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Preparation and Evaluation of Wrightia Tinctoria Extract Transdermal Patch and Antimicrobial Activity against Clinical Pathogenic Microorganisms

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Abstract

Investigate phytoconstituents in chloroform leaf extracts of *Wrightia tinctoria* while developing transdermal patches. Transdermal patches of *Wrightia tinctoria* herbal extract prepared with a solvent casting technique. Based on a physicochemical and in vitro drug diffusion investigation, numerous formulation parameters, drug-polymer ratios, and permeation enhancers were optimized, with the optimum formulation chosen for optimisation. The most effective formulation will be tested for antibacterial activity. This demonstrates the drug's uniform dispersion during the polymer ratio patch 1:4. Information gathered from the in-vitro diffusion profile chosen for formulation development. With varied reaction kinetics, the greater correlation coefficients (r2) indicate greater comprehension of the product diffusion and diffusion rate mechanism. These findings imply that drug release from this patch was controlled by diffusion. Based on in vitro diffusion and physicochemical investigations, 1:4 ratios (formulation 2) were determined to be the optimal formulation. Antimicrobial activity analysis was then performed on Formulation 2. The antimicrobial test findings proved that the patch effectively reduced bacteria growth. The results reveal that the *Wrightia tinctoria* plant chloroform extract has antibacterial action against Staphylococcus aureus.

Keywords: *Wrightia tinctoria,* Transdermal patch, antimicrobial studies.

Introduction

Plants are the medicines of nature used by people on this earth since prehistoric times for medicine and food. Currently, global efforts are being made to identify and encourage natural therapies in plants as a valid form of human drug delivery. Hidden in nature, the main objective behind it is the treatment of every disease. Nevertheless, the distribution of herbal drugs often needs adjustment to achieve continuous release, increase patient outcomes, etc. Earlier, herbal remedies did not attract researchers to change new drug delivery mechanisms due to development, standardization, storage and identification difficulties. However, modern drug delivery systems (NDDS) are now opening the way to introducing a novel herbal drug delivery system

for days of industrial development by using revolutionary toxicity prevention methods, increasing longevity, enhancing the bioavailability of herbal products, protecting against physical and chemical degradation. Every pharmaceutical company and firm strives to build a secure and safe drug delivery. Transdermal drug delivery can have beneficial systemic and local effects. Transdermal delivery a successful option to providing drugs that can maintain consistent plasma concentrations, reduce the frequency of doses associated with increased patient compliance, and prevent gastrointestinal intervention. Transmission of transdermal drugs is self-medication, allowing a drug to travel through the skin surface over a set period to achieve a systemic effect. Transdermal patches can be administered in soluble lipid-based material. TDDS prevent several issues linked to drug administration, like first-pass metabolism, enzymatic degradation, acid drug hydrolysis, digestive pain, medication volatility, adverse effects and therapeutic malfunction, and spread of disease hazards. The advantages include regulating prescriptions for patients, low cost, and controlled availability of drugs[1, 2]. Transdermal restrictions on drug delivery also have signs of skin inflammation, macromolecular contaminants, and the potential for ion compounds unsuitable for those with inflammation or decreased peripheral blood flow. For the first century, drugs topically integrated without enhanced transdermal immersion agents. Transdermal optimisation agents have been used to augment the absorption of simple drug molecules administered topically throughout the second century. The penetration of macromolecules in topically applied drugs of the third century has been allowed.

Polymers regulate drug release from the transdermal patch reservoir, allowing safe, continuous, and efficient drug delivery to the body. A Herbal formulation means a type of medicinal product consisting of one or more herbal extracts inaccurately assessed to provide specific medical, cosmetic, and other profits. [3, 4, and 5] Herbal formulations are developed by subjecting whole plants, separate or divided plants, and plant parts such as filtration, insulation, translation, extraction, cleansing, intensity or fermentation into therapies. They include herbal powdered ingredients, distilled tincture, spices, essential oils, pure liquids. *Wrightia tinctoria* is a central medicinal plant for treating various diseases in the Indian Medicines Program (Figure 1). The formulation is having an active pharmaceutical product with limited side effects. It can be put on the market, particularly as a highly potential anticancer medication for skin cancer. Its efficacy on cervical and lung cancer lines also suggests possible use as a chemotherapeutic medicine.

Clean unripe fruit juice was used to thicken the latex. The seeds, they say, are aphrodisiac and anthelmintic. When chewed with salt, the leaves have been used to alleviate toothache. The plant latex juice has been used in Nepal to avoid bleeding. Seeds contain dark red, sub-drying oil of therapeutic qualities. Bark and leaves used as traditional remedies for psoriasis, stomach pain, toothache and dysentery [6,7, and 8]. The present study was planned and evaluated to produce transdermal patches of herbal extract of *Wrightia tinctoria* using pectin as a natural polymer.

Plant description

- Taste: Bitter
- Colour: Green
- Odour: Pungent

Taxonomic classification

- Kingdom: Plantae
- Order: Gentianales
- Family: Apocynaceae
- Genus: *Wrightia*
- Species: tinctoria
- Synonyms: Kudi, Dudhi, Indrajao, Easter tree, Jaundice curative tree
- Application: Treatment of psoriasis, skin disorders, hepatitis and also used as a hair tonic.

