

DESIGN AND DEVELOPMENT OF INNOVATIVE VESICULAR DERMAL DELIVERY INTEGRATING SYNTHETIC AND HERBAL AGENTS FOR ENHANCED MANAGEMENT OF ANTIARTHRITIC ACTIVITY

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Abstract: *The formulation and evaluation of Transferosomes-loaded transdermal patches containing Clopidogrel bisulfate (CB) and Kalanchoe pinnata (KP) were explored for the management of rheumatoid arthritis (RA). Clopidogrel bisulfate is an antiplatelet drug with low oral bioavailability (50%) and undergoes extensive first-pass metabolism, necessitating frequent high doses. Although CB is not a traditional anti-inflammatory drug, its role in reducing platelet aggregation can indirectly influence inflammatory processes in RA. By minimizing platelet activation, it may help alleviate some inflammatory responses associated with joint damage. This study investigates the anti-inflammatory effects of clopidogrel. KP is known for its anti-inflammatory effects. It contains various bioactive compounds that can inhibit pro-inflammatory cytokines and enzymes involved in the inflammatory process, such as cyclooxygenase (COX) and lipoxygenase (LOX). This action helps in reducing swelling and pain associated with RA. The plant also exhibits immunomodulatory effects, which can help in balancing the immune response in RA patients. CB primarily functions through its antiplatelet action and indirect modulation of inflammation, while KP offers direct anti-inflammatory and immunomodulatory benefits, making their combination potentially effective for managing rheumatoid arthritis. Transferosomes, advanced vesicular carriers, were incorporated to enhance the transdermal delivery of CB, aiming to improve bioavailability, enhance therapeutic outcomes and patient compliance in RA treatment. Transdermal patches were then fabricated using different polymers to ensure sustained drug release. These patches deliver the drug directly into systemic circulation through the skin, bypassing first-pass metabolism. The patches were assessed for physical properties such as weight uniformity, thickness, flexibility, and folding endurance. In vitro drug release, anti-arthritis activity and anti-microbial activity were studied. The developed transdermal patches may increase the therapeutic efficacy and reduce the toxic effect of clopidogrel bisulfate. The findings suggest that the addition of KP was shown to enhance the anti-inflammatory effect, supporting a synergistic action that may provide better management of conditions like cardiovascular disease and inflammation. Transferosomal transdermal patches are designed to enhance drug delivery and bioavailability, overcoming the drawbacks of oral drug delivery.*

Keywords: *Kalanchoe pinnata, Clopidogrel bisulfate, Transferosomes, Anti-inflammatory and arthritic effects.*

INTRODUCTION

The concentration of the drug in the body must be maintained at therapeutic level for long period. To increase the drug concentration and to overcome the disadvantages of oral administration, it is necessary to find another route of administration. The skin has recently emerged as a promising route for drug delivery. Its surface area and ease of administration make it the best alternative for drug administration. The main disadvantage of transdermal drug delivery (TTD) system is that they have lower permeability to hydrophilic drugs and macromolecules have difficulty in penetrating the skin. Several types of methods have been developed to overcome this problem. Stratum corneum of the skin is the rate limiting membrane that allows only a selected drug to penetrate the skin. Therefore, to overcome this problem, it has planned to formulate transferosomes and produce them in the form of transdermal patches. Transferosomes, which are prepared from phospholipids and edge activators (surfactants). The surfactant is mainly a single chain surfactant with a larger radius of curvature that destabilizes lipid bilayers and increases the deformability of the vesicle. Surfactants have been used as leading activators. Compared to oral administration, transferosomes show improved skin permeability for some drugs and the right amounts of drugs are transferred effectively ¹. RA is a systemic autoimmune disease characterized by inflammatory arthritis and extra-articular involvement. It is a chronic inflammatory disorder caused in many cases by the interaction of genes and environmental factors, including smoking, that primarily affects synovial joints. It usually begins in the peripheral lymph nodes, is usually symmetrical, and progresses to involve proximal lymph nodes if left untreated. Inflammation of the joints over time leads to their destruction with loss of cartilage and bone erosion. RA where symptoms last less than six months is defined as early RA, and when symptoms have been present for more than six months, it is defined as established RA. If RA, left untreated, is a progressive disease that leads to increased morbidity and mortality. There is no laboratory test and the arthritis of rheumatoid, which is difficult to diagnose this disease in the first fits. A total clinical approach is required to establish diagnosis and prevent damage to common wounds. The patient treatment with rheumatoid arthritis requires tromarology therapy and non-haremacological therapy. Today, the care rate is the early treatment with anti-noisy drugs that change illness. Despite treatment, many patients advance to disability and suffering from the meaningful morbidity with time. Fair and un-farmological treatment (physiotherapy, patients and patient's tips) is required to improve clinical results ². KP is a medicinal plant widely used in folk medicine for the treatment of kidney stones, stomach ulcers, lung infection, rheumatoid arthritis, etc. *Kalanchoe pinnata* is neutralized in the temperate regions of Asia, Australia, New Zealand, the Antilles, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia and Hawaii. In many of these areas, such as Hawaii, it is considered as invasive species. In French Polynesia, *Kalanchoe pinnata* has been declared a threat to biodiversity. It is also widely distributed in the Philippines and is known as *katakataka* or *kataka-taka*, which is also an adjective meaning amazing or extraordinary. In India it grows in gardens and wild in the mountains from north-western India, the Deccan and Bengal ³. It has a wound healing property, Hepatoprotective & Nephroprotective activity, Antihistamine activity, Hypoglycaemic activity, Anti-inflammatory activity, Anti-depressant, Antioxidant, Anti-cancer, Diuretics and anti urolithiatic activities⁴. CB is a thienopyridine drug that initially has little or no inhibitory effect on platelets. Its active form (active metabolite) is produced by metabolism of prodrug via the hepatic cytochrome P450 system, more specifically by the enzyme CYP2C19. The active metabolite permanently and irreversibly inactivates the platelet G protein receptor known as P2Y12. P2Y12, normally activated by ADP, induces a conformational change in the surface GPIIb/IIIa molecule. This conformational change increases the affinity of GPIIb/IIIa for divalent binding molecules fibrinogen and VWF, thereby allowing platelet aggregation. Thus,

inactivation of this G protein-activated receptor by clopidogrel results in decreased platelet aggregation ⁵. CB has recently been shown to have anti-inflammatory properties. It may exert its beneficial effects by “cooling down” two important pathogenic pathways, platelet reactivity and inflammation. While consuming CB produces rashes. KP has anti-histamine property. While combining KP with CB will reduce causing of rashes ⁶.

