

# Physicochemical Investigation And Development Of Antimicrobial Herbal Cream Of Ficus Racemosa Linn. Leaf Extracts

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## Abstract

The Bark, fruit, leaf, root, latex and seeds of the Cluster fig, *Ficus racemosa* Linn belonging to family Moraceae has been widely used for the treatment of various diseases like leprosy, diarrhoea, diabetes, menorrhagia, stomachache, cholera, mumps, haemorrhoids, boils, parotitis, toothache, vaginal disorders, diarrhea, cancer, inflammatory and skin diseases etc. In this study, the extraction process was optimized using solvents with different polarities towards obtaining high antimicrobial potential of selected leaves. Antimicrobial potential of chloroform, acetone, ethanol, methanol and aqueous extracts and also subsequent re-extracted products obtained from latter four solvent extracts by methanol(70%) & ethanol(70%) was investigated. Leaves of the plant was pharmacognostically characterized which helps in identification of the plant species, dried powders were extracted with above mentioned solvents. phytochemical screening revealed that except chloroform extract, other four extracts contain flavonoids(glycosides), aqueous extract contains tannins, sugar and saponins, alcoholics contain steroids, terpenoids and phenolic compounds. Methanol(70%) & ethanol(70%) re-extracted fractions were found to contain most of the phytochemicals. All extracts were investigated for antimicrobial activities. Cream bases were prepared and evaluated. Herbal creams of selected extracts were prepared and antimicrobial potential was investigated by agar plate method comparing the activity with standard cream such as soframycin and clotrimazole. O/W cream of methanol(70%) extract dispersed in DMSO showed highest activity in terms of zone of inhibition against *Klebsiella pneumonia*(24.12mm), *Staphylococcus aureus*(23.42mm), *Candida albicans*(26.74mm) & *Aspergillus niger*(25.78mm). This study confirmed that the methanol(70%) extract incorporated in O/W cream showed better antimicrobial activity due to high content of phytochemicals.

**Keywords:** *Ficus racemosa*, extraction, Methanol(70%), O/W cream, antimicrobial activity.

## INTRODUCTION

Human beings began to believe that nature alone could provide the means to remove pain and disease and thus sought remedies in nature.[1] Nature is the great source of inspiration to the human beings who always tried to explore the secrets of nature, but have never accepted anything without verifying them timely.

pharmacological screening of some herbs used traditionally for the treatment of skin diseases have been identified and tested to establish the scientific proof against such utility.[2,3]

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DOI:  
10.47750/pnr.2023.14.01.25

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How to cite this article: Tankadhar Mishra et. al: PHYSICOCHEMICAL INVESTIGATION AND DEVELOPMENT OF ANTIMICROBIAL HERBAL CREAM OF FICUS RACEMOSA LINN. LEAF EXTRACTS, J PHARM NEGATIVE RESULTS 2023;14(1): 180-191.

Herbal medications in the form of paste of raw material are applied to the skin for therapeutic or protective functions against diseases or suffering of skin.[4] In folklore the tribal people use juice of herbs to treat the cuts and wounds. Farmers apply the fresh juice of leaves of herbs found in road side to stop bleeding and also healing of cuts. The selection of plants usually involves various ethnobotanical approaches as one of the routine methods that are employed in choosing the plant for pharmacological study. Considering the local/regional availability and basing on biodiversity and information of folklore use, the Cluster fig *Ficus racemosa* Linn belonging to family Moraceae was selected for physicochemical investigation and standardization of antimicrobial herbal cream of extracts of leaves. *Ficus racemosa* Linn is a common evergreen, moderate to large, spreading, lactiferous, deciduous tree, 15-18 m high, without prominent aerial roots and is native of many states of India and also many countries and sometimes cultivated for fruits.[5] In Ayurveda, bark, fruits, leaf, roots, latex and seeds of *Ficus racemosa* are medicinally used by Indian practitioners in different forms, sometimes in combination with other herbs.[6] Different parts of *Ficus racemosa* shows antibacterial, hepatoprotective, antitussive, antiulcer, wound healing, anthelmintic, antidiuretic effect, antidiarrheal, anticancer, anti-inflammatory activities etc. Decoction of leaves is used as a douche in dysmenorrhea, as a wash for wounds and ulcers.[7] Root and fruits are used in leprosy, diarrhoea, diabetes, circulatory and respiratory disorders and menorrhagia.[8] Latex is used to cure stomachache, cholera and mumps, haemorrhoids, boils, parotitis, orchitis, traumatic swelling, toothache, vaginal disorders.[9] Fruits are reported to contain sterols, triterpenoids, flavonoids, glycosides, tannins, carbohydrates.[10] The leaves of the plant are rich in triterpenoids (basically lanosterol), flavonoids, alkaloids and tannins.[11] The leaves also contain kaempferol, rutin, arabinose, bergapten, psoralenes, ficusin, coumarin, phenolic glycosides[12] and saponins.[13] Stem bark contains alkaloids, sterols and quercetin.[14] Latex contains a-amyrin,  $\beta$ -sitosterol, cycloartenol, cycloeuophordenol, 4-deoxyphorbol and its esters, palmitic acid, taraxerol, tinyatoxin, tirucalol, trimethyl ellagic acid.[15]