Pharmacological activity

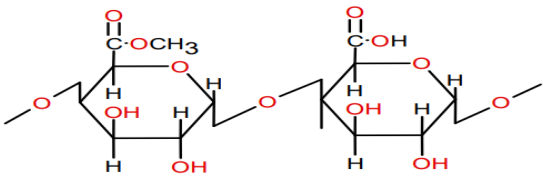
- Antimicrobial
- Anti-inflammatory
- Anti-hepatitis
- Antidiabetes
- Anticancer

Chemical constituents:14-alpha-methyl zymosterol, Wrightiadione, Lupeol, Lupenone, Wrightia, Quercetin, Stigmasterol,Campesterol.

Polymer description (Pectin)

- Synonyms: Poly-D-galacturonic acid methyl ester
- Common Name: *Citrus Pomelo* Fruit
- Binomial Name: *Citrus maxima*
- Family: Rutaceae
- Kingdom: Plantae

Chemical structure



- Colour: It is a brown-coloured powder.
- Solubility: Pectin dissolved at 85-90°C soluble in hot alkali, insoluble in the cold alkali, forming a yellow precipitate.

Commercial uses of *Citrus Pomelo*fruit

- Pectin used in the pharmaceutical and food industry (gelling agent and stabiliser)

Medicinal uses of *citrus Pomelo* fruit

- Pectin positively affects blood cholesterol.
- Pectin serves as a prophylactic toxic cation poisoning drug.
- Suitable to extract plum and mercury from the respiratory and gastrointestinal organs.
- Pectin and pectin combinations with other colloids commonly treat diarrheal disorders, especially in infants and children.
- Pectin was used as a binding agent and polymer in tablet formulation for controlled release.

Storage conditions

- Pectin should store at ambient temperature for further use[9]

Materials and Methods

Preformulation studies

Polymer isolation (pectin extraction)

Dry citrus fruit powder (50 g) mixed with 300 mL of purified water. The extraction water has been acidified by 40% citric acid, and the pH has remained at 3.2 and 1.2. The acidified blended peel powder material has been heated to 60°C for about 2 hrs. The mixture was then passed between double folded muslin cloths and cooled down to room temperature -isolation of pectin using ethyl alcohol as a precipitating agent of pectin. Concentrated pectin extracts then precipitated into 95 % ethanol, for which one volume of extract was added to various densities of ethanol with a continuous stirring time of 15 minutes. Citrus fruit extract and ethanol ratios were 1:0.5, 1:1 and 1:1.5. The solution was incubated aside for 2 hrs without mixing. Pectin sieved through four-layered muslin cloths and precipitates rinsed three times with ethyl alcohol to remove any remaining impurities. Ultimately, the residue was kept in a hot air oven at 35 ° C to 40 ° C for drying, and the yield percentage determined.

Y_{pec} (%) =p/b_i×100

Y_{pec} is the pectin yield in percentage, p is the quantity of extracted pectin (gram), and b_i is the initial amount of peel powder.[9]

Physical-chemical pectin sample characterisation of the pomelo citrus fruit peel

Qualitative Tests

The solubility of dry pectin in cold and hot water

Add ten ml of 95 % ethyl alcohol and 50 mL of purified water to 0.25 gm of pectin in two conical flasks separately. Vigorously shaken the mixture in the second flask to form a suspension and heated at 85-95°C for 15 minutes.

The solubility of pectin

- Added 5mLof pectin solution to 1mL of 0.1 NaOH in two separate conical flasks and heated the next flask at 85-90°C for 15 min.

Determination of pH

PH of the pectin solution determined using a pH meter at room temperature. The pH of the solution is 3.2, maintained with 40% citric acid.

Quantitative test

Equivalent weight (E.W):

A conical 250 mL flask of pectin (0.5 gm) was weighed and moistened with 5mLethanol. The mixture is supplemented by 1g of sodium chloride and 100 mL of distilled water, and a few drops of phenol red. The solution was then gradually titrated to a pale permanent pink colour with 0.1 M NaOH. The equivalent weight was calculated using the formula below.

$$EW = \frac{\text{Weight of pectin sample/volume of alkali } (cm^3)}{\text{Molarity of Alkali}} X100\%$$

Methoxyl content (MeO)

The neutralised solution derived from the weight determination used to determine the quality of methoxyl. Pectin saponification leads by the liberated acid titration performed as follows. I added 25mL 0.25 M NaOH to the neutralized solution. The mixture was stirred correctly and allowed to stand at ambient temperature for 30 minutes.25 ml of 0.25NHCl was added and titrated to the same endpoint with 0.1NNaOH. The percentage of methoxyl content determined using the equation follows:

$$MeO\% = \frac{\text{Volume of alkali (cm3)} \times \text{Weight}}{\text{Weight of pectin sample (mg)}} X100$$

Moisture value

An empty crisp was dried in an oven, cooled and weighed in a desiccator. It was transferred to a 5 gm pectin sample and put in a hot air oven (100°C) for 1 hr. After that, the Petri plate was removed, cooled and weighed in a desiccator. It has repeated the cycle once. The humidity content was calculated using the following equation:

$$\text{Moisture value\%} = \frac{\text{Weight of the Residue}}{\text{Weight of the sample}} X100$$

Content of anhydrouronic acid

The content of anhydrouronic acid measured using the previously defined values of equal weight and methoxyl content according to the equation

$$\text{Content of anhydrouronic acid\%} = \frac{176 * 100}{Z}$$

$$Z = \frac{\text{Weight of sample (mg)}}{\text{meq of Titration A} \times \text{meq of Titration B}}$$

Esterification

Esterification of extracted pectin measured using equation using data from determinations of methoxyl and CAAcontent.

$$\text{Esterification \%} = \frac{176 * MeO\% * 100}{31 * AUA\%}$$

Collection of *Wrightia tinctoria*leaves

Wrightia tinctoria leaves have collected from vellapara, Palakkad, Kerala.