MATERIALS AND METHODS:

Materials: Clopidogrel bisulfate, HPMC, PVP, Carbopol 940, Ethanol, Chloroform, PEG 400, Glycerol, Tween 80, Soya lecithin was procured from Lobachemie Pvt. Ltd, Mumbai India. Plant extract, Phosphate buffer and Distilled water were prepared at Pharmaceuticals laboratory, SVCP, Namakkal, India.

Methods:

Construction of Standard Curve for CB

Estimation of pure CB: It was estimated spectroscopically at 219 nm.

Preparation of 0.1 N HCl: Dilute 8.5 ml of concentrated HCl with water to produce 1000ml.

Preparation of standard drug solution

Stock solution: 100 mg of CB was transferred to a 100 ml volumetric flask and dissolved in 0.1 N HCl. Methanol was added to the flask to bring the volume up to the required level, resulting in a 1000 µg/ml concentration.

Standard solution: 10 ml of this solution was pipetted out and put into a 100 ml volumetric flask. The volume was adjusted using methanol to produce a solution of 100 µg/ml. From this solution, further dilutions were prepared with 0.1 N HCl. Thus, the final concentration ranges from 10-50 µg/ml as per Beer-Lambert's law. Moreover, the samples were analysed spectroscopically at their λ max 219 nm. The absorbance of each sample was plotted versus concentration. The measured absorbance was given in the (Table 1) ⁷.

Flow chart for preparation of standard drug solution: 100 mg of clopidogrel taken in 100 ml volumetric flask. Dissolved in 0.1 N HCl and shake well. Make the volume up to the mark using methanol (1000 µg/ml). Pipette out 10 ml of above solution and transfer into 100 ml volumetric flask. Volume was adjusted using methanol (100 µg/ml). Further dilutions were prepared with 0.1 N HCl to achieve required concentration (10-50 µg/ml) are shown in (Figure 1 and Figure 2).



Figure 1: Preparation of standard solution

Dilution chart:

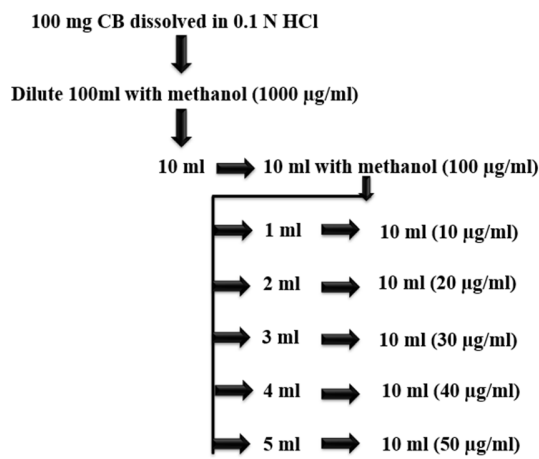


Table 1: Absorbance of clopidogrel bisulfate at 219 nm

S. No	Concentration (µg/ml)	Absorbance at 219 nm
1	0	0
2	1	0.2143
3	2	0.4156
4	3	0.6278
5	4	0.8321
6	5	1.0345

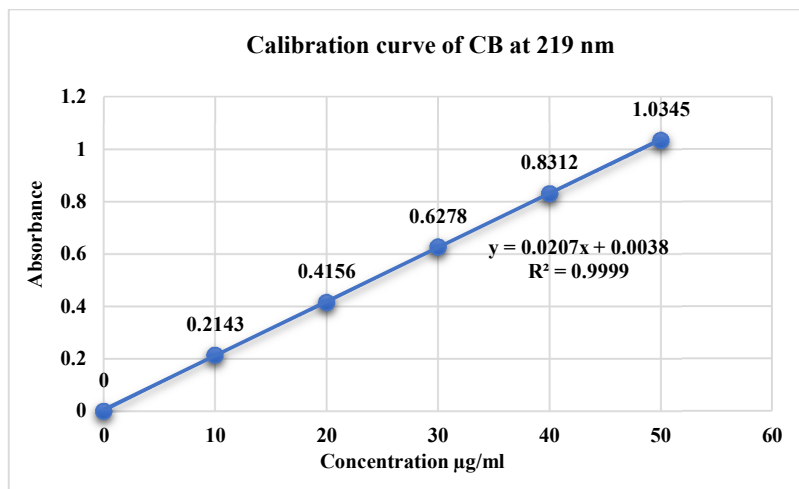


Figure 2: Calibration curve for clopidogrel bisulfate at 219nm

COLLECTION AND EXTRACTION OF PLANT MATERIAL:

The fresh leaves of *kalanchoe pinnata* were collected. After that, the plants were thoroughly cleaned with tap water, rinsed with distilled water, and allowed to air dry at room temperature for a day. Without adding water, *kalanchoe pinnata* leaves were crushed in a mortar. The Soxhlet extraction method was used to extract the dried powdered leaves of *kalanchoe pinnata* with a hydroalcoholic solvent for 24 hours are shown in (Figure 3 and 4). The particles were then filtered and dried at 40°C using a vacuum evaporator. Ultimately, the percentage yield of dried extract was computed^{7,8}.