The dried powders of leaves of *Ficus racemosa* Linn were investigated in a scientific way for physicochemical and antimicrobial aspects. An attempt was made to prepare and rationalize the cream of its more active extracts and correlate the activity with standard antimicrobial creams.

#### MATERIAL AND METHOD:

All the materials collected and used in the study were of analytical and pharmaceutical grade.

Collection and Pharmacognostic characterization:[16]

10 kg of the healthy Fresh leaves of varied size of *Ficus racemosa* Linn. were collected from the roadside from our village to Patnagarh, Dist- Bolangir, Odisha India, during

month of September 2018. The plant was authenticated by Mr. Pramod Kumar Panigrahi, Reader in Botany, Jawaharlal College, Patnagarh, Odisha. A voucher specimen was deposited in The Pharmaceutical College, Barapali, Odisha. The macroscopical studies were carried on freshly collected leaves towards size, shape, surface, color etc through visual observation. Microscopical studies were carried out by taking free hand sections of fresh leaves stained with safranin and fast green, mounted on stage of the microscope fitted with camera and observed under bright field for different microscopic anatomical characteristics. Powder microscopy was conducted to determine leaf constants [such as stomatal number, stomatal index, palisade ratio, size of starch granulate and presence of calcium oxalate(by microscopic method)]; physical constants [ash values(by combustion method), Solvent Extractive Values(by maceration), moisture content(by drying and variation in weight)] and presence of inorganic radicals(by chemical method).[17]

Extraction and Phytochemical analysis:[18]

The leaves were shade dried at room temperature, grinded using electrical grinder and passed through the sieve no.60 to get a coarse powder(Fig-1). 250 gm of coarse powder was defatted with 1 litre of petroleum ether(PE). The defatted mass was subjected to successive maceration for a 48 hour cycle at room temperature using solvents at increasing order of polarity. Defatted powders were macerated with one litre each of chloroform, acetone, ethanol, methanol and purified water one after other by drying the powder after each extraction. The filtrates were concentrated by using rotary evaporator till complete dryness at 40 °C. The extracts were denoted as [CL], [AC], [ET], [ML] and [AQ] respectively. Extracts were subjected to preliminary phytochemical analysis by dispersing in 6% DMSO.



Fig 1. Plant, leaf and powder of leaves of *Ficus racemosa* Linn

Biological screening:[19,20]

Biological screening of the extracts was done by using four bacterial strains[*Staphylococcus aureus*(Sa), *Propionibacterium acnes*(Pc), *Klebsiella pneumonia*(Kp) and *Pseudomonas aeruginosa*(Pa)] and four fungal strains[*Candida albicans*(Ca), *Candida tropicalis*(Ct), *Microsporum gypseum*(Mg) and *Aspergillus niger*(An)] collected from Department of Microbiology, Sambalpur University. Culture media selected for use were Nutrient agar and broth for Sa, Thioglycolate agar and broth for Pc, Sabouraud dextrose agar and broth for all fungi.[21] Agar

plates and broths were sterilized prior to use. Extracts were dissolved in 6% DMSO solution and sterilized by filtration using 0.45 µm Millipore filter. Biological screening of extracts was done by studying antimicrobial sensitivity (following disc diffusion method by determining zone of inhibition [ZOI] using paper discs containing 1000 µg of each extract as well as selected discs of standards procured).