Figure 1: Leaves of Wrightia tinctoria

Extraction for leaves of *Wrightia tinctoria*

The shade dried leaves subjected to size reduction and passed into sieve no: 20 and then 40. About 500g of the dried powder was extracted continuously in the Soxhlet apparatus with petroleum ether for 24 hrs to remove the waxy materials. Then it has extracted with distilled for 72hrs. After 72hrs, the water substance has evaporated to obtain the crude extract (7.4%w/v). The extract was dried under a vacuum oven [10].

Evaluation of phytochemical

Alkaloids

Dragendroff's test

The extract treated with potassium bismuth iodide solution. Orange-brown precipitate has formed, which indicate the occurrence of alkaloids.

Mayer's reagent
The extract treated with a potassium mercuric iodide solution reagent. The precipitate created, which shows the occurrence of alkaloids.

Wagner's reagent

The extract treated with a reagent (iodide and potassium triiodide solution) from Wagner. The reddish-brown precipitate formed, and there are alkaloids

Foam test (Saponin glycoside)
Shake the extract with water. The foam was produced, which indicates the existence of saponins.
Test for phenolic and tannins compounds

Ferric chloride test
Only a few drops of ferric chloride solution added to the aqueous sample. The dark black colour has developed in which tannins and phenolic compounds were present.

Bromine water test
The aqueous extract treated with bromine water. Discolouration of bromine water indicates the occurrence of phenolic and tannins.

Potassium permanganate test:
The aqueous extract treated with dilute potassium permanganate. Discolouration of the solution indicates the occurrence of phenolic and tannins.

Benedict's test (Test for reducing sugars)
0.5mL of extract 1mL water 5 to 8 drops of fehling solution was applied and Brick-red precipitate absence indicating the absence of sugar reduction.

Ninhydrin test (Test for amino acids)
Aqueous extract heated with % ninhydrin solutions on boiling water bath for 10 min. No purple color formed and shows the presence of amino acid. The aqueous extract is treated with solution NaOH and lead acetate solution, and boiled. No black precipitate is formed and shows amino acid.

Flavonoid's test
For the methanol extract, add potassium hydroxide solution and then 10% ammonia. Yellow colour precipitates developed and indicated the existence of flavonoids.

To the ethanol extract, add a small number of drops of lead acetate solution. The yellow colour precipitate formed, which indicates the existence of flavonoids.

Test for terpenoids
1.4gm of the extract-treated with0.5mL of chloroform and 0.5mL of acetic anhydride added to a concentrated sulphuric acid solution.No Red violet colour was obtained, which indicates the absence of terpenoids.

Test for steroids

Libermann- buchard test
We applied few drops of acetic anhydride and 1mL of the concentrated sulphuric acid through the side of the test tube to extract the chloroform solution and set it aside for a while. Browning formed at the junction, which indicates steroid presence.

Salkowski Test
To the extract, add chloroform solution, a small number of drops of concentrated sulphuric acid added to mix and allowed to stand. Greenish fluorescence was formed and confirmed the presence of steroids.

Libermann's reaction
Three ml of an extract mix with three ml of acetic anhydride, warm and cold, and add a small number of drops of concentrated sulphuricacid. The blue colour was formed and indicated the presence of steroids [11].

Test for Glycosides (Bontrager's test)
To the extract, add dilute sulphuric acid and filtrate extracted with little chloroform layer separated, added an equal volume of dilute ammonia. The red colour observed in the ammonical layer confirms the presence of glycosides.

Preparation of transdermal Patch
Four batches of herbal extract of *Wrightia tinctoria* Transdermal patches wasformulated on four different ratios using a drug and polymer (1:2, 1:4, 1:6 & 1:8). Weighed polymer concentrations were dissolved in water. The measured quantity of extract added to the mixture above and well mixed until a homogeneous mass formed. The measured amount of permeation enhancer and glycerin

then added. The amount of extract in all four batches was the same. The resulting mixture was poured into a Petri plate and air-dried at room conditions for 24hrs. With the help of a knife, the patches were peeled off from the Petri dish and kept in desiccators.[12] (Table 1)

Table 1: Formula for the herbal extract of wrightia tinctoria transdermal patches

Ingredients	**Formulation code**			
	F1	F2	F3	**F4**
***wrightia tinctoria* Extract (mg)**	40	40	40	**40**
Pectin (mg)	160	240	320	**400**
DMSO (ml)	0.3	0.3	0.3	**0.3**
Glycerin (ml)	0.3	0.3	0.3	**0.3**
Water	**q.s**	**q.s**	**q.s**	**q.s**

Preparation of calibration curve of herbal extract of *Wrightia tinctoria*

Accurately weighed quantity100mg herbal extract transferred to a 100mL standard flask and dissolved in a small amount of distilled water to make the standard stock solution of 1mg /mL up to volume. 1mL was taken from stock in a 10mL volumetric flask and made up the buffer volume; from this sample, 0.5mL to 3mL liquid was transferred to a 10mL volumetric flask required volume with further distilled water and the corresponding concentration range from 5 to 50 µg/mL. Using a U.V. spectrophotometer, the absorbance of these solutions was determined at 382 nm. The calibration curve has formed between the absorption and the concentration.