Figure 3: Soxhlet extraction of powdered leaves

Flow Chart for Extraction of Kalanchoe Pinnata Leaves

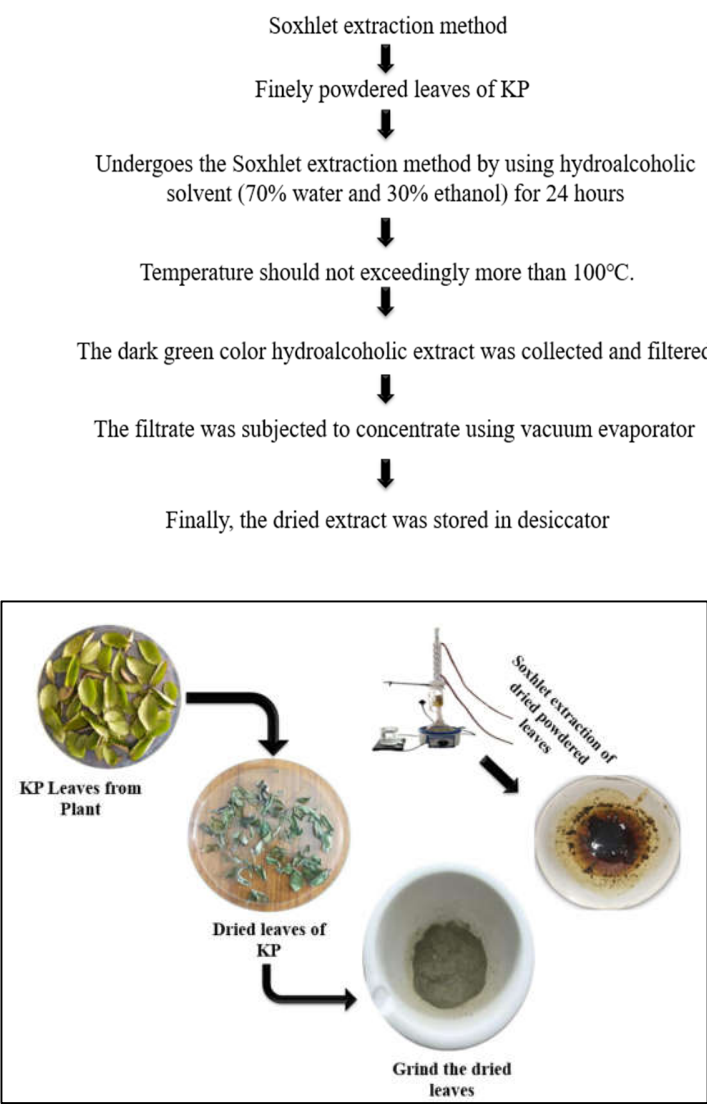


Figure 4: Diagrammatic representation of Collection and Extraction of Kalanchoe Pinnata Leaves

PROCEDURE FOR FORMULATIONS

Preparation of Transfersosomal Suspension

Transfersomes were prepared by the ethanol injection method and its composition are shown in (Table 2). weigh required amount of Phospholipid, edge activator and lipophilic drug and was dissolved in ethanol (organic phase). Stir the mixture continuously until a clear solution was obtained. The plant extract was dissolved in the phosphate buffer (aqueous phase). Both solutions are heated to 45-50°C. Now the ethanolic solution was injected dropwise in the aqueous phase while continuously stirring for the specified period.

Then the mixture was transferred to vacuum evaporator for the removal of ethanol and further sonicated for the reduction of particle size are illustrated in (Figure 5) ^{9,10}.

Flow Chart for Preparation of Transfersomal Suspension: Ethanol Injection method

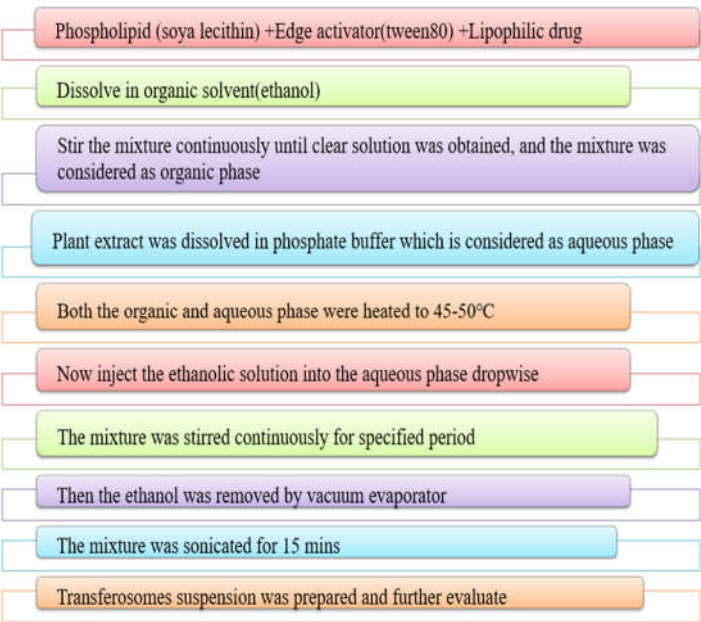


Table 2: Composition of Transfersomal Suspension

Components	Quantity	Function
Soya lecithin	80 mg	Lipid
Tween 80	0.2 ml	Edge activator
Drug	75 mg	Active ingredient
Ethanol	QS	Solvent
Plant extract	3 mg	Active ingredient
Phosphate buffer	QS	Aqueous phase

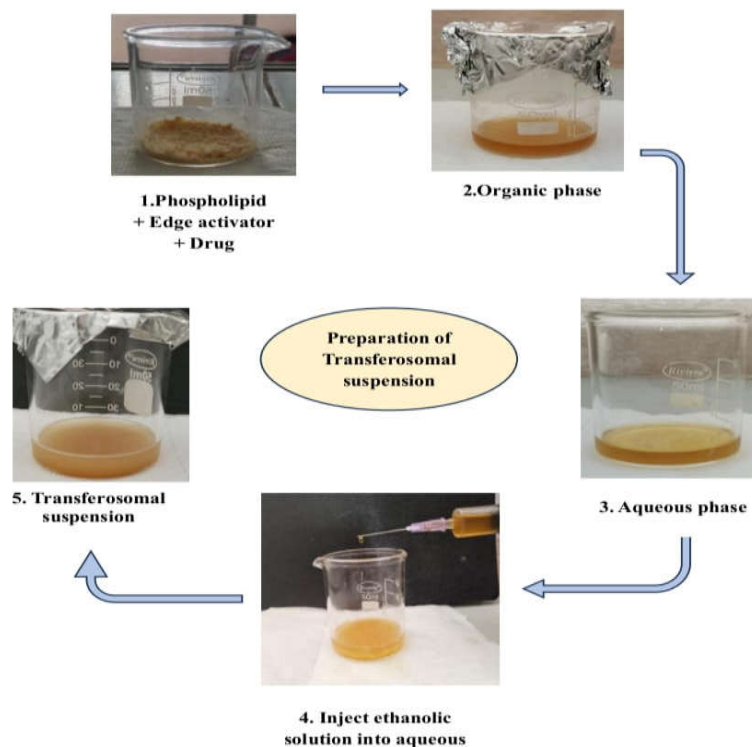


Figure 5: Formulation of the Transfersomal Suspension

FABRICATION OF TRANSDERMAL PATCHES

The solvent casting evaporation technique was used to produce the patches. A range of polymers were incorporated. Required amount of solvent Ethanol: Chloroform (1:1) were mixed with varying amounts of polymers. To produce a clear solution, the polymeric dispersion was agitated for roughly ten minutes using a magnetic stirrer. To the aforesaid solution, weighed amounts of Tween 80 and polyethylene glycol 400 were added. Using a magnetic stirrer, suitable amount of above prepared Transfersome suspension was well combined over a few minutes. The resulting homogeneous solution was poured into a petri plate and placed in an inverted funnel to help regulate solvent evaporation and prevent patch cracking. They set this aside for the night. After being taken off the plate, dried pieces were sliced and placed in desiccator ¹¹.

