

Preparation and Evaluation of Transdermal patch using three Ficus bark Extracts

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Abstract- Transdermal drug delivery system is an adhesive patch applied to the skin that contains medication that is intended to be absorbed into the bloodstream through the skin. It has advantages over conventional dosage forms like reduce side effect, control and steady delivery, easy application, reduces the frequency of administration. In the study an attempt was made to prepare a transdermal patch using the bioactive extracts of three Ficus species- *F. Benghalensis*, *F racemosa* and *F religiosa*. These plants are known to have chemical constituents which have wound healing, antimicrobial and antioxidant activity. A patch with these extracts can help overcome the side effects of synthetic medicines. Extracts showed presence of phenols, carbohydrates flavonoids, anthraquinones and saponins. The individual extracts were standardised by estimation of total phenol and flavonoid content. The combined aqueous and methanolic extracts combination contained 1.12% and 1.129% total Polyphenol content and 0.55% and 0.64% flavonoid content respectively. Using Combination of three water extracts and three methanolic extracts, patch was prepared and evaluated for physico- chemical parameters. The physico- chemical parameters of prepared transdermal patch were satisfactory. The patch prepared with methanolic extracts of the barks showed polyphenol and flavonoid content. Hence the extracts can be successfully formulated as transdermal patch.

Key word: *Ficus benghalensis* Linn., *Ficus religiosa* Linn, *Ficus racemosa* Roxb, transdermal patch, Phytochemical screening

Introduction:

The plants of genus 'Ficus' are, found throughout the world as moderate woody plants or trees. They have a vast traditional role in indigenous system of medicine like Ayurveda, Siddha, Unani and Homoeopathy. [1]

The most common species of Ficus are *F. Benghalensis*, *F racemosa* and *F religiosa*. Extracts of these plants have properties that render them capable of promoting accelerated wound healing compared with placebo control. [2] They have also been screened for antimicrobial properties. Phytochemical investigations of some Ficus species have reported phenolic compounds as their major components having enormous potential as sources for antimicrobial drugs.

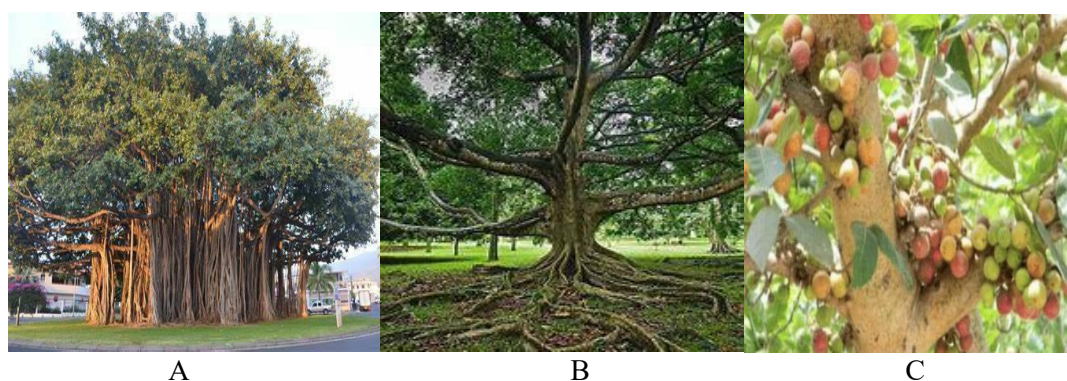


Photo No:1,2,3 Three barks of A- *Ficus benghalensis* Linn., B- *Ficus religiosa* Linn, C- *Ficus racemosa* Roxb.

Ficus benghalensis

Ficus benghalensis, large evergreen tree, belongs to the family Moraceae. It is commonly known as "Indian Banyan Tree". [3] Phytochemical investigation of *F. benghalensis* showed the presence of ketones, flavonoids, flavanols, sterols, pentacyclic triterpenes and triterpenoids, furocoumarin, esters [4]. According to Ayurveda, *Ficus benghalensis* is known to be astringent to bowels; useful in the treatment of ulcers, vomiting, vaginal complaints, fever, different kinds of inflammations, and leprosy. According to Unani system of medicine, the latex of this plant is tonic. The latex also lessens the inflammations, hence useful in piles, nose- diseases, gonorrhoea, etc. The aerial root is found

to be useful in syphilis, biliousness, dysentery, and in treating the inflammation of liver, etc. The bark is astringent and tonic and used in diabetes and leucorrhoea, lumbago, sores, ulcers pains and bruises.[4] *Ficus benghalensis* is known to have Antihyperglycemic and antidiabetic activity, antihyperlipidemic and hypercholesterolemia activity, Anti-inflammatory activity, analgesic activity, antibacterial activity, antifungal activity, larvicidal activity, antidiarrheal activity, antimutagenic activity, antioxidant activity, cytotoxic activity, hepatoprotective activity, antiarthritic activity, antiallergic potential wound healing potential, immunostimulatory effect etc.[5]

Ficus religiosa:

