbath at 40^{0} C to form a colloidal solution.
- Add Propylene glycol (1 ml) and Ethanol (2 ml) in a separate vessel and heat at 40^{0} C.
- Organic solution added to aqueous solution.
- Undergo Sonication.[3]

Preparation of Transdermal patch of *Aristolochia indica* and *Boerhavia diffusa*

- Add chloroform (7.5 ml) and ethanol (7.5 ml) in the ratio 1:1.
- HPMC (180 mg) is added and dissolved by stirring.
- Glycerin (0.1 ml) and PEG 400 (0.1 ml).
- Ethosomes of *Aristolochia indica* and *Boerhavia diffusa* leaf extract (15 mg) is diluted in few drops of ethanol, adds to the above solution and placed in magnetic stirrer for 30minutes.
- Add few drops of Menthol.
- Pour in to a Petri dish and kept overnight for drying.[4][5]

Evaluation test for transdermal patches

Organoleptic characteristics

The physical appearance of developed patch was evaluated by using a naked-eye examination for its appearance, colour, clarity, flexibility, and smoothness.[6]

Uniformity of weight

Three matrix systems were taken and they were weighed individually. The readings obtained were recorded and average weight was determined.

Folding endurance

A strip of specific area is to be cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be fold at the same place without breaking gave the valueof folding endurance.

Percentage elongation break test

The Percentage Elongation Break is to be determined by noting the length just before the break point, the percentage elongation can be determined from the below mentioned formula.

Elongation Percentage = [L1 – L2 / L2] × 100

Where,

L1 = Final length of each strip

L2 = Initial length of each strip

Thickness of the patch

The thickness of the drug loaded patch is measured in different points by using a digital micrometer screw guage and determines the average thickness and standard deviation for the same to ensure the thickness of prepared patch. [4][5]

TR = PSR + (HSR × LC)

Where,

TR = Total reading

PSR = Pitch scale reading

HSR = Head (circular) scale reading

LC = Least count

Percentage moisture content test

This test was also carried to check the integrity of films

under dry conditions. The individual transdermal films (of specified area) were kept in a desiccator containing fused anhydrous calcium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72 hrs.[7] The percentage moisture content was determined by using the following formula

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

Skin irritation test

The prepared patch of *Aristolochia indica* and *Boerhavia diffusa* was placed on the skin and tapped on the place. The patches are placed on for 20-30 minutes. The area of skin that was tested will be evaluated after the patches are removed.[8]

Measurement of pH

pH of the patch was measured by using digital pH meter. The pH of the patch must ideally near to normal pH of skin to avoid any irritations.

Flatness test

Patches were cut into three longitudinal strips, with the length of each strip was measured and the difference due to non-uniformity in flatness calculated using percentage constriction, with 0% constriction equal to 100% flatness.[9]

$$\text{Percentage Constriction} = \frac{\text{Final length of each strip}}{\text{Initial length of each strip}} \times 100$$

RESULTS

Collection and Authentication of leaves of *aristolochia indica* and *boerhavia diffusa*

The leaves of *Aristolochia indica* and *Boerhavia diffusa* were collected and authenticated. These leaves were subjected to further evaluation.

Extraction

Table No 1: Observation table of color and percentage yield of extract.

Extract	Colour	Weight (%w/w)
Aristolochia indica ethanolic extract	Green	5
Boerhavia diffusa ethanolic extract	Green	7

In vitro anti-inflammatory activity test

Table No. 2: Comparison of Percentage inhibition of Aspirin and Ethanolic extracts of Aristolochia indica, Boerhavia diffusa and their Combination.

Sl No	Sample	Concentration (µg/ml)	Absorbance at 230 nm	Percentage inhibition (%)
1	Control		2.133	
2	Standard	100	2.191	201.60
		200	2.817	205
		400	2.820	210.20
		800	2.859	245.10
		1000	2.994	264.90
3	*Aristolochia indica*	100	2.285	201.67
		200	2.323	205.03
		400	2.881	254.28
		800	2.915	257.13
		1000	3.389	299.11
4	*Boerhavia diffusa*	100	2.301	203.08
		200	2.926	258.25
		400	2.739	241.74
		800	2.976	262.66
		1000	3.456	305.03
5	Combination of	100	2.584	228.06
	Aristolochia indica and	200	2.686	237.18
	Boerhavia diffusa	400	2.950	260.37
		800	3.178	280.49
		1000	3.583	316.24

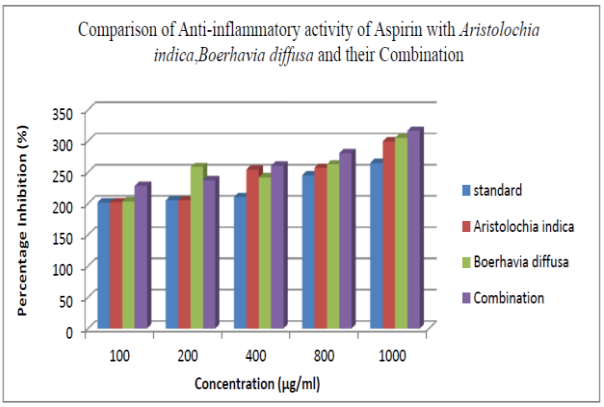

Fig. 01: Comparison of Anti-inflammatory activity of Aspirin with Aristolochiaindica and Boerhavia diffusa

Formulated herbal anti inflamatory transdermal patch

Anti inflammatory transdermal patches using leaf extracts of *Aristolochia indica* and *Boerhavia diffusa* were prepared.