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Flow Chart for Fabrication of Transdermal Patches

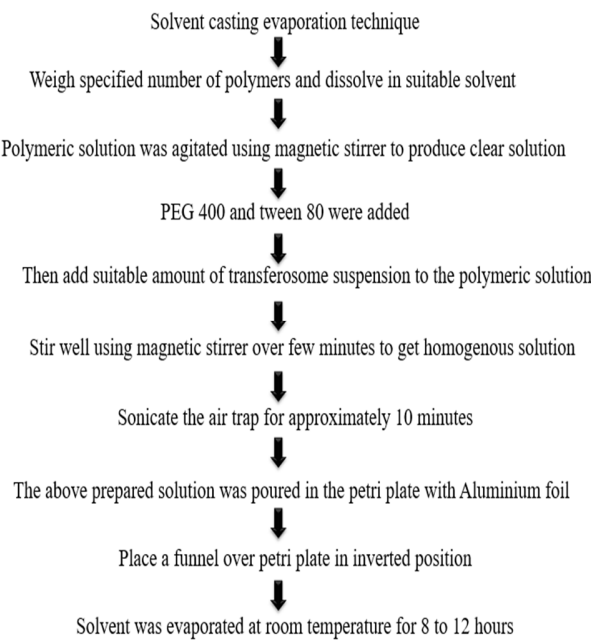


Table 3: Composition of Transdermal Patches

Ingredients	Patch A	Patch B	Patch C
Hydroxy propyl methyl cellulose	1g	0.8g	1.25g
Poly vinyl pyrrolidone	0.75g	-	-
Carbopol 940	-	0.1g	-
PEG 400	0.3ml	0.3ml	-
Tween 80	-	-	0.75ml
Ethanol: chloroform	12.5ml: 12.5ml	12.5ml: 12.5ml	12.5ml:12.5ml
Transferosomal suspension	10ml	10ml	10ml

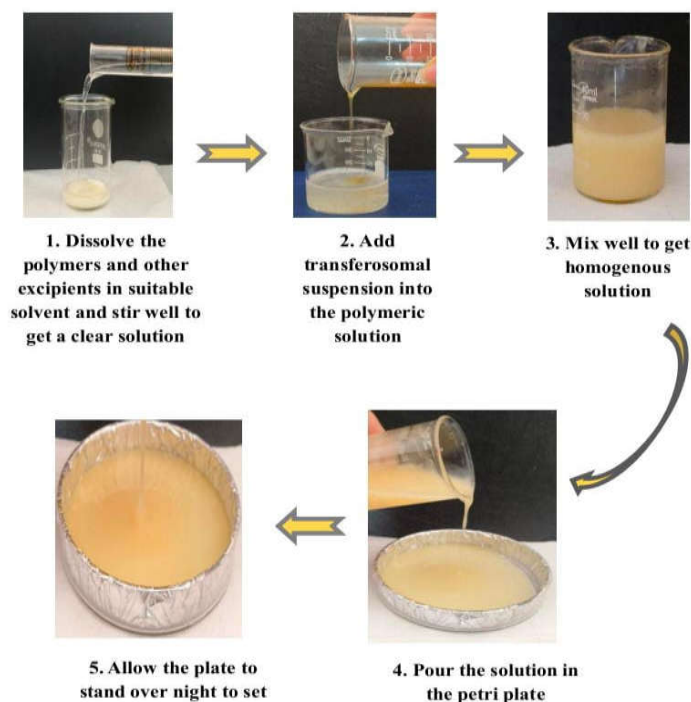


Figure 6: Formulation Of Transfersomes- Loaded Transdermal Patches

EVALUATION AND STANDARDIZATION:

Estimation of Plant Extract: Pharmacognostical Studies

Morphological Studies: The drug is studied for its morphological characters like colour, odour, texture.

Physio- Chemical Parameters: The drug is subjected to various physiochemical parameters such as loss on drying and ash value.

a) Determination of Loss on drying: Take two grams crude powder in evaporating dish and dried it in oven at 105° C until its constant weight was obtained. After drying the weight was noted and loss on drying was calculated. The percentage was calculated based on sample taken initially.

$$\text{Loss on drying \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

were,

Initial weight = Weight of the sample before drying, Final weight = Weight of the sample after drying

- b) **Ash value:** 2 gram of crude drug was weighed accurately and transfer it into a crucible. Before transferring the crude drug, the crucible must be weighed and noted. Then the crucible with the drug was incinerated by increasing temperature. It was incinerated until it becomes red hot until free from carbon. Then the crucible was cooled and weighed. Finally, the ash content was calculated.

$$\text{Ash content \%} = \frac{\text{Weight of Ash}}{\text{Initial weight of Sample}} \times 100$$

were,

Weight of Ash = Residue weight after complete incineration of the sample, Initial Weight of Sample = Weight of the sample before incineration¹³.

Phytochemical Analysis

1) Test for Carbohydrates

Molisch's test: Few drops of Molish reagent were added to the aqueous solution of sample and then add concentrated hydrochloric acid to sidewall of the test tube. Formation of purple colour coloured ring at the junction of two phases confirms the presence of carbohydrate.

2) Test for Reducing sugar

Fehling's Test: Equal volumes (1 mL each) of Fehling's A and Fehling's B solutions were mixed. To this mixture, 2 mL of the aqueous sample solution was added, and the solution was heated in a water bath for 5–10 minutes. The formation of a reddish-brown precipitate indicated the presence of reducing sugars.

Benedict's Test: To 4 mL of the aqueous sample solution, 1 mL of Benedict's solution was added and heated to boiling. The appearance of a green colour confirms the presence of reducing sugars.

3) Test for Alkaloids

Mayer's test: To the sample solution add few drops of Mayer 's reagent. Formation of white precipitate confirms the presence of alkaloids.

4) Test for Saponins

Forth test: To the aqueous solution of sample add 5ml of distilled water and it was mixed vigorously. Formation of foam indicates the presence of saponin.

5) Tests for Flavonoids (Quercetin)

Lead Acetate test: Take few drops of 10 percent lead acetate and add it into the drug extract. There will be a development of yellow coloured precipitate which confirms the presence of Flavonoids.

Sodium Hydroxide test: Continuous increasing amount of Sodium Hydroxide to the extract gives yellow colour and disappeared after the addition of acid ^{14,15}.