Ficus religiosa, commonly known as peepal is one of the oldest trees in Indian literature [6] Phytochemical screening of aqueous extract of *Ficus religiosa* showed the presence of carbohydrates saponins, phenols, flavonoids, tannins and terpenoid. While the methanolic extract showed the presence of Carbohydrates, Saponins, Phenols, Flavonoids, Proteins, Tannins, Terpenoids. [7] *Ficus religiosa* is used in folk medicine to treat asthma, cough, sexual disorders, diarrhea, hematuria, earache and toothache, migraine, eye troubles, gastric problems and scabies. Leaf decoction possesses analgesic attribute for toothache. [15] *Ficus religiosa* is known to have Antidiabetic activity, Anti-inflammatory activity, Analgesic activity, Antioxidant activity, Anticonvulsant activity, antimicrobial activity, Wound healing activity, Antiamnesia activity, Ant acetylcholinesterase activity, Proteolytic activity, Bronchospasm activity, Immunomodulatory activity, Antitumor activity, Antiulcer activity, Antifungal activity, Anthelmintic activity. [8]

Ficus racemosa:

Ficus racemosa is also known as *F. glomerata*. [9] The leaves contain triterpenoids, tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoids, fucosin, coumarin, phenolic glycosides and saponins. Fruits are reported to contain sterols, triterpenoids, flavonoids, glycosides, tannins, carbohydrates. β -sitosterol, gluanol acetate, hentriacontane, tiglic acid of taraxasterol, lupeol acetate, gallic acid, ellagic acid and α -amyrin acetate. Stem bark contains steroids, alkaloids, tannins, gluanol acetate, stigmasterol, β -sitosterol, β -sitosterol-D-glucoside, gluanol acetate, and quercetin, β -sitosterol, β -amyrin, and lupeol acetate have been isolated from the bark of *F. racemosa*. [10] *Ficus racemosa* Linn has been widely used in traditional medicine for various ailments. Its bark, fruit, leaves, roots, latex, and seeds are used medicinally in a variety of forms, sometimes in combination with other herbs. Roots are reported to be used in dysentery, pectoral complaints, diabetes, applied in mumps, other inflammatory glandular enlargements and hydrophobia. The bark is known to be highly efficacious in threatened abortion and also recommended in urological [11] *Ficus racemosa* Linn is reported to have Anti - Diuretic activity, Anthelmintic, Antibacterial, Antipyretic, Wound healing, Ant filarial, Antidiarrheal, Anti-inflammatory, Antiulcer, Analgesic, Hepatoprotective, Antitussive. [12]

Transdermal Drug Delivery System

A transdermal patch, also known as a skin patch, is an adhesive patch applied to the skin that contains medication that is intended to be absorbed into the bloodstream through the skin. This promotes the healing of a body part that has been harmed.

Transdermal patch: Is an adhesive, drug containing devices of defined surface area that deliver a pre-determined amount of drug to the surface of intact skin at a pre-programmed rate [13]

The basic components of transdermal patch consist of polymer matrix / Drug reservoir, active ingredient (drug), permeation enhancers, pressure sensitive adhesive (PSA), backing laminates, release liner, and other excipients like plasticizers and solvents [13]

Synthetic polymers like Polyvinylchloride, polyethylene, polyvinyl alcohol, polypropylene, polyamide, polyacrylate, polyurea, polyvinylpyrrolidone, polymethylmethacrylate etc have also been successfully used in preparation of TDDS. [13]

Other excipients Various solvents such as chloroform, methanol, acetone, isopropanol and dichloromethane are used to prepare drug reservoir. In addition, plasticizers such as butylphthalide, triethyl citrate, polyethylene glycol and propylene glycol are added to provide plasticity to the transdermal patch [13]

During the past couple of decades, transdermal of drug delivery system (TDDS) is gaining more global acceptance and honor with the demonstration of percutaneous absorption of a large number of drugs.

Transdermal delivery system, such as ointments, patches, and gels, have long been used for skin concerns. In recent years, many novel transdermal applications, such as nano emulsions, liposomes, lipid nanoparticles, and microneedles, have been reported. Transdermal patch is gaining popularity.

Advantages of transdermal patch [13]

- Reduce side effect.
- Control and steady delivery.
- Improved compliances.
- Easy application.
- Protect wound from environment.
- Smother continuous dosing over extended period of time
- Effective method for administering by bio active compounds through the epidermis
- It reduces the frequency of administration

Hence in the present study preparation of transdermal patch using combination of extracts derived from barks of *Ficus benghalensis*, *Ficus racemosa* and *Ficus religiosa* for wound healing is proposed.

MATERIALS AND METHOD**COLLECTION AND AUTHENTICATION OF SAMPLE**

The three selected barks are *Ficus benghalensis* Linn, *Ficus racemosa* Roxb, *Ficus religiosa* Linn, L were collected from Indus Herbs, Basavanagudi, Bengaluru, Karnataka. They were then authenticated at Central Ayurveda Research Institute, Bengaluru.

PREPARATION OF EXTRACTS

Fresh barks of *Ficus Benghalensis*, *Ficus religiosa*, *Ficus racemosa* were dried in Hot Air oven at 40°C for 3 days, powdered and sieved to pass through #10 mesh. All the powders were stored in air tight containers for further use.