Study of re-extracted mass:

[ML] extract (showed profound antimicrobial effect) and other three extracts such as [ET], [AQ] and [AC] (showed antimicrobial activity to some lesser extent) were mixed and

re-extracted by dissolving in Methanol: water(70:30) and Ethanol : water(70:30) with the aim to extract most of the phytoconstituents from all four extracts into the above selected solvent mixtures. The extract thus obtained were denoted as [ML-70] and [ET-70] and were subjected to phytochemical analysis and biological screening to compare the ZOI with the individual extracts.

Formulation and evaluation of cream bases:

Two cream bases each of O/W type (Table 1) and W/O type (Table 2) were selected, prepared and evaluated.

Table 1. Formula of O/W cream base taken for study:[22]

O/W cream base[C-01]		O/W cream base[C-02]	
Ingredients	Amount % w/w	Ingredients	Amount % w/w
<b><u>Oil phase</u></b>			
White soft paraffin	15.0	Stearic acid	05.0
Liquid paraffin (heavy)	06.0	Isopropyl myristate	02.0
Cetostearyl alcohol	07.2	Glyceryl monostearate (S E type)	10.0
Cetomacrogol 1000	01.8		
<b><u>Aqueous Phase</u></b>		<b><u>Aqueous Phase</u></b>	
Popylene glycol	10.0	Glycerin	10.0
Sodium metabisulphite	0.2	Purified water	73.0
Purified water	59.8		
Total	100 gm		100 gm

Table 2. Formula of W/O cream base taken for study:

W/O cream base[C-03]		W/O cream base[C-04]	
Ingredients	Amount % w/w	Ingredients	Amount % w/w
<b><u>Oil phase</u></b>			
Heavy liquid paraffin	25.0	Liquid Paraffin (heavy)	50.0
Bees wax	12.0	Lanolin	05.0
Isopropyl myristate	10.0	Stearic acid	10.0
Petroleum jelly	10.0		
Paraffin wax	05.0		
<b><u>Aqueous Phase</u></b>		<b><u>Aqueous Phase</u></b>	
Borax	01.0	Triethanolamine	01.25
Purified water	35.0	Purified water	33.75
Total	100 gm		100 gm

Method of Preparation of bases:

● Oil phase: Solids were melted in a beaker on water

bath. Soft followed by liquid items were added and mixed by stirring and heated up to 75 ° C.

● Aqueous phase: Ingredients were dissolved in about

50% of purified water in a conical flask and heated to 75 ° C. Rest of purified water was also heated to 75 ° C in another conical flask.

● Aqueous phase was added slowly but continuously to oil phase with stirring at high speed using a mechanical stirrer for about 20 minutes at 75 ° C followed by low stirring after being removed from water bath until the temperature has fallen to about 35 ° C. The prepared cream was reverted to liquefied mass after 6 hours. Finally the cream was cooled and filled in collapsible tubes and also in jars as per the requirement.

Evaluation of cream bases:

Prepared cream bases were evaluated for different parameters for selection of suitable bases for development of herbal creams such as Psychosensory evaluation, Rheological evaluation, Physical evaluation and Thermodynamic evaluation.

Psychosensory evaluation: Cream bases packed in collapsible tubes were evaluated manually by 20 student volunteers of either sex with their own willingness for different parameters such as extrudability, consistency, spreadability, appearance, color and homogeneity and observations are noted in Table 4.