EVALUATION TEST FOR TRANSDERMAL PATCHES

1. Physical appearance: All the prepared patches were physically examined for their colour, clarity, flexibility, and homogeneity.

2. Thickness of the patch: The thickness of the drug-loaded patches was determined using a screw gauge micrometre at three different points on each patch. The average thickness and standard deviation values were then calculated for each drug-loaded patch.

3. Weight uniformity: Three randomly selected patches from each formulation were used for the weight variation test. Each film from the batch was weighed individually, and the average weight was then calculated.

4. Folding Endurance: The folding endurance of the prepared films was determined manually. A strip of film (5 × 5 cm) was cut and repeatedly folded at the same spot until it either broke or developed visible cracks. The number of folds the film could withstand before failure was recorded as its folding endurance¹⁶.

5. Drug content uniformity: Each patch from different formulations (patch size of 1 cm², equivalent to 25 mg of drug) was dissolved in phosphate buffer (pH 7.4) and continuously shaken for 24 hours using a magnetic stirrer to extract the drug. After filtration and dilution with phosphate buffer, the percentage of drug content was measured spectrophotometrically at a wavelength of 219 nm¹⁷.

6. Percentage Moisture content: This test was conducted to evaluate the integrity of the films under dry conditions. Individual transdermal patches (of a specified area) were placed in a desiccator containing fused anhydrous calcium chloride at room temperature. The films were weighed at regular intervals of 24, 48, and 72 hours. The percentage moisture content was calculated using the following formula¹⁸.

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

7. Percentage moisture uptake: The patches were accurately weighed and placed in desiccators containing 100 mL of a saturated potassium chloride solution, which maintains a relative humidity (RH) of 80–90%. After three days, the patches were removed and reweighed. The study was conducted at room temperature. The percentage moisture absorption was calculated using the following formula:

$$\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

8. Water vapour transmission rate: Glass vials with a 5 mL capacity were thoroughly washed and dried to a constant weight in an oven. Approximately 1 g of fused calcium chloride was placed in the vials, and polymer patches of 1.44 cm² were fixed over the brim using adhesive tape. The vials were then weighed and stored in a humidity chamber at 80-90% RH for 24 hours. The vials were removed and weighed at 24-

hour intervals for three consecutive days to measure the weight gain. The water vapor transmission rate (WVTR) was calculated using the following formula:¹⁶

$$\text{Water vapour transmission rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time} \times \text{Area}} \times 100$$

9. Flatness study: The flatness study was conducted to evaluate whether the prepared transdermal patches maintained a smooth surface and did not constrict over time. Three longitudinal strips were cut from different portions of the film, and their initial lengths (l_1) were measured. The variation in length due to non-uniformity in flatness was determined by calculating the percent constriction, where 0% constriction corresponds to 100% flatness. The percent constriction was calculated using the following formula:

$$\% \text{Constriction} = \frac{l_1 - l_2}{l_1} \times 100$$

Where,

l_1 is the initial length of the strip

l_2 is the final length after the study.

10. Surface pH: The patches were placed in glass tubes in contact with 0.5 mL of double-distilled water and allowed to swell for 1 hour. A combined glass electrode was then brought near the surface of the swollen patch, and pH readings were recorded after allowing an equilibration period of 1 minute¹⁷.

11. In-vitro % drug release: The in vitro drug release of the transdermal formulations was evaluated using a Franz diffusion cell, a reliable method for predicting drug transport across the skin from topical formulations. The receptor compartment of the diffusion cell was filled with phosphate-buffered saline (pH 7.4), and synthetic cellophane membrane was used as a diffusion barrier. The prepared formulations were applied to the membrane in the donor compartment and uniformly spread onto the cellophane membrane. The assembly was maintained at $37.0 \pm 2.0^\circ\text{C}$ with continuous stirring at 50 rpm. Samples (1.0 ml sample) were withdrawn at specific time intervals (0, 5mins, 15mins, 30mins, 45mins, 1, 2, 3 and 4hours) and replaced with an equal volume of fresh medium to maintain the receptor phase volume at 30 ml. The withdrawn samples were analyzed spectrophotometrically at 219 nm to determine the drug release¹⁸.

13. Compatibility studies: The compatibility of the drug with excipients was assessed using Attenuated Total Reflectance-Fourier Transform Infrared (AT-FTIR) spectroscopy. A minute quantity of the liquid sample was directly analyzed on the instrument. The FTIR spectra of the individual drug, excipients, and their physical mixture were compared to detect any potential incompatibilities. To ensure accurate results, multiple scans were performed, yielding smooth spectra at a specified resolution¹⁹.

14. SEM Analysis: Scanning Electron Microscopy (SEM) analysis was performed using a Specify Model SEM instrument, where transdermal patches were prepared, mounted on electrically conductive mounts, and coated with a thin layer of gold to enhance conductivity. Imaging was performed at multiple magnification levels to capture detailed surface morphology and microstructural features in various regions, ensuring comprehensive representation of the sample. This protocol enabled the examination of surface topography and microstructure, providing valuable insights into the properties of the transdermal patches²⁰.

15. Particle size measurement and Polydispersity Index: Particle size measurement and polydispersity index is one of the most important characterisations of the nano formulations. In any nano lipoidal formulation, size of the droplet / particle can be directly related to the stability, drug release as well as in vitro and in vivo performance. In transferosomal formulation, the size of the droplet can range less than 100nm. Generally, Dynamic light scattering (DLS) method is used for the determination of droplet size. In this technique the measurement of transition diffusion coefficient is carried out by observing the interaction between the laser beam and dispersion ²¹.

16. In-Vitro Anti-Arthritic Activity

In vitro Anti- Arthritic assay was conducted by Protein denaturation method.

1. Test solution (0.5ml): It consist of 0.05ml of test solution of various concentrations (100-500µg/ml) and 0.45ml of BSA (5% aqueous solution)

2. Test control solution (0.5ml): It consist of 0.05ml of distilled water and 0.45ml of BSA (5% aqueous solution).

3. Product control (0.5ml): It consist of 0.05ml test solution of various concentrations (100 - 500µg /ml) and 0.45ml of distilled water.