Preparation of Aqueous and methanolic extracts

200 g of powdered bark materials were subjected to refluxation using 800ml of water and methanol as solvent for 12 hrs. All the three barks were extracted individually and filtered. The crude extracts were then subjected to rotary evaporation at 60°C to get a semisolid extract. The percentage yield was calculated and extracts were stored in refrigerator for further use. Results are tabulated in Table No: 02

PRELIMINARY PHYTOCHEMICAL SCREENING

The extracts of three barks were individually subjected to phytochemical screening to identify the nature of chemical constituents present in them by following chemical tests. Results of phytochemical screening for individual drugs have been tabulated in Table No:03

Test for Carbohydrates:

100 mg solvent free extract was dissolved in 5mL of distilled water and filtered. (20mg/ml)

Molisch's test: To 2ml filtrate ,2 drops of alcoholic α -naphthol and 1ml conc. H₂SO₄ (along the sides of test tube) was added. A violet ring indicates presence of carbohydrates.

Benedict 's test: To 0.5ml filtrate ,0.5ml Benedict 's reagent was added. It was then boiled for 2 min. A Green/yellow/red color indicates presence of carbohydrates.

Fehling 's test: To 1ml each of Fehling 's solution A & B ,1ml filtrate was added, boil in water bath. A red precipitate indicates presence of carbohydrates.

Tests for Amino acids:

100 mg solvent free extract was dissolved in 5ml of distilled water and filtered.

Ninhydrin test: To 2ml filtrate, 2 drops of Ninhydrin solution was added. A purple- colored solution. indicates presence of amino acids.

Tests for Proteins:

100 mg solvent free extract was dissolved in 5ml of distilled water and filtered.

Biuret test: To 2ml filtrate,1 drop of 2% copper sulphate sol, 1ml of 95% ethanol and KOH pellets were added. A pink colored solution (in ethanolic layer) indicates presence of proteins.

Millon's test: To 2ml filtrate, few drops of Millon's reagent were added. A white precipitate indicates presence of proteins.

Test for Polyphenols:

1 g of solvent free extracts were dissolved in 50 ml distilled water (20mg/ml).

Iodine test: To 1ml extract few drops of dil. Iodine solution was added. A transient red color indicates presence of tannins.

Ferric chloride test: To Extract few drops 5% ferric chloride solution was added. Dark green/bluish black color indicates presence of tannins.

Lead acetate test: To plant extract ,3ml of 10% lead acetate solution was added. A white precipitate indicates presence of tannins.

Tests for Flavonoids:

1 g of solvent free extracts were dissolved in 50 ml distilled water (20mg/ml)

Lead acetate test: To 1ml plant extract, few drops of 10% lead acetate solution was added. A yellow precipitate indicates presence of flavonoids.

Shinoda test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc. HCl were added. Appearance of magenta color after few minutes indicated the presence of flavonoids.

Alkaline reagent test: To 1ml extract, 2ml of 2% NaOH solution and few drops dil. HCl were added. An intense yellow color, becomes colorless on addition of diluted acid indicating presence of flavonoids.

Tests for Sterols:

100 mg of plant extract was treated with 10 ml of chloroform and filtered

Salkowski 's test: few drops of concentrated sulfuric acid were added to filtrate shaken and allowed to stand, appearance of red color in the lower region indicates the presence of sterols.

Liebermann-Burchard's test: the semisolid plant extract was dissolved in 2ml acetic anhydride, 1-2 drops of conc. H₂SO₄ (along the side of test tube) were added. Appearance of reddish-brown ring indicates presence of sterols.

Test for Saponins:

1 grams of solvent free extracts were dissolved in 50 ml distilled water (20mg/ml)

Foam test: 2 ml of extracts were shaken with 5ml of water. if foam persisted for 10 mins it indicates presence of saponins.

Test for Alkaloids:

50gm solvent free extract was mixed with few ml dil. Hcl and then filtered. The following tests were done to the filtrate.

Mayer 's test: too few ml filtrate, 1-2 drops of Mayer's reagent (along the sides of test tube) was added. A creamy white/yellow precipitate indicates presence of alkaloids.

Hager 's test: To few ml filtrate, 1-2 ml Hager 's reagents was added. A creamy white precipitate indicates presence of alkaloids.

Wagner 's test: To Few ml filtrate, 1-2 drops of Wagner 's reagent (Along the sides of test tube) was added. A brown/reddish precipitate indicates presence of alkaloids.

Test for Glycosides:

Detection of Anthraquinone glycosides

Bontrager's test: The extracts were first hydrolyzed. The hydrolyzed extracts were shaken with benzene, the benzene layer was collected and treated with ferric chloride solution and dilute hydrochloric acid and extracts were shaken with dilute ammonia, a pink to cherry red color in the ammoniacal layer indicated the presence of anthraquinone

aglycone.

Detection of Cardiac Glycosides

Legal test: Dissolve 1g plant extract in pyridine ,2% Sodium nitroprusside solution and 10% Sodium hydroxide was added. A pink colored solution indicates presence of glycosides.

Keller-Kallani test: 100 mg solvent free extract was dissolved in 5ml of distilled water and filtered. To 1ml filtrate, 1.5ml glacial acetic acid, 1 drop of 5% ferric chloride and conc. H₂SO₄ (along the side of test tube) were added. A blue-colored solution (in acetic acid layer) indicates presence of cardiac glycosides.

Baljit test: To 2ml extract, a drop of Baljit's reagent (1% aqueous solution of picric acid and 10% solution of sodium hydroxide. Both are mixed before use) was added. A yellow-orange color indicates presence of cardiac glycosides.

STANDARDIZATION OF EXTRACTS:

The extracts of *Ficus Benghalensis*, *Ficus religiosa*, *Ficus racemosa* obtained by refluxation with water and methanol were evaluated for content of different Phyto-chemicals like polyphenols and flavonoid.