Rheological evaluation: Cream bases were evaluated for spreadability, viscosity and extrudability and observations are recorded in Table 4. Spreadability was measured by the method using simple apparatus suggested by Mutimer et al.[23] in which easiness of spreading without excessive pressure was observed. Viscosity of cream bases was measured by Brookfield Viscometer (DV II+ Pro model) using spindle number S- 64 at 20 rpm at a temperature of 25 °C. Extrudability is measured by the force required to move the cream out of its tube. Mutimer et al. in their investigation described a simple method for extrusion pressure measurement. The yield value was determined from the graph and rheogram is shown in Fig 4.

Physical evaluation: Cream bases can be evaluated for various physical parameters such as pH, Softening range, Bleeding and Globule size analysis and observations are noted in Table 4. pH of prepared creams was measured by using Systronics digital pH meter. Softening range was determined in the melting point apparatus by recording the lower temperature at which the cream started melting and the higher temperature at which the cream melted completely. Bleeding[24] was determined by observing the inner surface of cross section of tube being stored for a week for presence or absence of bleeding due to partial separation of phases. Dispersed phase globule size measurement of creams was carried out by microscopic method using micrometer.[25]

Thermodynamic evaluation: Creams were evaluated for Phase separation, for stability through Freeze-thaw cycling and storage at different higher temperatures. Phase separation study was done to determine the temperature at which two phases separate completely. Freeze-thaw cycling

for study of stability was done by storing the creams at refrigerated temperature (4 °C) and 45 °C repeatedly for six cycles ( at each temperature of not less than 48 hours). The creams were evaluated visually for appearance, phase separation and for dispersed phase globule size distribution. Stability of cream bases were studied by storing the tubes for one month at different temperatures like 25 °C, 30 °C and 40 °C and observed for stabilization of globule size of dispersed phase.

Preparation and evaluation of medicated creams:

Both [ML-70] and [ET-70] extracts which showed higher ZOI in comparison to the individual extracts were incorporated in the selected cream base. Incorporation of extract in the base was done by trituration taking varied % of extract (5, 10, 15 and 20%). Cream base and selected medicated creams were subjected to toxicological studies and all medicated creams were studied for antibacterial potential through determination of ZOI and the effect was compared to the standard antibacterial cream(soframycin skin cream containing Framycetin sulphate 1%) and antifungal cream(Surfaz cream containing Clotrimazole 1%).

Toxicological study was conducted for prepared cream base and medicated creams containing 20% extracts. Draize rabbit patch test is done on abraded skin using three rabbits each. The abraded skin was occluded for 24 hours and then removed for scoring of irritancy, erythema or oedema.[26] Human patch test was done by applying the creams on skin of inner forearm of students on their own willingness. The skin was occluded for 24 hours and then removed for scoring of irritancy, erythema or oedema.

Selection of final formulation and further evaluation:

[ML-70] extract having 15% of extract which contained most of the phytoconstituents including flavonoids and phenolics and having higher ZOI against the bacterial and fungal strains was selected and studied for wound healing potential.

Wound healing activity studies:[27]

Infected Wound Model in albino rats(150-180gm) was utilized to evaluate wound healing activity. On wounding day, animals were anesthetized using subcutaneous injection of ketamine (1 ml/kg) and diazepam (1 ml/kg). The rats were depilated on the back. Full thickness circular excision wounds sized about 300 mm<sup>2</sup> were created along the markings using toothed forceps, scalpel, and scissors. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. After achieving complete haemostasis the wound was inoculated with standard strain of *S. aureus* culture (24 hrs). After 24 h of contamination with *S. aureus* suspensions (1×10<sup>6</sup> CFU/mL), the experimental animals were randomized into five groups (1-3) (6 rats per group). Gr-I Served as control and received the cream base; Gr-II Served as reference antibacterial standard and received Soframycin skin cream(Aventis); Gr-III [ML-70] cream with 15% extract. The animals were

placed singly in separate cages. Treatments of infected wounds commenced on the 2nd day to allow establishment of infection on the wound. The wound area was measured with a translucent paper and a 1 mm<sup>2</sup> graph sheet on day 0, day 4, and thereafter every fourth day until wound closure is completed. Wound contraction was calculated as a percentage of the original wound size.