4. Standard solution (0.5ml): It consist of 0.05ml of Piroxicam (100-500µg /ml) and 0.45ml of BSA (5% aqueous solution).

The pH of the solutions was adjusted to 6.3 using a small amount of 1 N HCl. The samples were then incubated at 37 °C for 20 minutes, followed by heating at 57 °C for 3 minutes. After cooling, 2.5 mL of phosphate buffer (pH 6.3) was added to each sample. The absorbance was measured at 219 nm using a pure blank. Piroxicam, used as the standard reference drug, was treated similarly for absorbance determination. The percentage inhibition of protein denaturation was calculated for all formulations (F1 to F3) using the following formula ²².

$$\text{Inhibition \%} = \frac{\text{Absorbance of the Control} - \text{Absorbance of the sample}}{\text{Absorbance of the Control}} \times 100$$

17. Anti-Microbial Activity by Well diffusion method: The antimicrobial properties of the transdermal patches were evaluated using the agar well diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines for bacterial and yeast testing. The antibacterial activity of the Clopidogrel and Kalanchoe pinnata loaded transferosomes of various formulations (F1-F3) was determined by measuring the inhibition zones against Gram-positive specifically *Staphylococcus aureus* (2075). Bacterial suspensions were prepared in sterilized normal saline. Two plates with three wells with a 9 mm diameter were created using a sterile cork borer, and an overnight bacterial culture (plate with positive bacterial culture) was evenly spread over sterile Mueller–Hinton agar using a cotton swab. The three patch formulations were cut according to the well diameter and were introduced into the wells of the positive plate and a standard disk also placed in the plate along with the formulation. Plate was incubated at 37 °C for 24 hours, after which bacterial growth inhibition zones were measured in millimetres to find the resistance activity of the patches ²³.

RESULT AND DISCUSSION

Pharmacognostical Studies: The Pharmacognostical studies consist of the following parameters before collection and preparation of plant extract should be done.

a) Morphological study

Colour: Dark greenish in colour; Odour: Characteristic odour; Shape: Pendulous and bell-shaped blooms

b) Physio-chemical parameters: Physio-chemical parameters such as loss on drying and ash value were determined for the powdered drug and the result were presented in (Table 4).

Table 4: physiochemical characteristics of *kalanchoe pinnata*.

S. No	Physiochemical constants	Result (%w/w)
1	Loss on drying	2.12
2	Ash value (total ash)	8.50

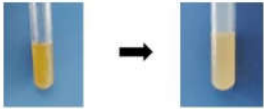
PHYTOCHEMICAL ANALYSIS OF *KALANCHOE PINNATA* LEAVES

The quantitative analysis of aqueous solution of *kalanchoe pinnata* for the presence of various chemical constituents (preliminary chemical screening) and the results are shown in the (Table 5 and 6).

Table 5: Preliminary chemical screening on aqueous solution of *kalanchoe pinnata*

S. No	Name of the test	Observation	Inference
1.	Test for carbohydrate		
	Molisch's test	Formation of purple colour ring at junction of the two phases	Presence of carbohydrates
2.	Test for reducing sugar		
	Fehling's test	Appearance of reddish-brown colour precipitate	Presence of reducing sugar
3.	Test for alkaloid		
	Mayer's test	White precipitate is formed	Presence of alkaloids
4.	Test for saponins		
	Froth test	No formation of foam	Absence of saponins
5.	Test for flavonoids		
	Lead acetate test	White precipitate was obtained	Presence of flavonoids
	Sodium hydroxide test	Appearance of yellow colour	Presence of flavonoids

Table 6: Results of Preliminary chemical screening of *K. Pinnata*

Molisch's test	Fehling's test	Mayer's test
		
Lead acetate test	Sodium hydroxide test	
		

PREFORMULATION STUDIES

Compatibility studies: In the FTIR spectra of a pure drug and a formulation F1, F2, and F3 with ingredients (different polymers like HPMC, Carbopol, and PVP) and other excipients, the peaks of major functional groups of Clopidogrel Bisulfate, which are present in the spectrum of a pure drug, are observed. This means there are no interactions between the drug and other ingredients in a physical mixture, and the drug is compatible with other ingredients are illustrated in (Table 7) and (Figure 7)

Table 7: ATR-FTIR Spectrum wave numbers and their functional groups for a) Drug, b) Plant, Formulations (c) F1, (d) F2, (e) F3

S.NO	Wave numbers(cm ⁻¹)	Functional groups
(a)	3354.50	N-H stretching
	2972.89	C-H stretching
	1752.98	C=O bending
	1179.90	SO ₃ H
	1042.88	C-O stretching
	878.76	C-H bending
(b)	3331.69	O-H stretching
	1635.64	C=O stretching
(c)	3365.38	O-H stretching
	2975.55	C-H stretching
	1655.27	C=O stretching
	1044.83	C-O stretching
	878.68	C-H bending
(d)	3365.34	O-H stretching
	1044.68	C-O stretching
	878.54	C-H bending
(e)	3361.21	O-H stretching
	1044.53	C-O stretching
	878.54	C-H bending