Estimation of total polyphenol content by Folin Ciocalteu colorimetric method

Total phenolic content was estimated by using "Folin-Ciocalteu" assay method.

Chemicals used: Folin-Ciocalteu phenol reagent (1:1), 3.5% sodium carbonate,

Standard: Gallic Acid

Procedure:

- 50 mg of standard gallic acid was dissolved in 50 ml distilled water to get concentration of 1mg/ml. From above stock solution 1 ml was taken and diluted to 10 ml to get a concentration of 100µg/ml. From this 0.2, 0.4, 0.6, 0.8 and 1 ml were taken in 25 mL volumetric flasks.
- 1.25 ml of Folin Ciocalteu phenol reagent, 2.5 ml of 3.5 % sodium carbonate solution were added and volume was made to 25 ml using distilled water to get concentration of 20, 40, 60, 80, 100 µg/flask
- These were then kept aside at room temperature for 30 mins.
- The absorbance for the standards were recorded against the reagent blank at 765 nm
- Graph was plotted by taking absorbance of standard Gallic acid on Y axis and concentration of Gallic acid on X axis.

Sample preparation:

Samples were prepared in water to get concentration of 1mg/ml using distilled water.

- 1ml of sample was taken, 1.25 ml of Folin Ciocalteu phenol reagent, 2.5ml of 3.5 % sodium carbonate solution was added and volume was made up to 25 ml using distilled water. The contents were mixed and volumetric flasks were kept aside for 30 mins at room temperature. All the samples were made in the similar manner
- **Blank reagent:** 1.25 ml Folin Ciocalteu reagent, 2.5 ml of 3.5% sodium carbonate and volume was made to 25 ml using distilled water.
- The absorbance for the samples were recorded against the reagent blank at 765 nm
- The gallic acid content was determined by interpolation from Standard linear graph.

From the graph $y=mx+c$ value is obtained Where y is absorbance at 765 nm and x is total phenolic content in the different extracts and using this, we determine the concentration of gallic acid present in the sample according to the absorbance.

Then the total phenol content is determined using the formula

$$T = C \cdot XV/M.$$

Where - T is the total phenolic content in mg· g⁻¹ of the extracts as GAE, C is the concentration of gallic acid established from the calibration curve in mg· ml⁻¹, V is the volume of the extract solution in ml and M is the weight of the extract in g.

The total phenol content was expressed as mg of GAE/ gram of the extract. The absorbance of different concentration of gallic acid at 765nm is shown in Table No:04 The linearity graph is recorded in Graph No:1 Total phenol content of the extracts as mg/g GAE is represented in Table No: 05 and Graph No :2

Estimation of total Flavonoid content by Aluminum chloride colorimetric method

The total Flavonoid content of the three Ficus barks extract was determined by Aluminum chloride colorimetric method using Rutin as standard and details are recorded in Graph No:3 and Table No:06.

Materials:

Rutin: John baker Inc. Colorado, U.S.A.

Potassium acetate: Thomas Baker (Chemicals) pvt. limited, Mumbai.

Aluminum chloride: Finer limited, Ahmedabad.

Acetic acid: 99.8% Spectro chem pvt. ltd, Mumbai. Methanol: 99.5% purity, Thomas baker (Chemicals) pvt. limited, Mumbai.

Preparation of Reagents: 10% Aluminum chloride:

10g of Aluminum chloride was dissolved in 100ml of distilled water. (Produce steamy clouds of hydrogen chloride gas) by placing the flask in an ice bath in a fume hood. 1M Potassium acetate: 9.82g of Potassium acetate was dissolved in 100ml of distilled water.

Preparation of Extracting solvent: methanol: water: acetic acid (140:50:10)

Preparation of standard solution: 200mg of Rutin was dissolved into 100ml of extracting solvent, to obtain a concentration of 2mg/ml (stock solution A)

Solution B - This solution B was prepared by taking 1 ml of above solution A & made up the volume to 10 ml with extracting solvent so as to obtain a concentration of 200µg/ml.

Preparation of sample solution:

a) (fbw, frw, fw, combination of water extract).50mg each drug was dissolved into 25 ml of extracting solvent, to obtain a concentration of 2mg/ml (stock solution A)

Solution C - This solution B was prepared by taking 3 ml of above solution A & made up the volume to 10 ml with extracting solvent so as to obtain a concentration of 600µg/ml.

b) Fbm, frm, fm combination of methanolic extract) 50mg of each drug was dissolved into 25 ml of extracting solvent, to obtain a concentration of 2mg/ml (stock solution A)

Solution D - This solution B was prepared by taking 6 ml of above solution A & made up the volume to 10 ml with extracting solvent so as to obtain a concentration of 1200µg/ml.