Phosphate buffer preparation pH 7.4

PH 7.4 phosphate buffer has prepared as per the method described in Indian pharmacopoeia. 1996 using disodium hydrogen phosphate and sodium hydroxide. The pH has adjusted to 7.4 before quantitative estimation.

Physicochemical evaluation of *Wrightia tinctoria* Transdermal Patch

Formulated patches have been subject to preliminary assessment testing. Patches with any flaws in the air or variation in thickness, weight uniformity were excluded from further studies.

Weight uniformity

It did so by measuring five separate batch patches taking the uniform size at random, and estimating the average weight of three of them. The experiments were carried out on a dried patch for 4 hr at 60°C before testing.

Patch thickness

The Patch thickness has been measured with the use of an optical vernier calliper at various patch stages. Three randomly selected patches are used for each formulation. The mean values for the thickness of a single patch determined.

Drug content determination

The patches were taken and attached to a beaker containing 100 mL of distilled water. The medium was stirred with a magnetic bead for five hr. The solution was then purified and then tested for drug content with sufficient dilution at 382 nm spectrophotometrically.

Folding stamina

It was determined to fold repeatedly, and it split. The number of times the patch could fold without breaking gave the importance of pliable stamina.

A moisture content uptake

The patch was correctly weighed and put in aluminium chloride-containing desiccators. The patch was picked out and weighed after 24 hr. The level of absorption of moisture measured as the difference between the final and original weights.

A moisture content level

The patch weighed and kept in calcium chloride-containing desiccators. The patch was removed after 24hr and weighed.

Determination of surface pH

The patches were permitted to swell, keeping them in contact with 1 mL of deionised water at

room temperature for 2 hr, and the pH was noted.

Percent elongation

A patch sample stretches as tension is applied, and this was referred to as a strain. The strain is the deformation of the patch divided by the original size of the sample. In general, patch elongation increases with the rise in plasticiser content.

Cellophane membrane treatment

Cellophanemembranewasboiledinthedistilled waterfor1hr,washed with fresh distilled water three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3percent sodium sulphite and soaked in distilled water for 2 min at 60°C, followed by acidified with 0.2% sulphuric acid. Finally, the membrane was dipped in a boric buffer (pH 9) until it used for permeation study.

Drug permeation studies

The *in-vitro* diffusion rate of herbal extract of *Wrightia tinctoria* transdermal patches was evaluated by an open-ended tube using distilled water as diffusion medium up to 8 hr studies. The cellophane membrane was bound at one end of the tube and then immersed in a receiver compartment containing 200 mL of 7.4 buffer solution, which was stirred at medium speed and maintained at 37°C±2°C samples taken at regular time intervals and replaced by a fresh diffusion medium at the same volume. The samples were analyzed using a visible spectrophotometer at 382nm.

Diffusion kinetics

Data obtained from in-vitro diffusion studies fitted into different kinetic equations. Using in-vitro release studies data obtained. (Zero-order, Higuchi equations and the first order). Drug release mechanism determined by the use of KorsmayerPeppas equations. Kinetics Zero Order: cumulative sum of medicines released plotted against time (C = K0 t). This kinetic explains the release from the formulations of concentration-independent products. Kinetics of first order: first order as a cumulative percentage of the remaining drug vs time. This kinetics describes the drug release from the formulations based on concentration.LogC = LogCo – kt / 2.303

Higuchi's Model: The model of Higuchi as a cumulative percentage of drug released versus square root of time. Q = Kt1/2/2. This model describes the release of the drug from a swellable matrix based on Fickian diffusion as a square root of the time-dependent cycle.

KorsmayerPeppas Equations: determine the mechanism of the release of drugs, the first 60 % of the release of drugs plotted in Korsmeyer et al.[9].

Antifungal activity

The antibacterial activity of given formulations evaluated using the disc diffusion method. *Aspergillus spp* inoculums prepared using sterile potato dextrose broth. Potato dextrose agar double strength media were made by autoclaving 0.760 g in 100ml. Inoculum inoculates the potato dextrose agar plates using a sterile cotton swab. *Wrightia tinctoria* chloroform extract and antibiotic discs placed on agar under aseptic conditions. Agar plates incubated for 30 min at the refrigerator to diffuse the formulation into the agar, and finally, plates incubated at 37°C for 48hrs. The Himedia zone reader used to measure Antibacterial activity.[13]

Antibacterial activity

Chloroform extract antibacterial activities were studied using the diffusion of discs: *Escherichia coli*, *Staphylococcus aureus*, and inoculums prepared using broth media with nutrients. Sterile double strength muller Hinton agar media prepared 7.6 gm in 100 ml. Use sterile cotton swabs; inoculate the test bacteria on the Mueller Hinton agar plates. *Wrightia tinctoria* chloroform extract mounted on sterile plates. Disks were dried aseptically for the elimination of solvents under laminar air flow. Dried disks mounted on the crop surface inoculated Mueller Hinton agar plates and plates incubated for 24 hours at 37 ° C. Usage of the media zone reader to test the antibacterial activities.[14,15]

Results and Discussion:

Studies on the pre-reformulation

Pectin isolation from *citrus pomelo* peel:

When pectin present in *citrus pomelo* fruit peel has extracted by citric acid-based method, the maximum yield of 44.1% obtained at 70°Cand pH 3.2. Previous studiesreported that extraction from *C.limetta* resulted in 32.42% and 15.92%, respectively.