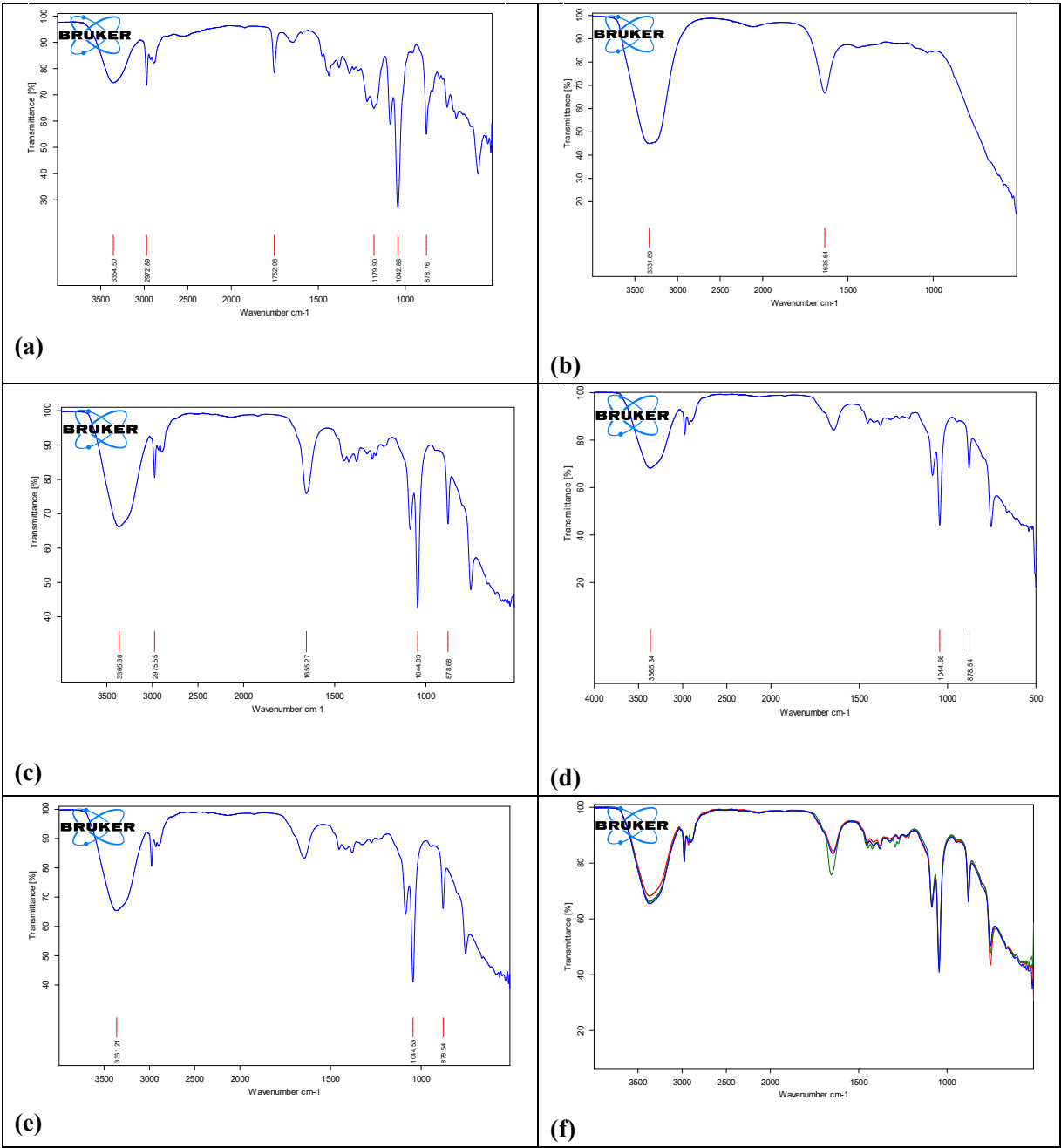
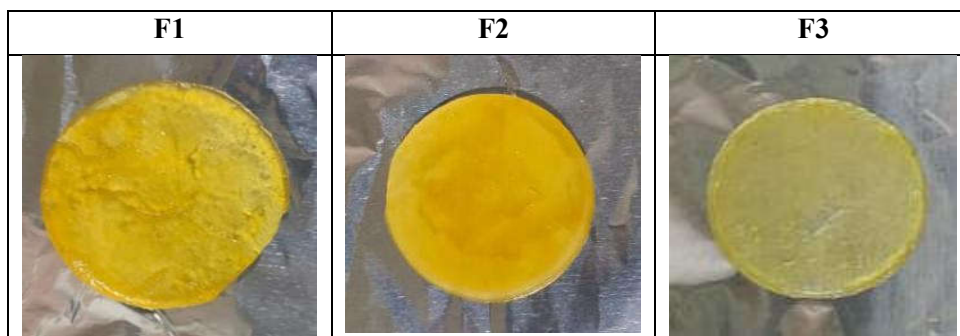


Figure 7: ATR-FTIR spectrum for a) Drug, b) Plant, Formulation ((c) F1, (d) F2, (e) F3)

EVALUATION OF PATCHES

Formulations F1, F2, and F3 were successfully formulated with the respective procedure and then the formulations F1, F2, and F3 were evaluated for their stability, so the basic standardization parameters were analysed.

Figure 8: Illustration of Transdermal patch



Standardization test results for transferosomes loaded transdermal patches

Physical appearance: All three formulation (F-F3) has Good transparent, Flexibility, Non-sticky and Homogeneous activity

Estimation of Thickness test, pH Measurement, Weight uniformity test and Folding endurance test

Table 8: Mean $\pm$ SD, n=3

Formulation code	Average thickness (mm)	Average Surface pH	Average Weight uniformity	Average Folding endurance
F1	0.403 ± 0.01	5.7 ± 0.1	0.161 ± 0.03	30 ± 1
F2	0.511 ± 0.01	6.2 ± 0.1	0.199 ± 0.01	34.6 ± 1.5
F3	0.441 ± 0.02	5.9 ± 0.1	0.179 ± 0.01	32.3 ± 1.5

Estimation of % Moisture content test and % Moisture Uptake test

Table 9: Mean $\pm$ SD, n=3

Formulation code	Average moisture content (%)	Average moisture uptake (%)
F1	4.12 ± 0.1	3.27 ± 0.13
F2	3.21 ± 0.09	3.03 ± 0.15
F3	3.39 ± 0.03	3.88 ± 0.11

Estimation of Drug content test, Water vapour transmission rate**Table 10: Mean $\pm$ SD, n=3**

Formulation code	Average drug content (%)	Average WVTR (mg cm/cm ² 24h)
F1	88.64 $\pm$ 0.51	0.0034 $\pm$ 0.0003
F2	92.53 $\pm$ 0.49	0.0043 $\pm$ 0.0004
F3	90.89 $\pm$ 0.25	0.0039 $\pm$ 0.0001

Flatness study**Table 11: Flatness study**

Parameter	F1	F2	F3
Flatness	100%	100%	100%

% In-Vitro diffusion study**Table 12: In-vitro % drug diffusion study for prepared patch F1, F2 & F3 at 219nm, Mean $\pm$ SD, n=3**

Time	Average % Drug release F1	Average % Drug release F2	Average % Drug release F3
15 mins	6.1 $\pm$ 0.22	8.59 $\pm$ 0.22	8.14 $\pm$ 0.24
30 mins	13.19 $\pm$ 0.4	15.94 $\pm$ 0.09	15.48 $\pm$ 0.53
45 mins	26.9 $\pm$ 0.11	28.92 $\pm$ 0.93	29.83 $\pm$ 0.85
1 hr	36.91 $\pm$ 1.01	49.20 $\pm$ 0.28	44.66 $\pm$ 1.14
2 hrs	47.08 $\pm$ 0.16	60.42 $\pm$ 0.9	57.36 $\pm$ 0.36
3 hrs	50.31 $\pm$ 0.33	69.98 $\pm$ 0.55	62.62 $\pm$ 1.7
4 hrs	53.98 $\pm$ 0.82	78.80 $\pm$ 0.84	65.57 $\pm$ 0.90

In-vitro anti-arthritic activity**Table 13: In-vitro Anti-arthritic study by % inhibition of protein denaturation, mean $\pm$ SD, n=3**