Procedure:

a) 0.1, 0.2, 0.3, 0.4, 0.5, 1.0 ml of standard solution (Stock B solution) were taken separately into test tubes and diluted suitably with methanol to get 2.0 ml. To each of these tubes 0.1ml of 10% AlCl₃ solution; 0.1 ml of 1M Potassium acetate solution was added, followed by 2.8 ml distilled water, the contents were mixed. All tubes were allowed to stand at room temperature for 30 min.

b) Sample solution was prepared by taking 1ml of each of the sample solution in place of standard solution and diluted with methanol to get 2.0 ml. To this 0.1 ml of 10% AlCl₃ solution; 0.1 ml of 1M Potassium acetate solution and 2.8 ml of distilled water were added. The contents mixed, allowed to stand at room temperature for 30 min.

c) A blank determination with 2 ml of methanol instead of sample/standard treated similarly, was maintained.

d) Absorbance of the reaction mixture was measured against reagent blank at 415 nm using UV ((Shimadzu pharamspec-1700 UV-visible) spectrophotometer.

e) Total Flavonoid content of each of the extract, was calculated as mg % of Rutin using the standard calibration curve.

f) Linearity data for absorbance of different concentration of rutin with aluminum chloride at 415nm is shown in Table No: 07

g) Graphical representation of the same is seen in Graph No:4

Preparation of Transdermal patch

Table No: 01 Ingredients used in preparation of transdermal patch

Sl. NO	Ingredients	Uses	Quantity
1	HPMC -15cps LR	Polymer	250mg
2	PEG -400	Plasticizers	2-3ml
3	Distill Water	Solvent	10ml
4	Glycerin purified LR	Lubricant	2-5 drops

		/Humectant	
5	Three Aqueous and methanolic extract of Ficus bark (1:1:1 combination)	Wound healing agent	150mg

Procedure:

1. 250 mg of HPMC was weighed and dissolved in 10ml of water in beaker
2. 150mg PEG-400 was weight and added to above solution
3. 1:1:1 Aqueous and methanolic of extract of *Ficus benghalensis*, *Ficus religiosa*, *Ficus racemosa* was added and subject to mixing for 30 mins using magnetic stirrer.
4. The mixture was further sonicated to remove the entrapped bubbles.
5. The viscous mixture was then poured into a petri plate previously greased with glycerine.
6. An inverted funnel was placed with glycerine on the Petri plate to prevent dust from settling on it.
7. The solution was found to solidify into a translucent film after allowing to dry in Petri plate for 20-24hours. This gave the transdermal patch which was stored in clean labelled aluminium foil until further studies were done.

EVALUTION OF TRANSDERMAL DRUG DELIVERY SYSTEM

Physicochemical characteristics of transdermal patch: All the prepared patches of both water and methanolic extract combination of the three Ficus barks *Ficus Benghalensis*, *Ficus religiosa*, *Ficus racemosa* were evaluated for the following

Physicochemical characteristic

- Physical evaluation
- Thickness of patch
- Weight uniformity
- Folding endurance
- Percentage of moisture content
- Phenol content in patch
- Flavonoid content patch

Physical evaluation: All-prepared patches were examined visually for by colour, clarity, flexiability and smoothies the results are tabulated in Table No: 8

Thickness of patch: Three transdermal patch of each formulation were taken and the thickness of the patch was measured using screw gauge at different places. The average patch thickness is tabulated in Table No:8

Weight uniformity: Three patch of each formulation were taken and weighed individually by using single pan balance and average weight of the patch were calculated and was computed the results are tabulated in Table No:8

Folding Endurance: Three transdermal patch of each formulation of size (2×2 cm) were cut by using sharp blade. Folding endurance was determined by repeatedly folding a small strip of patch at the same place till it broke. The number of times, the film could be folded at the same place without breaking gave the value of folding endurance. The mean value of three readings were taken and were computed the results are tabulated in Table No:8

Determination of moisture content: The transdermal patch was weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture content (%) was determined by calculating moisture loss (%) using the formula the results are tabulated in Table No:8

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

STANDARDISATION OF FORMULATIONS.

The formulations were standardized by estimating the Total Phenol content and total flavonoid content

Total Phenol Content of The Formulations:

The total phenol content in all the formulations was estimated by “Folin ciocalteu” method as described under - 1ml of the formulations were mixed with 10ml of water to get Concentration Of 1mg/MI. The Solutions Were Filtered and Phenol Content Was Estimated. The Results Are Tabulated in Table No:09

Total flavonoid content of the formulations

The total flavonoid content in the formulation was estimated by Aluminum chloride colorimetric method as described under -1ml of the

Formulation was mixed with 10ml of water to get concentration of 1mg/ml. the solution was filtered and flavonoid content was estimated the results are tabulated in Table No:09

RESULTS AND DISCUSSION**PREPARATION OF EXTRACTS:**

The three barks of Ficus- *Ficus benghalensis*, *Ficus religiosa*, *Ficus racemosa* were extracted using water and methanol by Refluxation method. The percentage yield of extracts are given below:

Table No. 02: Percentage yield of extracts:

Sl.no	Plant	% Water extract	% Methanolic extract
1	<i>Ficus benghalensis</i>	8.4	3.69
2	<i>Ficus religiosa</i>	12.8	4.58
3	<i>Ficus racemosa</i>	15.2	6.8

PRELIMINARY PHYTOCHEMICAL SCREENING:

All the extracts were subjected to preliminary phytochemical screening to find out nature of chemical constituents present in the extracts. The results of phytochemical screening are tabulated in Table No. 03. All the extracts were found to contain phenols, flavonoids, and saponins.