Physical and chemical characterisation of pectin sample from the fruit peel of citrus pomelo

Qualitative analysis

The pectin colour obtained from fruit *C.Pomelo* was brown. The solubility of dry pectin in water and alkali demonstrated. (Table 2)

Table 2: Qualitative tests for pectin

Parameters	Results
Colour	Brown
The solubility of dry pectin in cold water	Insoluble, forms suspension
The solubility of dry pectin at 85-90°C	Mixture dissolves
The solubility of pectin in cold alkali	Pectin forms a yellow precipitate
The solubility of pectin in hot alkali	Dissolved
pH	3.2 at60-75°C

Quantitative analysis

The equivalent weight in mg/mL was noticed to be 438.59 mg /mL, while the methoxyl content was observed to be 4.415%. The pectin methoxyl content usually varies between 0, 2 and 12 %, depending on extraction mode. The methoxyl content in the current study was less than 7%, meaning that the pectin is low-ester and therefore suitable for consistency. The amount of *C.pomelo* fruit in anhydrouronic acid was 53%. (Table 3)

Table 3: Quantitative test for pectin

Equivalent weight(mg/ml)	**438.59**
Methoxylcontent(%)	4.415
Moisture content (%)	98.13
AUA (%)	56.49
DE (%)	3.117
Ash content (%)	32
Methoxylcontent(%)	4.415

Phytochemical evaluation of *Wrightia tinctoria*

The existence of phytochemical tests indicated that alkaloids, saponins, tannins, phenolic compounds, flavonoids and steroids. (Table 4)

Table 4: Phytochemical constituents

S. No.	Chemical Constituents	Herbal extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phenolic	+
5.	compounds	+
6.	Flavonoids	+
7.	Steroids	+
8.	Glycosides	+
9.	Amino acids	+
10.	**Reducing sugar**	+

Standard curve of herbal Extract of *Wrightia tinctoria* transdermal patches

Transdermal patches of Wrightia tinctoria were measured using U.V. The spectrophotometric approach uses the phosphate buffer pH7.4 to calculate absorbance at 382 nm. Beer's Lambert's 2-10µg / ml rule. The coefficient of correlation was 0.999 (Figure 2 and Table 5).

Table 5: Standard graph of herbal extract of *Wrightia tinctoria*

S. No.	Concentration(mg/ml)	Absorbsance(nm)
1	0	**0.00**
2	10	**0.2166**
3	20	**0.4085**
4	30	**0.6155**
5	40	**0.8098**
6	**50**	**1.0031**

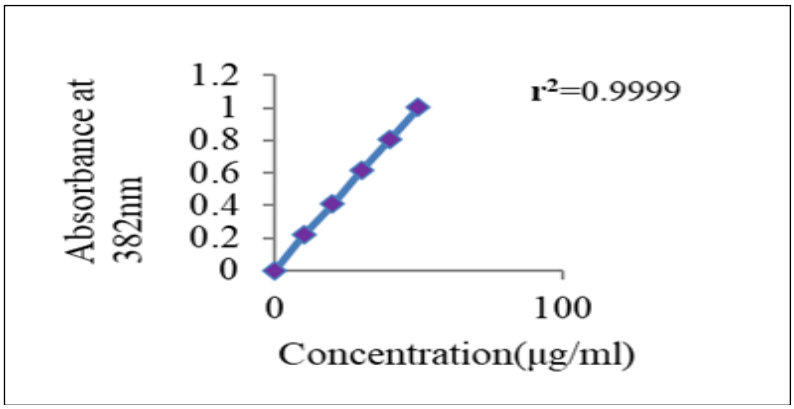


Figure 2: Standard values of herbal Extract of *Wrightia tinctoria*

Evaluation of transdermal patches

The results obtained from the uniformity of weight,the thickness of the patch, content uniformity,folding endurance,percentage moisture content, percentage moisture uptake, determination of surface pH,per cent elongation are given in table 6 and 7.

Physical appearance

Colour: Yellow

Taste: Bitter

The four (F1, F2, F3, F4) batches of extract loaded patches with different ratios of polymers were subjected to various physicochemical evaluations. Based on thickness, uniformity of weight, folding endurance, percentage moisture uptake and moisture content and tensile strength, the formulation F2 was selected for further studies. (Figure 3)

Figure 3: Prepared transdermal patch from herbal extract of *Wrightia tinctoria*

Table 6: Physicochemical evaluation of herbal extract of *Wrightia tinctoria* Transdermal patches

Formulation code	Formity of weight (g)	Thickness (mm)	Drug content (%)
F1	0.34±0.14	0.43±0.73	85.56±0.92
F2	0.42±0.85	0.45±0.23	88.69±0.56
F3	0.45±0.10	0.56±1.13	83.98±0.16
F4	0.33±0.43	0.35±0.33	82.03±0.22