**RESULT & DISCUSSION:**

Results of Physico-chemical investigation:

Fresh leaves were collected from the local area and studied for macroscopical characteristics. Leaves were simple, ovate, ovate-lanceolate, surface was observed as glabrous with membranous texture. Color of upper surface of leaf was found as dark green, and lower surface light green. Leaf apex was acute and leaf base symmetric, margin seen as wavy. Size of leaves was measured and found 12.95 ±1.73cm long and 7.08±0.94cm broad(n=20).The stipule was found 2.2 cm long, ovate-lanceolate, petiole - 3.8-7cm, angular and reddish brown. Microscopic study was conducted. Lamina of the transverse section showed a prominent hypodermis beneath the upper epidermis. Midrib consisted of well developed collenchymas below upper epidermis and above lower epidermis. Vascular bundles were observed as bicollateral, crescent shaped having patches of perimedullary phloem and 3-4 secondary vascular bundles above the primary vascular bundle. Xylem vessels were observed with reticulate thickening. Ground tissue consisted of loosely arranged polygonal parenchymatous cells having calcium oxalate prisms and cluster crystals. Starch grains were scattered throughout the ground tissue. Anomocytic stomata were observed. Mesophyll was observed below the palisade cells and were composed of 3-4 layers of loosely arranged parenchymatous cells with scattered calcium oxalate

crystals. Two layers of radially elongated palisade cells were seen below the hypodermis. Trichomes were observed as covering, long, unicellular or bicellular, few having a hooked-top.

Leaf constants were observed. Leaves contain about 43 stomata per sq. cm., stomatal index was 34.3, palishade ratio varied from 5.5 to 6.8. Starch granulates and calcium oxalates were visualized in the mounted leaf powder. Ash values were found to be 11.97±0.26% w/w as total ash, as 0.81±0.02% w/w as acid insoluble ash , 2.12±0.06% w/w as water soluble ash , and as 8.34±0.19% w/w as sulphated ash. Extractive values were obtained as 0.62±0.02%(w/w) of PE, 2.14±0.03%(w/w) of CL, 2.81±0.04%(w/w) of Ac, 6.29±0.07%(w/w) of ET, 9.30±0.12% (w/w) of ML and 8.32±0.06% (w/w) of AQ. Moisture content was determined as 6.63±0.08% (w/w) which showed that the powder was less hygroscopic. Inorganic elements such as calcium, sodium, iron, sulphate and chloride were present.

Five extracts were made using different solvents. % yield of [CL] was 1.26%, [AC]-2.54%, [ET]-4.63%, [ML] being the highest as 7.68% and [AQ]-6.17%. The % yield of re-extracted products were much higher with 16.68% of Methanol(70%) and 13.43 % of Ethanol(70%) extracts.

Chloroform extract showed presence of steroids, terpenoids and carotenoids. Most of the phytoconstituents were present in other four extracts. [ET] & [ML] contained alkaloids, glycosides, flavonoids, steroids, terpenoids, tannins, phenolics, carotenoids and coumarins. [AQ] shown the presence of glycosides, including saponin and flavonoid glycoside, saponins, tannins etc. [AC] contained alkaloids, glycosides, flavonoids, steroids, phenolics and carotenoids. On re-extraction using methnol and ethanol in mixture with water as (70:30), it was observed that most of the phytoconstituents were present in these latter extracts.(Table 3)

Table 3: Phytochemical screening of leaf powder of Ficus racemosa Linn.

Phyto chemicals	[CL]	[AC]	[ET]	[ML]	[AQ]	[ML-70]	[ET-70]
Alkaloids	-	+	+	+	-	+	+
Glycosides	-	+	+	+	+	+	+
Saponin glycosides	-	-	+	+	+	+	+
Flavonoid glycoside	-	-	+	+	+	+	+
Saponins	-	-	-	+	+	+	+
Flavonoids	-	+	+	+	-	+	+
Steroids	+	+	+	+	-	+	+
Terpenoids	+	-	+	+	-	+	-

Reducing sugars	-	-	-	+	+	+	+
Carbohydrates	-	-	-	-	+	+	+
Tannins	-	-	+	+	+	+	+
Amino acids & Proteins	-	-	+	+	+	+	+
Phenolic compounds	-	+	+	+	-	+	+
Carotenoids	+	+	+	+	-	+	+
Coumarins	-	-	+	+	-	+	+

