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FORMULATION AND EVALUATION OF PHYTOSOME LOADED ANTIPYRETIC TRANSDERMAL PATCH OF *ALTERNANTHERA BRASILIANA* (L.) KUNTZE

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ABSTRACT
Alternanthera brasiliana (L.) Kuntze or Brazilian joy weed is a perennial herbaceous plant belonging to the *Amaranthaceae* family. It all the plant parts have been used medicinally however the leaves are of prime importance as it is used in folkloric treatment of malaria, pains, infections and diabetes. In the present work deals with the preparation of phytosome by using ethanolic extract of *Alternanthera brasiliana* (L.) Kuntze leaves and their evaluation such Entrapment efficiency, Scanning Electron Microscopy (SEM analysis), Particle size determination and Zeta potential. *Alternanthera brasiliana* (L.) Kunzte and formulation and evaluation of phytosome loaded transdermal patches.

KEYWORDS: *Alternanthera brasiliana* (L.)Kuntze, Ethanolic leaves extract, Phytosome, SEM, Zeta potential, Transdermal patches.

INTRODUCTION
Native to South and Central America, *Alternanthera brasiliana* (L.) Kuntze is a flowering plant of the Amaranthaceae family. Penicillin or joy weed are common names for the perennial herbaceous plant *Alternanthera brasiliana* (L.) Kuntze in Brazil.[1] All parts of the plant have been used medicinally, but the leaves are particularly significant because they are a common folk remedy for diabetes, aches, infections, and malaria. In North East India, it has historically been used as a hemostatic. It has a round to polygonal stem, lengthy internodes, and swollen nodes at which opposing leaves attach. It is characterized as perennial, prostrate, and branchy.[2]

Traditionally it is used as hemostatic in north east India. It is described as perennial, prostrate and branchy, presenting a circular to polygonal stem, long internodes and swollen nodes at which opposite leaves attach. The inflorescence is cymes, composed of hermaphrodite, actinomorphous and monocyclic flowers.[3]

Figure 01: Alternanthera brasiliana (L.) Kuntze.

MATERIALS AND METHODS
Preparation of *Alternanthera brasiliana* (L.) Kuntze leaf extract
The plant materials (leaves) of *Alternanthera brasiliana* (L.) Kuntze were collected and then shade dried and coarsely powdered. 30 g of coarsely powdered leaves was packed in Soxhlet apparatus and extracted by using 1000ml ethanol (80%) (approx. 2days). The extract was collected. The extract was then filtered through Whatmann No. 1 filter paper and concentrated.[4]

Preparation of Phytosome of *Alternanthera brasiliana* (L.) Kunzte Ethanolic leaves Extract (ABEE)
Rotary Evaporation Technique

- 300mg of plant extract and soya lecithin (1:1) were dissolved in chloroform in a rotary round bottom flask
- Stirred for 2 hr at a temperature < 40˚C
- Thin-film of the sample was obtained to which n-hexane was added, continuously stirred until a monolayer of phospholipid was obtained
- Added Phosphate buffer 6.8 and lyophilized
- Dried powder were collected, packed in an amber-coloured glass bottle and stored at room temperature
- Phytosomes complex (F1, F2, and F3) was prepared by rotary evaporation method in the ratios of (1:1, 1:2, 1:3) by varying the polymer concentration.[5][6][7]

Table 01: Ingredients for the formulation of phytosome.

SL. No.	Formulation Code	Ratio Drug: Soya lecithin	Chloroform (ml)	N-hexane (ml)	Phosphate buffer 6.8 (ml)
1	F1	1:1	20	15	5
2	F2	1:2	20	15	5
3	F3	1:3	20	15	5

Figure 02: Rotary vacuum evaporator.

Figure 03: Phytosome.

Evaluation of Phytosome
Evaluation

- SEM Analysis (SCANNING ELECTRON MICROSCOPY
- Particle size
- Zeta Pottential
- Drug Entrapment Efficiency

SEM(Scanning Electron Microscopy) analysis
Scanning electron microscopy study was done to determine the surface morphology, size and shape of prepared phytosomes. The freeze dried Phytosomes was subjected for Scanning electron microscopy and photographed.[8][9]

Particle size
The particle size of phytosomes was measured by particle size analyzer **(Microtrac).**

Zeta potential
Zeta potential (complete charge generated by medium) defines the charge of phytosomes in emulsions. Zeta potential may be negative, positive, or neutral depending on the composition of the phytosomes. ZP could reflect the stability of phytosomes in a medium; in fact, charged particles repel each other enough to maintain stability. Phytosome emulsion with a zeta potential ±30 mV is known to be stable. ZP measurement of the optimized phytosome suspension was done by using the zeta sizer.

For the measurement, 1ml of the sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette and the ZP was measured.