Table 7: Physicochemical evaluation of herbal extract of *Wrightia tinctoria* transdermal patches

Formulation code	Folding Endurance (Nos)	Moisture Uptake (%)
F1	230±0.76	2.01±0.08
F2	249±0.23	2.85±1.03
F3	251±0.36	3.56±1.03
F4	239±0.72	2.56±0.65

Table 8: Physicochemical evaluation of herbal extract of *Wrightia tinctoria* transdermal patches

Formulation code	Moisture Content (%)	Surface pH
F1	2.8±0.07	7.1±0.10
F2	3.4±0.22	7.3±0.72
F3	3.9±0.36	7.2±0.72
F4	1.7±0.46	7.0±0.33

Table 9: Physicochemical evaluation of herbal extract of Wrightia tinctoria Transdermal patches

Formulation code	Per cent Elongation (% mm)	Tensile Strength (Kg/mm^2)
F1	85±0.32	5.32±0.55
F2	91±1.11	6.36±0.87
F3	86±1.08	6.7±0.87
F4	82±0.23	5.5±0.86

In-vitro drug diffusion

The in-vitro drug release studies for formulations F1toF4 were conducted. More medicines have been released in the pH 7.4 phosphate buffer Fig. 4 shows that the formulation F2 has released 71 per cent in 12 hr. The figure shows that all formulations have delayed release characteristics. In the case of F2 formulation, drug release was higher using pH 7.4 phosphate buffer solution. The release date is shown in Table 8.

Table 10: *In vitro* drug diffusion study

Time(hr)	% Cumulative Drug Release			
	F1	F2	F3	**F4**
1	1.82	1.53	1.34	**1.37**
2	3.98	3.99	4.69	**3.46**
3	5.33	5.78	5.86	**6.28**
4	9.12	11.28	10.32	**15.68**
5	22.92	31.99	19.85	**22.45**
6	34.72	36.98	28	**24.48**
7	36.21	38	32.69	**26.98**
8	42.4	44.66	40.95	**33.95**
9	44.65	56.96	52.38	**39.29**
10	50.13	62.89	58.69	**43.68**
11	58.40	66.82	60.88	**47.65**
12	**66.6**	**71.01**	**65.32**	**49.52**

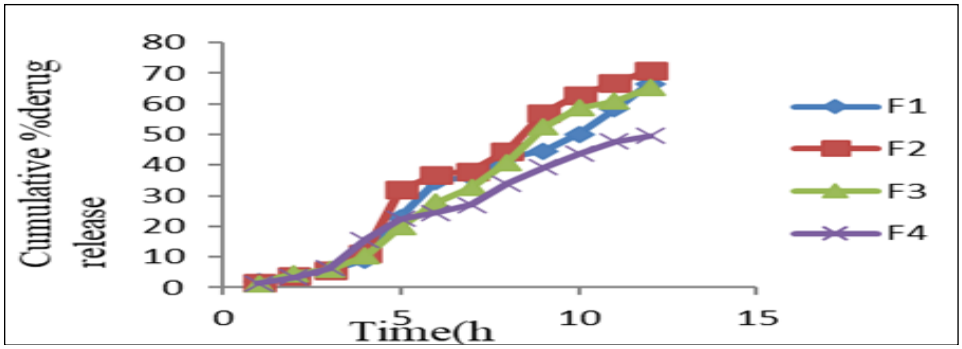


Figure 4 In-vitro drug diffusion studies

The kinetics of drug release

Data obtained from the *in vitro* dissolution analysis from different formulations were adapted to the arithmetical models. Kinetic models included the equations Zero order, First order, Higuchi equation and Korsmeyer - Peppas. Nine and ten summarise the release kinetics of the best formulation (F2). Fig 5,6,7,8 displays the model relevant data; for the best formulation studied along with their R^2 values, K constant and n exponential value, F2 followed either Zero-order or Korsmeyer – Peppas model. Values of the 'n' were found to be between 0.83-0.95, indicating the Non-Fickian dissolution-controlled drug release

Table 11: Release kinetics of formulation 2

Time(hr)	%CDR	Log % CDR	Log time	SQRT time
1	1.53	0.184	0	**1**
2	3.99	0.600	0.3010	**1.4142**
3	5.78	0.761	0.4771	**1.7320**
4	11.28	1.052	0.6020	**2**
5	31.99	1.505	0.6989	**2.2360**
6	36.98	1.567	0.7781	**2.4494**
7	38	1.579	0.8450	**2.6457**
8	44.66	1.649	0.9030	**2.8284**
9	56.96	1.753	0.9542	**3**
10	62.89	1.798	1	**3.1622**
11	66.82	1.824	1.0413	**3.3166**
12	**71.01**	**1.851**	**1.0791**	**3.4641**

Table 12: Kinetic parameters for drug release

Formulation code	Zero order		First order		Higuchi		Korsmeyer - peppas		n values
	r^2	K_0	r^2	K_0	r^2	K_0	r^2	K_0	
F_2	0.9397	5.855	0.7123	0.002	0.8182	0.904	0.9603	1.807	0.909

Drug release kinetics (Figure 5, 6, 7 and 8)