(+)sign stand for presence and (-ve) sign stand for absent of phytoconstituents

Findings of biological screening:

The antimicrobial sensitivity test of microorganisms (bacteria and fungi) in terms of zone of inhibition was carried by disc diffusion method. Paper discs were prepared for each selected extract containing 1000 µg / ml and subjected to zone of inhibition study. [ML] showed better zone of inhibition (11.13 – 17.46 mm) against various bacteria. [ET] showed average value of 7.18-15.14mm, [AQ] showed average value of 7.86-12.08 mm and [AC] showed average value of 7.13-15.93 mm. Chloroform extract showed the lowest zone of inhibition. Similarly [ML] showed better zone of inhibition (14.13 – 17.73 mm) against various fungi. [ET] showed average value of 7.14-14.21mm, [AC] showed average value of 10.41-16.83 mm, [AQ] showed average value of 7.53-9.16 mm and

Chloroform extract showed the lowest zone of inhibition against fungal strains.

After re-extraction it was observed that both Methanol(70%) and ethanol(70%) extracts showed higher ZOI in comparison to respective individual values. [ML-70] showed the ZOI more than 20mm with all the strains with highest against Pb(21.87mm) and lowest against Mg(19.78mm). Similarly, [ET-70] showed the ZOI in the range of 18.34mm(Ct) to 20.76mm(Kp). These increase in antimicrobial activities shown by these latter extracts might be due to the presence of higher level of phytoconstituents after re-extraction.(Table 4 & Fig 2) Both extracts([ML-70] and [ET-70]) were taken for preparation of medicated creams

Table 4: Zone of inhibition (in mm) shown by extracts against microbial strains:

Sr. No.	Microbial Strains		Plant Extracts						
			Zone of inhibition in mm[n=3]						
			[CL]	[AC]	[ET]	[ML]	[AQ]	[ML-70]	[ET-70]
1	<i>S. aureus</i>	Sa	6.34 ±0.13	7.13 ±0.14	11.53 ±0.21	15.46 ±0.01	11.23 ±0.17	21.64 ±0.03	20.35 ±0.08
2	<i>P. acnes</i>	Pb	6.53 ±0.21	15.93 ±0.04	7.87 ±0.15	11.13 ±0.05	7.86 ±0.04	21.87 ±0.17	19.69 ±0.21
3	<i>K. pneumoniae</i>	Kp	6.23 ±0.21	10.86 ±0.07	15.14 ±0.09	17.46 ±0.05	12.08 ±0.11	21.34 ±0.07	20.76 ±0.01
4	<i>P. aeruginosa</i>	Pa	6.00	11.41 ±0.12	7.18 ±0.14	15.22 ±0.13	11.58 ±0.18	20.24 ±0.06	20.13 ±0.11
5	<i>C. albicans</i>	Ca	6.46 ±0.06	16.42±0 .05	14.21±0 .03	17.73±0 .04	9.16±0. 14	20.98 ±0.12	18.69 ±0.23

6	<i>C. tropicalis</i>	Ct	6.94 ±0.18	16.83±0.16	10.89±0.12	16.96±0.13	7.53 ±0.21	20.64 ±0.04	18.34 ±0.17
7	<i>M. gypsum</i>	Mg	6.23 ±0.21	10.53±0.17	8.36±0.05	14.13±0.11	8.83±0.08	19.78 ±0.02	19.02 ±0.19
8	<i>A. niger</i>	An	6.24 ±0.21	10.41±0.20	7.14±0.11	15.01±0.09	7.86±0.14	20.03 ±0.14	18.86 ±0.21

\*The results were expressed as mean± standard deviation.