Table No: 03 Phytochemical composition of aqueous and methanolic extracts of *Ficus bengalensis* Linn, *Ficus religiosa* Linn, *Ficus racemosa* Roxb

PHYTOCHEMICALS	Tests	FBW	FRW	FW	FBM	FRM	FM
Alkaloids	Mayer's test	-	-	-	-	-	-
	Wagner's test	-	-	-	-	-	-
	Dragendroff's test	-	-	-	-	-	-
	Hager's test	-	-	-	-	-	-
Carbohydrates	Molish's test	+	+	-	-	-	+
	Benedict's test	+	+	-	-	-	+
Glycosides	Borntreger's test	-	-	+	+	-	-
	Modified Borntreger's test	-	-	-	+	+	+
	Liebermann Burchard's test	-	-	-	+	+	+
Saponins	Foam test	+	+	+	+	+	+
Fixed oils and fats	Stain test	-	-	-	-	-	-
Resins	Acetone water test	+	+	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+
	Iodine test	+	+	+	+	+	+
Flavonoids	Alkaline reagent Test	-	+	-	+	+	+
	Lead acetate test	+	+	+	+	+	+
	Shinoda test	-	+	-	+	+	+
Proteins and amino acids	Xanthoproteic test	+	+	+	+	+	+
	Ninhydrin test	-	-	-	-	-	-
	Biuret test	-	-	+	+	+	+
	Millon's test	-	-	+	+	+	+
Mucilage	Ruthenium red test	-	-	-	-	-	-

	Swelling test	-	-	-	-	-	-
Anthocyanins	Hydrochloride test	-	-	-	-	-	-

Key (+) present, (-) Absent.

FBW- *Ficus bengalensis* water extract,

FRW- *Ficus religiosa* water extract,

FW- *Ficus racemosa* water extract

FBM- *Ficus bengalensis* methanolic extract,

FRM- *Ficus religiosa* methanolic extract,

FM- *Ficus racemosa* methanolic extract

The water extract of all tree barks showed saponins, flavonoids, tannins and carbohydrates

The methanolic extract all the barks showed saponins, tannins, anthraquinones and carbohydrates.

Standardization of extracts

The standardization of extracts was carried out by estimation of Total Phenol Content and Total flavonoid content.

Estimation of total polyphenol content by Folin Ciocalteu colorimetric method:

The data for linearity curve of gallic acid for determination of polyphenol content is represented in Table No:04 and the graph for the same is shown in Graph No:1.

The Total Phenol content of extracts are reported in Table No. 05.

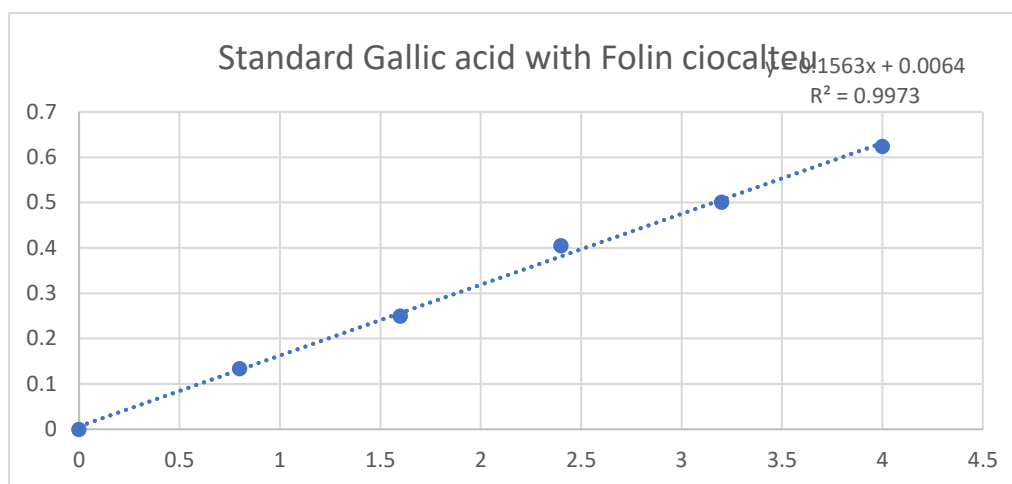
Graph No 02 shows the comparison of Total Phenol content of extracts and combination.

Water extract of *Ficus religiosa* was found to have highest Total Phenol content among the individual extracts.

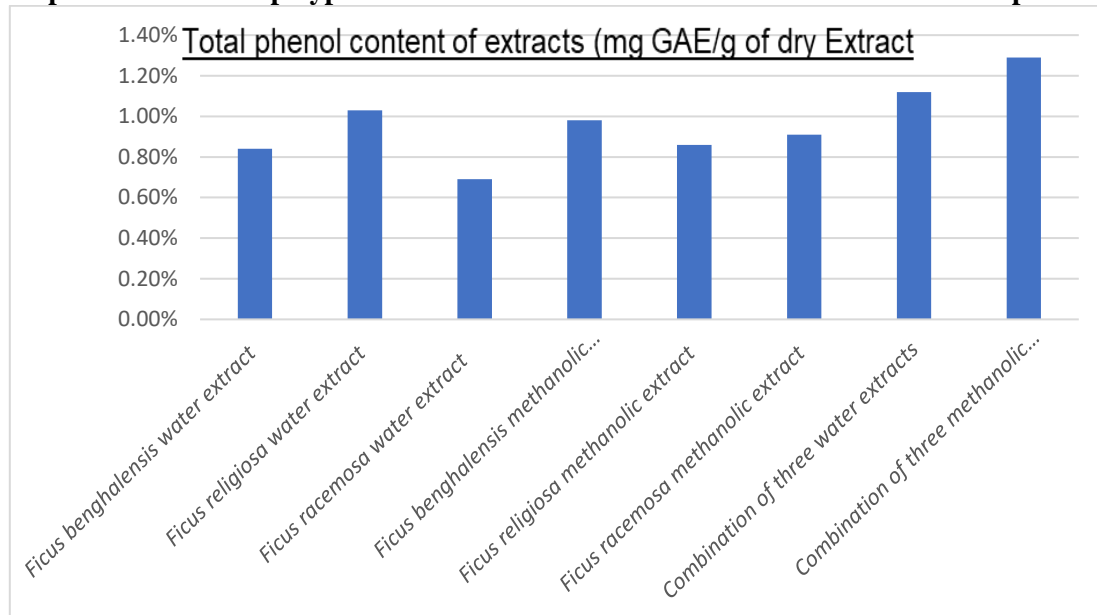
Combination of three methanolic extracts was found to be highest among the combinations.