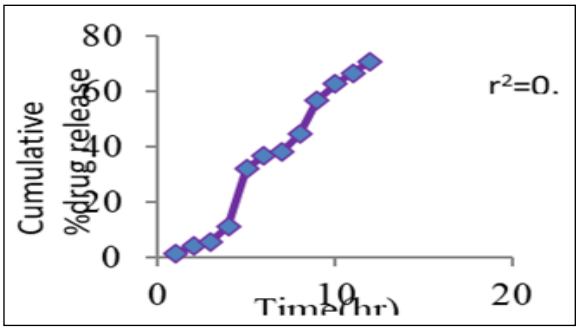


Figure 5: Zero order kinetics

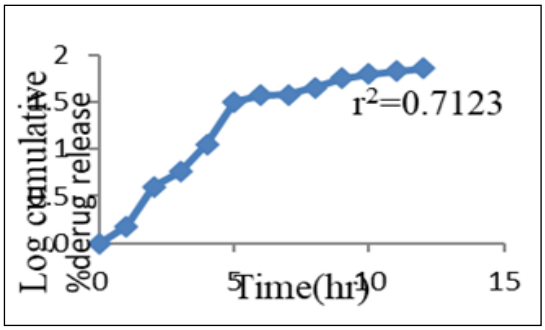


Figure 6: first order kinetics

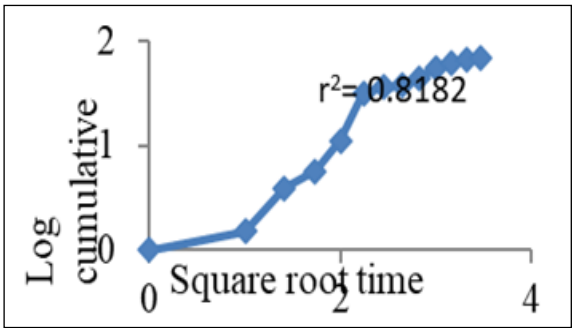


Figure 7: Higuchi model

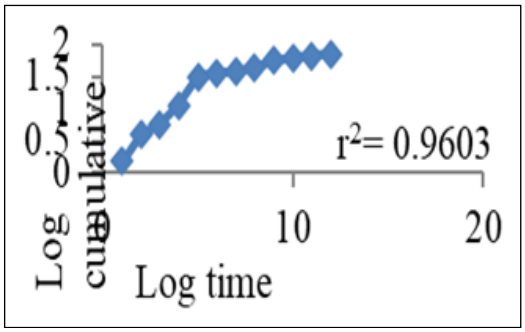


Figure 8: Korsmeyer - peppas

Antifungal activity of *Wrightia tinctoria*

Wrightia tinctoria chloroform extract herbal extract activity studied using the disc diffusion process. Aspergillus spp inoculum was prepared using dextrose broth(Figure11).

Antibacterial activity of *Wrightia tinctoria*

Chloroform extract antibacterial activities were studied using the disc diffusion method. Escherichia coli, staphylococcus aureus, inoculum made with medium nutrient broth (Figure 12)

Table 11: Antifungal activity of herbal extract of Wrightia tinctoria

Extract/Standard drug	Zone of Inhibition (mm)
	Candida albicans (n=4)
Chloroform	R
Clotrimazole	2±5

(R) Resistance

Table 12: Antibacterial activity of herbal extract of Wrightia tinctoria

Extract/Standard drug	Zone of Inhibition (mm)	
	Escherichia coli (n=3)	***Staphylococcus aureus* (n=3)**
Chloroform	R	**15±5**
Cephalexin	**2±5**	**25±5**

(R) Resistance

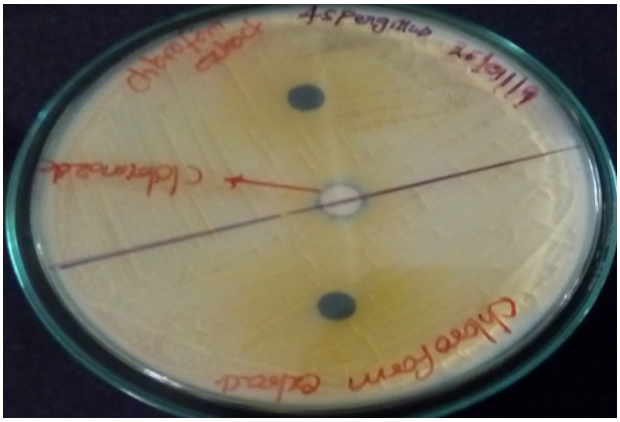


Figure 9: Antifungal activity of Wrightia tinctoria

Chloroform extract not showing activity against human pathogenic *Aspergillus spp*