Drug Entrapment Efficiency
Entrapment efficiency (EE) describes the amount of phytochemical that is embedded in the phytosomes. It is determined by ultracentrifugation method. Here the Phytosomes were centrifuged at 12000 rpm for 45 min to separate the phytosomes from unentrapped drug. Concentration of free drug as the supernatant was determined by measuring the absorbance at 462nm using UV-Visible spectrometer.[10][11]

$$Entrapment\ efficiency\ (\%) = \frac{amount\ of\ substance\ entarped}{\text{total amount of substance added}}\ X\ 100$$

Formulation Studies
Formulation of Antipyretic Phytosome Loaded Transdermal Patch
Alternanthera brasiliana (L.) Kuntze Antipyretic transdermal patch was prepared by adding phytosomes as the main ingredient in the official formula for transdermal patch preparation by solid dispersion method. After completion of formulation, it was evaluated for its physicochemical parameters such as: [12][13][14]

- Organoleptic evaluation
- pH evaluation
- Uniformity of weight
- Percent of moisture content
- Folding endurance
- Percentage elongation break test

Ingredients for the Formulation of Transdermal Patches

Table 2: Ingredients for the Formulation of Transdermal Patches.

SL NO	INGREDIENTS	ROLE
1	Phytosome	Active pharmaceutical ingredient
2	Chloroform	Dissolution of polymer
3	Ethyl cellulose	Patch forming polymer
4	HPMC	Film former
5	Ethanol	Solvent
6	Glycerin	Improves elasticity
7	Poly ethylene glycol 400	Plasticizer
8	Menthol	Permeation enhancer

Quantity of ingredients for Transdermal Patches of code F1, F2, F3, F4, F5

Table 3: Quantity of ingredients for the Formulation of Transdermal Patches.

INGREDIENTS	F1	F2	F3	F4	F5
• Phytosome	50 mg	50 mg	50 mg	50 mg	50 mg
• Chloroform	6 ml	6 ml	6 ml	6 ml	6 ml
• Ethyl cellulose	50 mg	100 mg	150 mg	200 mg	250 mg
• HPMC	450 mg	400 mg	350 mg	300 mg	250 mg
• Ethanol	6 ml	6 ml	6 ml	6 ml	6 ml
• Glycerin	0.001 ml	0.001 ml	0.001 ml	0.001 ml	0.001 ml
• PEG 400	0.3 ml	0.3 ml	0.3 ml	0.3 ml	0.3 ml
• Menthol	15 mg	15 mg	15 mg	15 mg	15 mg

Procedure for the Formulation of Transdermal Patches

- HPMC was dissolved in ethanol and chloroform in the ratio 1:1 using magnetic stirrer
- Then menthol and ethyl acetate were added to HPMC solution and homogenized using a magnetic stirrer
- Then PEG 400 was added to the mixture
- Ten mg of phytosome were added to the solution and glycerin is added
- And the mixture was homogenized using magnetic stirrer for 15 min until the dope solution formed
- 10 ml resulted solution was then poured into a plate and oven-dried at 30 ^{0}C for 24 hrs.
- The resulted plates were then taken and placed into desiccator for future evaluation.[15]

Fig. 04: Magnetic stirrer.

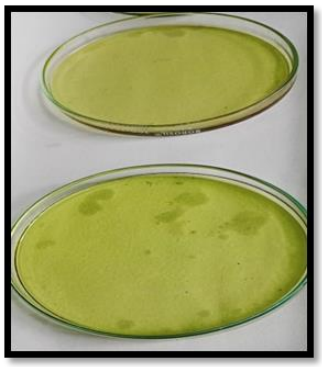


Fig. 05: Batter for patch.

Evaluation of Antipyretic Transdermal Patches

Quality of the patch is assessed in terms of parameters like uniformity of weight, folding endurance, percentage elongation break test, thickness of patch.[16]

Organoleptic Evaluation

The organoleptic evaluation was conducted by observing the color, odour, and texture of the patches.

pH Evaluation

The patches were cut (1 x 1 cm2) and immersed into 1 ml of distilled water for 2 hr at room temperature. A filtration process was then employed for removing excess water from the test tube. The pH meter was placed at three different places at the swollen part of the patch.

Uniformity of weight
For each formula, each of the three patches was weighed, and the average weight was then calculated.

Percentage of Moisture Content
The percentage of moisture content of the patches was evaluated by weighing the patches and then placed them into desiccator containing activated silica at room temperature for 24 hrs. After 24 h the patches were re-weighed.

The percentage of moisture content was calculated by the following formula

$$\text{Moisture content } (\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \text{ X } 100$$

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 give the value of folding endurance.