Table No: 04 Absorbance of Standard Gallic acid at 765nm

Sl no	Concentration ($\mu\text{g/ml}$)	Absorbance at 765nm
1	0.8	0.134
2	1.6	0.250
3	2.4	0.405
4	3.2	0.501
5	4.0	0.624

Graph No: 01 Linearity graph of standard Gallic acid with Folin chocolate**Table No: 05 Total phenol content of extracts**

Sl no	Extracts	Total phenol content in mg
1.	<i>Ficus benghalensis</i> water extract	0.84 mg
2.	<i>Ficus religiosa</i> water extract	1.03 mg
3.	<i>Ficus racemosa</i> water extract	0.69 mg
4.	<i>Ficus benghalensis</i> methanolic extract	0.98 mg
5.	<i>Ficus religiosa</i> methanolic extract	0.86 mg
6.	<i>Ficus racemosa</i> methanolic extract	0.91 mg
7.	Combination of three water extracts	1.12 mg
8.	Combination of three methanolic extract	1.29 mg

Graph No: 02 The % polyphenol content of GAE of extracts and combination respectively**Estimation of total Flavonoid content by Aluminum chloride colorimetric method:**

Flavonoid are one of the secondary constituents deleted in Ficus barks. Quantification of Flavonoids in the extracts and their combination was done by Aluminium chloride colorimetric method.

Linearity for standard Rutin with $AlCl_3$ is show in Table No:06.

Linearity Graph is Represented in Graph No:3;

Flavonoid content as Rutin equivalent is recorded in Table No:07.

Graph No 04 shows the comparison of Total Flavonoid content of extracts and combination.

Methanolic extract of *Ficus religiosa* was found to have highest Total Flavonoid content among the individual extracts.

Combination of three methanolic extracts was found to be highest Flavonoid content among the combinations.

Table No: 06 Absorbance of standard Rutin with Aluminum chloride at 415nm

Sl.no	Concentration ($\mu\text{g/ml}$)	Absorbance at 415nm
1	0.1	0.229
2	0.2	0.472
3	0.3	0.742
4	0.4	0.998
5	0.5	1.172

Graph No: 03 Rutin Calibration curve

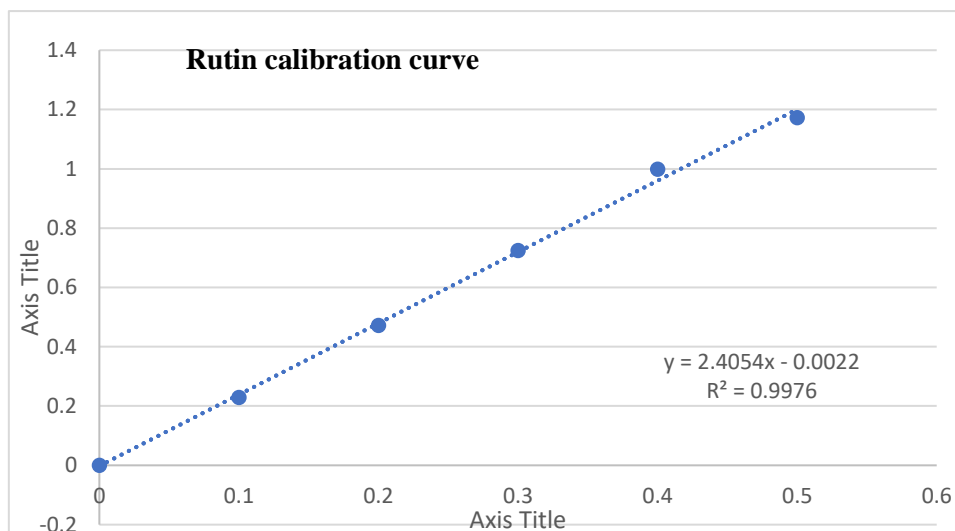
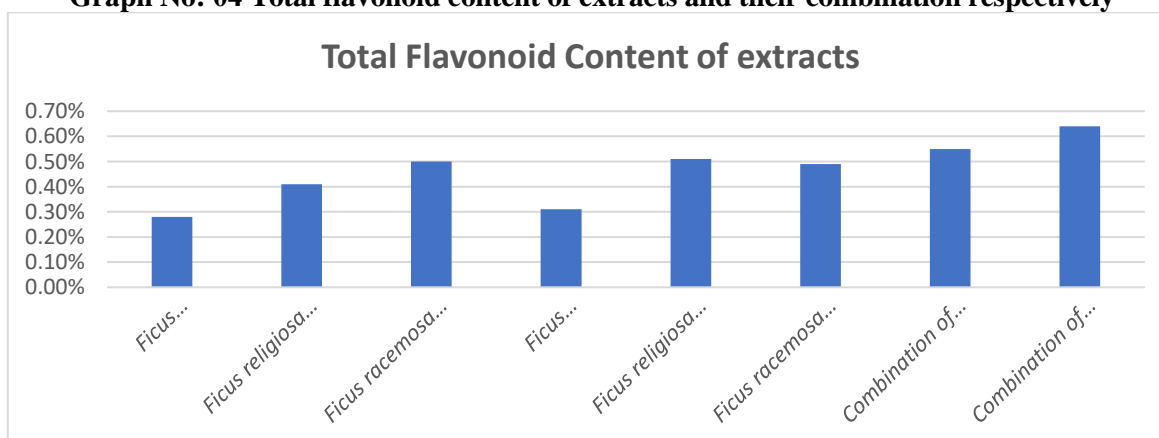