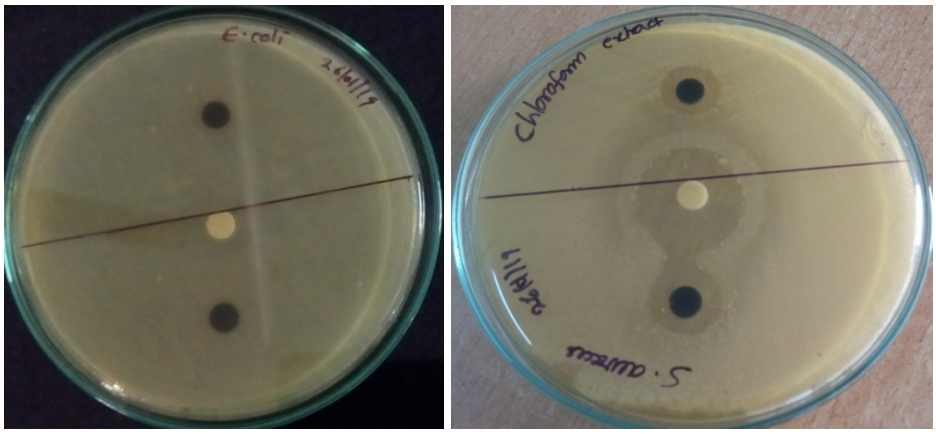


Figure 10 Antibacterial activities of *Wrightia tinctoria* transdermal patches

Chloroform extract not showing activity against human pathogenic *E. coli.* (B) Chloroform extract showing significant activity against human pathogenic *staphylococcus aureus*

Conclusion

Wrightia tinctoria extracts transdermal patches prepared by solvent casting method. The various formulation parameters, polymer-drug ratios and permeation enhancers were optimised to get thin, transparent, smooth, stable and high permeable transdermal patches. The best formulation was selected from the optimisation based on physicochemical evaluation and *in vitro* drug diffusion study. All four batches evaluated for percentage moisture uptake, moisture content, thickness, folding endurance, percentage drug content, per cent elongation, in-vitro, drug diffusion studies and adhesive strength. It indicates the homogenous dispensing of a drug during the patch of drug-polymer ratio 1:4. The data obtained from the *in vitro* diffusion profile of selected formulations fitted with different kinetic equations to determine the drug diffusion and diffusion rate method showed by higher correlation coefficients (r^2). Diffusion polymer-drug ratio 1:4 is zero-order, and the Korsmeyer-Peppas model was indicating non-fickian distribution. These resultsdemonstrate the medicine diffusion from this patch was diffusion controlled. Above the results of *in vitro* diffusion and physicochemical studies, 1:4 ratios (formulation 2) have concluded as the best formulation. Then the formulation twosubjected to a screening of antimicrobial activity. The antimicrobial screening result showed profoundly inhibits the microbial growth around the patch. The results indicate that the *Wrightia tinctoria* plant chloroform extract have antimicrobial activity against *staphylococcusaureus.*

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References

1. Zhang L, Mao S. Application of quality by design in the current drug development. *Asian J Pharm Sci*. 2017;12(1):1-8.
2. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol*. 2008;26(11):1261-1268.
3. Devi VK, Jain N, Valli KS. Importance of novel drug delivery systems in herbal medicines. *Pharmacogn Rev*. 2010;4(7):27-31.
4. Atmakuri LR, Dathi S. Current trends in herbal medicines. J Pharm Res., 2010; 3:109-113.
5. Niculescu-Duvaz I, Springer CJ. Antibody-directed enzyme prodrug therapy (ADEPT): a review.Advanced Drug Delivery Reviews. 1997; 26:151-72.
6. Kothari MJ, Londhe AN. Ethnobotany of Human HealthCare of Chikhaldara, amaravathi district in MaharashtraState. In J.K. Maheshwari Ed. Ethnobotany and MedicinalPlants of the Indian subcontinent, scientific publisher,Jodhpur, India, 2000.
7. Akihisa T, Ishtiaque A, Singh S, Tamura T, Matsumoto M.14α-Methylzymosterol and other sterols from *Wrightiatinctoria*

seeds. Phytochemistry. 1988; 27(10), 3231-3234.

8. Sivarajan VV and Balachandran I. Ayurvedic Drugs and their Plant Sources, Oxford and IBH Publishing Co. PVT. Ltd. New Delhi. 1999. 267-269.

9. Shilpa V P, Manjula Dev M S, Muddukrishnaiah K. Formulation and evaluation of matrix-based sustained-release tablets of quetiapine fumarate using citrus pomelo fruit peel as a natural polymer. Pharmacological and Pharmaceutical Reports.2018; 1(3):1-12.

10. Jolly, C.I, Mechery N.R, Comparative pharmacognostical, physicochemical and Antibacterial studies on seeds of Holarrhena antidysenterica Wall and Wrightia tinctoria R.Br. *Indian Journal of Pharmaceutical Sciences*. 1996: 58(2): 51–54.

11. George v,koshy A.S ,Pushpangadan P,Tryptanthrin from wrightia tinctoria .Fitoterapia,19996;67(6);553.

12. Ren C, Liang F, Lei L, Quang W, Sihai L, LiGang Z, Zhonggui H. Design and invivo evaluation of indapamide transdermal patch. Int J Pharm. 2009; 370:129-35.

13. Abbas HS, Krishnan A, Kotakonda M. Antifungal and antiovarian cancer properties of α Fe_2O_3 and α Fe_2O_3/ZnO nanostructures synthesised by *Spirulina platensis*. IET Nanobiotechnol. 2020;14(9):774-784.

14. Singh, Sumita. Antimicrobial, synergistic activity and antioxidant studies on multidrug resistance human pathogen using crude extract of Azadirachta indica Leaf and Withaniasomnifera Rhizome. *Journal of Plant Pathology & Microbiology*.2015.

15. Muddukrishnaiah K, Shilpa VP. Marine anaerobic bacterial diversity for the production of antimicrobial agents. Environ Dis 2017; 2:99-102.