Percentage Elongation Break Test
The percentage elongation is to determine by noting the length just before the breakpoint.
The percentage elongation can be determined from the below mentioned formula:
ELONGATION PERCENTAGE= [L1-L2 / L2] × 100

Where L1 is the final length of each strip
L2 is the initial length of each strip

RESULT AND DISCUSSION

Preparation of Phytosomes of ABEE

- The phytosome was prepared by using the rotary evaporation method
- Phytosomes complex (f1, f2, f3) was prepared by rotary evaporation method in the ratios of (1:1, 1:2, 1:3) by varying the polymer concentration.

Figure 6: Phytosome Obtained.

Evaluation of Phytosome

- **Entrapment Efficiency (%)**

Table 4: Entrapment Efficiency of Phytosome.

SL. No.	Formulation Code	Ratio Drug: Soya lecithin	Entrapment Efficiency (%)
1	F1	1:1	93%
2	F2	1:2	98%
3	F3	1:3	97%

Drug entrapment efficiency = **98 %**

- From the prepared Phytosomes, the drug entrapment efficiency was tested.
- The entrapment efficiency of F2 was found to be higher and F2 was selected as the optimized phytosome.

Scanning Electron Microscopy (SEM)

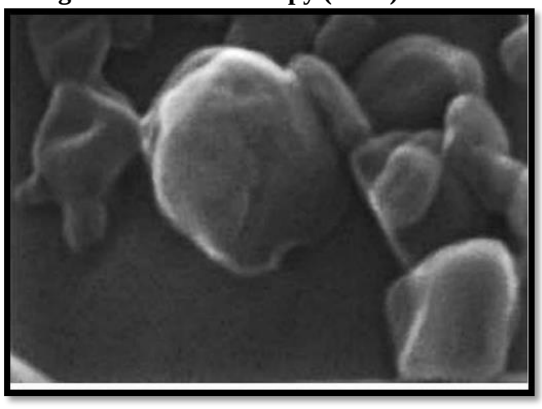
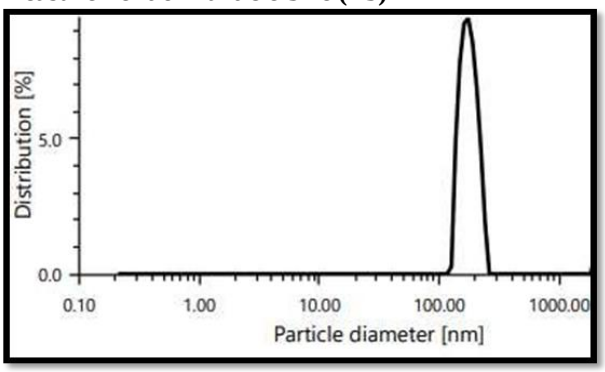


Fig. 7: SEM Image of Optimized Phytosome.

The SEM image shows that the surface morphology of the optimized phytosomes indicates they are spherical in shape.

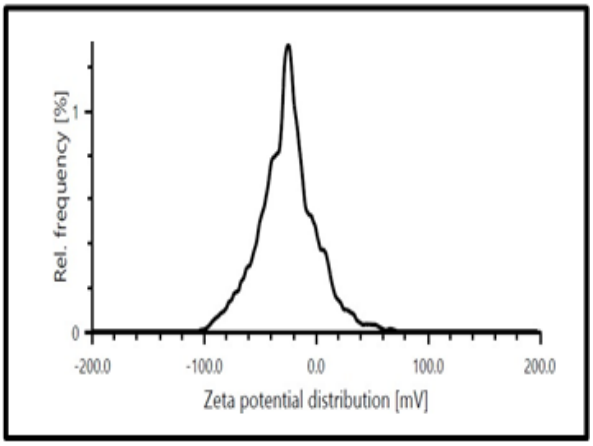
Measurement of Particle Size (PS)



Graph No 1: The particle size of the phytosome
Particle size = 176.88 nm

The particle size of the optimized phytosome was found to be **176.88** nm. This was in accordance with the particle size range of Phytosomes.

ZETA Potential



Graph No: 02: Zeta potential graph of prepared phytosomes.

Zeta potential of the optimized phytosome was found to be **-23.6 mv**. This indicates that the sample is highly stable and do not form aggregates.

Formulation of Antipyretic Transdermal Patches

Formulation of Antipyretic transdermal patch was done by solid dispersion method. The patches obtained were subjected to evaluation parameters including

- Organoleptic evaluation
- pH evaluation
- Uniformity of weight
- Percent of moisture content
- Folding endurance
- Percentage elongation test

Fig. 8: Prepared Patches.