Table No:07 Determination of Flavonoid Content of extracts and combination respectively

Sl no	Extracts	Total flavonoid content in mg
1	<i>Ficus benghalensis</i> water extract	0.28 mg
2	<i>Ficus religiosa</i> water extract	0.41 mg
3	<i>Ficus racemosa</i> water extract	0.50 mg
4	<i>Ficus benghalensis</i> methanolic extract	0.31 mg
5	<i>Ficus religiosa</i> methanolic extract	0.51 mg
6	<i>Ficus racemosa</i> methanolic extract	0.49 mg
7	Combination of three water extracts	0.55 mg
8	Combination of three methanolic extracts	0.64 mg

Graph No: 04 Total flavonoid content of extracts and their combination respectively



Preparation of Transdermal patch

A Transdermal patch was prepared using HPMC as polymer, PEG 400 as plasticizer and water as solvent. This patch was prepared by Solvent casting method.

The picture of prepared patch is shown below in Photo No. 04 and 05.



Photo No:04 Transdermal patch of water extract



Photo No:05 Transdermal patch of methanolic extract

EVALUTION OF TRANSDERMAL DRUG DELIVERY SYSTEM

Physicochemical characteristics of transdermal patch:

All the prepared patches of both water and methanolic extract combination of the three *Ficus* barks *Ficus Benghalensis*, *Ficus religiosa*, *Ficus racemosa* were evaluated for the following Physical evaluation. The results are shown in Table no: 08

The prepared patch was found to have acceptable physicochemical characters.

TPW-Transdermal patch of water extract.

TPM-Transdermal patch of methanolic extract.

Table No:8 – Results of Physicochemical characteristics of Transdermal patch

Parameters	TPW	TPM
Color	Transplant brown	Transplant brown
Clarity	Clear	Clear
Flexibility	Flexible	Flexible
smoothness.	Smooth	Smooth
Thickness of patch	0.241mm	0..236mm

Weight uniformity	58mg	42.6mg
Folding endurance	16.3	16
Percentage of moisture content	3.56%	3.66%

Total phenol and flavonoids content of formulations

All the formulations were evaluated for Total phenol content using —Folin Ciocalteu method. And flavonoid content by aluminum chloride method. The methodology was described essentially the same as in method above. The results are tabulate in Table No:09. Total phenol content of the formulations was found to range from 0.105-0.155 mg GAE/mg of dry extract. The transdermal patch of water extracts (TPW) and the transdermal patch of methanolic extract (TPM) was found to contain highest Total phenol content of 0.155 mg GAE/g of dry extract. The least was found to be in tpw – 0.105 mg GAE/g of dry extract

All the formulations were evaluated for total flavonoid content by Aluminum chloride method. Spectrophotometric determination of flavonoid content of three Ficus spices and combination of water and methanolic extract the results are tabulated in Table No : 09 Total flavonoid content of the formulations was found to range from 0.07-0.09 mg of dry extract. The transdermal patch of water extracts (TPW) and the transdermal patch of methanolic extract (TPM) 0.09 mg was found to contain highest of dry extract. The least was found to be in tpw – 0.07mg of dry extract.

Table – 9 Total phenol and flavonoids content of formulations

Formulations	Phenol content mg	Flavonoid content mg
Transdermal patch water extract of three Ficus spices	0.105	0.07
Transdermal patch methanolic extract of three Ficus spices	0.155	0.09

CONCLUSION:

In the present study, an attempt was made to prepare A herbal Transdermal patch using the bioactive extracts of *Ficus bengalensis* Linn, *Ficus religiosa*, and *Ficus racemosa*. The transdermal patch using HPMC as a polymer incorporating combined-aqueous and combined-methanolic extracts of three Ficus barks were prepared. That transdermal patch of combined-aqueous and combined-methanolic extracts, were then standardized for the polyphenol content and flavonoid content, the patch, which combined aqueous extract showed a polyphenol content of 0.105mg and flavonoid content of 0.07 mg per patch of 2×2 cm. The patch with combined methanolic extract showed poly phenol content of 0.155mg and flavonoid content of 0.09mg per patch of 2x2 cm. Hence this patch can be used as an alternate to conventional dosage forms and help us to overcome the Sid effects of conventional dosage form.

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