Formulation code	% Inhibition of protein denaturation Concentration 100 (μ g/ml)			Average (μ g/ml)
	Trial-1	Trial-2	Trial-3	
F1	35.83	38.95	36.58	37.12 $\pm$ 1.62
F2	44.87	43.32	42.89	43.69 $\pm$ 1.04
F3	39.86	40.23	40.29	40.12 $\pm$ 0.23

Piroxicam (STD)	39.64	39.77	38.98	38.69 ± 1.04
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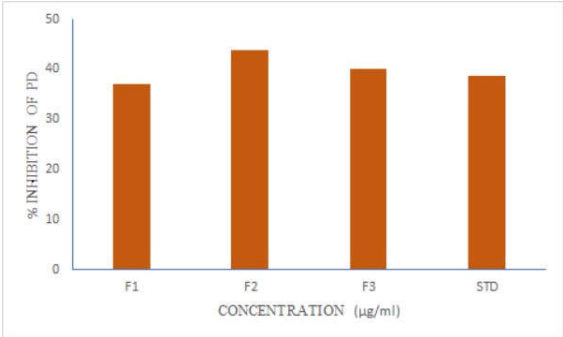


Figure 9: Effect of Formulations in Anti-Arthritic activity

The effects of these formulations of F1, F2 and F3 on inhibition of protein denaturation is shown in (Table 13) and (Figure 9). Most of the researches have reported that protein denaturation is one of the causes of Rheumatoid arthritis. These formulations offer significant protection against protein denaturation. The maximum percentage inhibition was observed in F2 formulation compared to F1 and F3 formulation. Compared to standard piroxicam gel, it exhibits exhibits a moderate variance.

Anti-Microbial study by Well diffusion method

The antimicrobial activity of the Clopidogrel and Kalanchoe pinnata loaded transferosomal patches were assessed by measuring the zone of inhibition of Gram-positive (Staphylococcus aureus) after 24 h in a bacterial culture. All the three-formulation inhibited S. aureus. The transferosomes loaded patches (F2 and F3) showed anti-microbial action against S. aureus which was more than the standard drug (Amoxicillin) . Based on these findings, the Clopidogrel and K. Pinnata loaded transferosomal formulation exhibits the antimicrobial activity are shown in (Table 14) and (Figure 10).

Table 14: Results of Anti-Microbial study

Bacterial strain	Zone of inhibition (mm)			Standard disc (Amoxicillin)(mm)
	F1	F2	F3	
Staphylococcus aureus	6mm	19mm	14mm	9mm



Figure 10: Effect of Formulations in Anti-Microbial activity

SUMMARY

Clopidogrel bisulphate is an antiplatelet drug which is inhibitor of platelet aggregation and also reduce the inflammation that prevent ischemic stroke and thrombosis. Although clopidogrel is not a traditional anti-inflammatory drug, its role in reducing platelet aggregation can indirectly influence inflammatory processes in RA. By minimizing platelet activation, it may help alleviate some inflammatory responses associated with joint damage. In this study we investigated that clopidogrel bisulphate has anti-inflammatory activity.

Kalanchoe pinnata is one of the medicinal plants that is used as anti-inflammatory, anti-diabetic, analgesic, anti-microbial, anti-histaminic, anti-tumours insecticidal, sedative, muscle relaxant, cytotoxic and anti-oxidant properties. This is prominent for their haemostatic and wound healing properties.

Clopidogrel bisulphate has a drawback of increased risk of haemorrhage rashes, pruritus, thrombotic, thrombocytopenic purpura and interaction with another administered drug.

In our study we found that by combining the Kalanchoe pinnata with Clopidogrel bisulphate produce synergistic activity and reduce the risk of haemorrhage and rashes and improve anti-inflammatory property.

Transdermal drug delivery system has a more advantages of avoiding first pass metabolism, reduce the side effects, improve the bioavailability and therapeutic outcomes, enhance patient compliance and painless procedure compared to oral administration.

Transdermal patch is a medicated adhesive patch applied to the skin and delivers required dose into the blood stream. But transdermal drug delivery system has a lower permeability to the hydrophilic drug and macromolecules have difficulty in penetrating the skin. To overcome this problem the transferosomes are formulated and produced them in the form of transdermal patches. Transferosomes is a novel drug delivery system and it is an ultra-deformable liposome that can penetrate well through the skin's lipid bilayers by increasing drug absorption. Upon normal patches the transferosomal patches can deliver the drug to specific skin layers and reduce its systematic side effects. Transferosomes may protect the drug from degradation and ensuring effective delivery of the therapeutic agents. A highly promising approach to overcome the clopidogrel limitations is to develop a lipid based novel drug delivery system through topical route.

The transferosome transdermal patch was developed for the delivery of Clopidogrel and Kalanchoe pinnata by using the various polymers HPMC, PVP, Carbopol in various combination.

The ATR-FTIR results shows that there is no any interaction between drug and polymer. The results of prepared transferosome transdermal patch persist optimum in its homogeneity, pH, drug content, viscosity, thickness, and moisture content.

In-vitro % drug release at 4 hours was performed in all formulations the results indicates that formulation F2 produced the highest drug release when compared to other formulations and it shows that it has a good diffusion characteristics and sustained drug delivery to the targeted site through topical administration. Apart from demonstrating better drug release property, the formulation F2 has also shown remarkable anti-arthritic and anti-microbial activity while comparing to F1 and F3 formulations. This study indicates that the formulation of transferosome transdermal patch may be the best option for their highest delivering a variety of drugs for topical administration.

CONCLUSION

In conclusion, the study on clopidogrel bisulfate and Kalanchoe pinnata-loaded transferosomal transdermal patches for rheumatoid arthritis (RA) management highlights the promising potential of this innovative drug delivery system. The combination leverages the anti-inflammatory and immunomodulatory properties of K. pinnata with the antiplatelet effects of clopidogrel, aiming to enhance therapeutic efficacy while reducing systemic side effects. Transferosomes are novel drug delivery systems capable of delivering both hydrophilic and lipophilic drugs. As these systems are designed for transdermal drug delivery, they can enhance patient compliance by offering a topical application. An ideal formulation should provide a slow drug release over an extended period, minimizing the need for frequent application and reducing systemic absorption. Based on the drug release, anti-arthritic and anti-microbial results, formulation F2 was chosen as the most optimal formulation.

FUTURE DIRECTIONS

Future studies should focus on further optimising the transferosomal formulation, exploring the potential of this combination therapy in other inflammatory diseases and further clinical trials are recommended to validate its efficacy and safety.

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