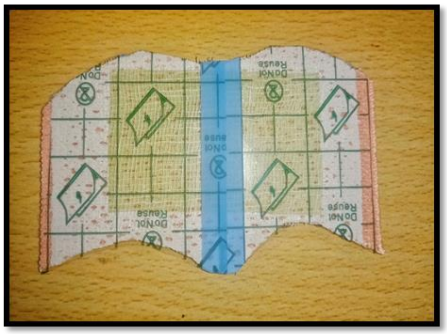


Fig. 9: Phytosome Loaded Transdermal Patch.

Evaluation of Phytosome Loaded Antipyretic Transdermal Patch

1. Organoleptic Evaluation

The organoleptic evaluation of the five formulas showed that all of the formulas generated a smooth surface texture, dry, elastic, yellow, menthol-odour, and transparent properties.

Table 5: Organoleptic evaluation of transdermal patch.

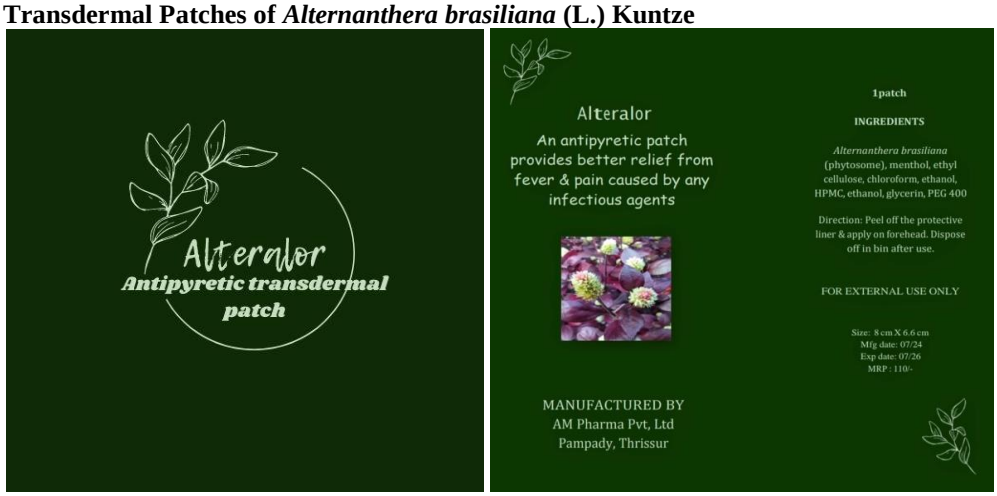
SL. No.	Characteristics	Observations
1	Color	Pale green to yellowish
2	Odour	Menthol odour
3	Surface	Smooth
4	Texture	Dry & elastic
5	Appearance	Transparent

2. Overall evaluation of phytosome loaded antipyretic transdermal patch of ABEE.

Table 6: Overall Evaluation of Phytosome Loaded Antipyretic Transdermal Patch of ABEE.

Sl. No.	Evaluation Parameters	F1	F2	F3	F4	F5
1	p H	5	5	6	6	5
2	Uniformity of weight (g)	0.56	0.55	0.53	0.52	0.50
3	Percentage moisture content (%)	6.29	5.55	3.70	1.88	1.69
4	Folding endurance (times)	98	90	85	72	60
5	Percentage elongation test (%)	3.84	3.56	2.87	2.35	1.87

Labelling of Transdermal Patches of *Alternanthera brasiliana* (L.) Kuntze



SUMMARY

- The overall summary of the thesis deals with the studies in the plant species of *Alternanthera brasiliana* (L.) Kuntze belonging to the family, *Amaranthacea.*
- Rotary vacuum evaporator was used for the formulation of phytosome and the formulation code F2 was found to be high entrapment efficiency and it was selected as the optimized phytosome.
- This optimized phytosome was evaluated for SEM analysis, zeta potential and particle size determination. And the optimized phytosome was incorporated into transdermal patch formulation.
- Solid dispersion method was carried out for the formulation of transdermal patches by varying concentration of HPMC and ethyl cellulose.
- Then the evaluation such as organoleptic, moisture content, uniformity of weight, pH, and folding endurance was performed.
- And the result showed amount of HPMC directly proportional to the moisture content. Because they have the ability to absorb the moisture content and also the uniformity of weight. pH of the patches was lied in the pH range of the skin. Folding endurance number gives the mechanical property. The folding endurance number was increased with increasing HPMC level.
- These results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

CONCLUSION

- The use of the medicinal plant as a primary health care was popular from ancient time because plants provide many health benefits for humans.
- This research work was carried out to develop a Phytosome loaded Antipyretic transdermal patch by using the leaves of *Alternanthera brasiliana* (L.) Kuntze. It is a perennial herbaceous plant which is widely found around the world and it is well known for their traditional use.
- The administration of the medicament in the form of Phytosome loaded transdermal formulation will be more effective and convenient for the drug delivery. And it will produce rapid onset of action at the desired site.

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