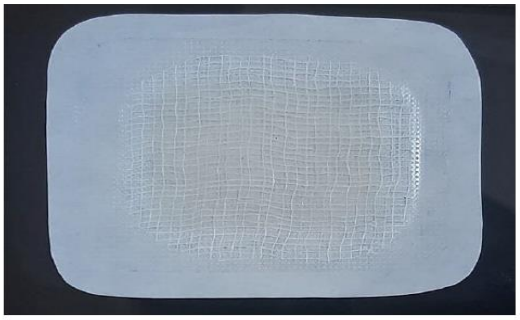

Fig. 02: transdermal patches using leaf extracts of Aristolochia indica and Boerhaviadiffusa.

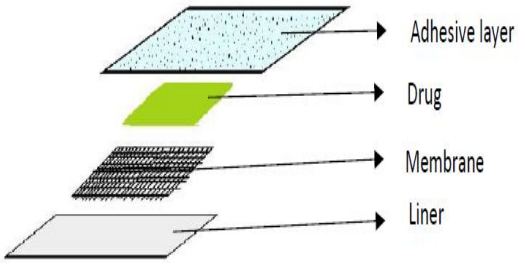

Fig. 03: 3D Model of transdermal patches using leaf extracts of Aristolochia indica andBoerhavia diffusa.

Evaluation test for transdermal patches

- **Organoleptic characteristics**

The physical appearance of developed patch was evaluated by using a naked-eye examination for its 0appearance, colour, clarity, flexibility, and smoothness.

Table No. 03: Organoleptic characterestics.

Sl. No.	Physical appearance	Observation
1	Appearance	Jellified Preparation
2	Colour	Yellowish green
3	Clarity	Opaque
4	Flexibility	Yes
5	Smoothness	Good

- **Uniformity of weight**

Table No. 04: Uniformity of weight.

Weight of individual patch (g)	Average weight of patch (g)
0.250	0.249
0.251	
0.250	
0.249	
0.248	

- **Folding endurance**

The number of times the film was folded at the sample place without breaking = value offolding endurance.
Number of times the film folded at the same place without breaking = 130 times, sofolding endurance = 130 times.

- **Percentage elongation break test**

Initial length of strip (LI) = 4cm
Final length of strip (L2) = 4.3cm
Therefore, percentage elongation =7.5%

- **Thickness of patch**

Determination of least count of screw gauge (LC)Linear scale Division, LSD = 1mm
Number of full rotation given to screw = 4mm
Distance moved by the screw = 4mm
Therefore, pitch P = 4/4 = 1mm
Number of division of circular scale = 100
Therefore, LC = 1mm/100
= 0.01mm

Table No. 05: Thickness of patch.

Sl no	Pitch scale Reading PSR (mm)	Observed HSR(n)	Correction Zero Error	HSR + ZeroError	Corrected HSR (n x LC)	Total Reading
1	0.2	4	+4	8	0.04	0.24
2	0.1	2	+4	6	0.02	0.12
3	0.2	4	+4	8	0.04	0.24

PSR = Pitch SCALE ReadingHSR = Circular Scale Reading
Total Reading = PSR + Correction HSRAverage thickness of patch = 0.2 mm

- **Percentage moisture content**

Initial Weight = 0.250 gFinal Weight = 0.243 g
Therefore, % Moisture content = 2.8 %

- **Skin irritation test**

The skin irritation test for patches of *Aristolochia indica* and *Boerhavia diffusa* wasperformed.
Itchiness, Redness, Rashes, Burning were not observed.

- **Measurement of pH**

PH of the prepared patch from *Aristolochia indica* and *Boerhavia diffusa* leaf extract was found to be 5.6. This lies in the normal pH range of the skin and with time no skin irritation was observed.

- **Flatness test**

Initial length of the strip = 6.5 cm
Final length of the strip = 6.4 cm
Hence, The flatness of the patch was found to be 98.46 %.

CONCLUSION

As there is less information available on anti-inflammatory action against the bites and stings of various organisms of the leaf extract of *Aristolochia indica* and *Boerhavia diffusa*, hence the morphological studies, microscopical studies, physicochemical parameters and chemical tests were performed for the authentication of the purity of the plant as well as identification of anti-inflammatory effects of the leaves. The invitro anti-inflammatory activity is carried out by Inhibition of Albumin Protein Denaturation Test. On evaluating the result, Albumin Denaturation Test illustrated that the extracts of *Aristolochia indica and Boerhavia diffusa* has greater percentage inhibition. Transdermal patches with ethosomal preparation of the leaf extract of *Aristolochia indica and Boerhavia diffusa* were prepared. The nano formulation (Ethosomes) of the extracts contributed to the increase in absorption of the drug through the skin.

Conflict of interest

Authors declare no conflict of interest.

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