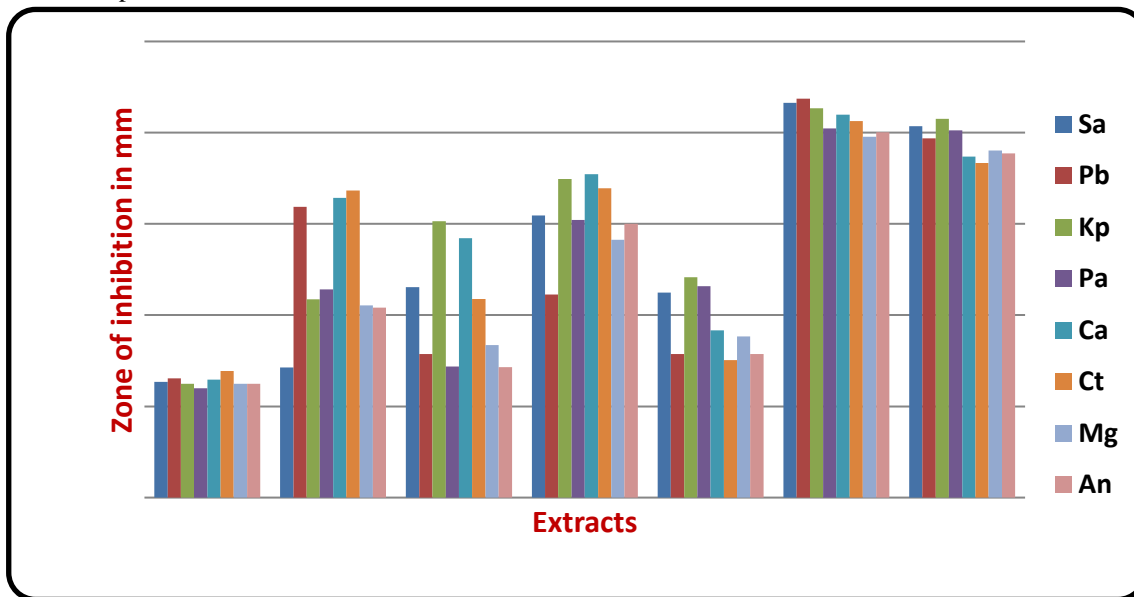


Fig 2. Histogram depicting the comparative zone of inhibition of the extracts of leaves of Ficus racemosa Linn.

Evaluation of cream bases:

Cream bases were evaluated for different parameters to select the better formulations for further study. Average findings of 20 students on psychosensory observations are noted in Table 5. Findings of rheological, physical and thermodynamic evaluation are noted in Table 6. The

rheogram is depicted in the graph.(Fig 3) From the study of globule size analysis after being stored at varied temperature and passed through freeze-thaw cycling, the comparison of frequency distribution of globules of cream bases in the range of 10-20 µm was made(Fig 4)

Table 5: Observations of psychosensory evaluation:

Cream base	Extrudability*	Consistency <sup>■</sup>	Spreadability*	Appearance & Colour	Homogeneity
C-01	3.8±0.24	A	3.7±0.37	Milky white	++
C-02	1.5±0.36	D	2.7±0.68	Light Yellowish white	++
C-03	2.9±0.15	C	2.8±0.21	Yellowish white	++
C-04	0.6±0.98	D	0.7±0.13	Light yellow	—

[ ● 3 - 4: Very good ; 2 - 3: Good ; 1 - 2: Fair ; 0 - 1: Not fair.]

[ ■ a :soft & good ; b: Soft yet stiff ; c: Firm & stiff d: Not satisfactory]

[ ++ : Homogenous —: Non-homogenous]

Table 6: Data showing findings of rheological, physical and thermodynamic evaluation:

Parameters	C-01	C-02	C-03	C-04
Spreadability (gm. cm./sec)[n=3]	154.8	126.3	133.3	65.8
Viscosity ( cps ) [n=3]	28400	34900	21430	14610
Extrudability	++++	++	+++	+
Yield value	411.5	135.9	501.0	-
pH	5.2	4.8	5.2	6.2
Softening range in °C	46– 50	47 – 51	47 – 51	46– 52
Bleeding	No	Yes	No	Yes
Phase separation at °C	76	63	71	54
Phase separation after freeze-thaw cycling	No	Partial	No	Complete

++++ : Excellent ; +++ : Good ; ++ : Fair ; + : Poor

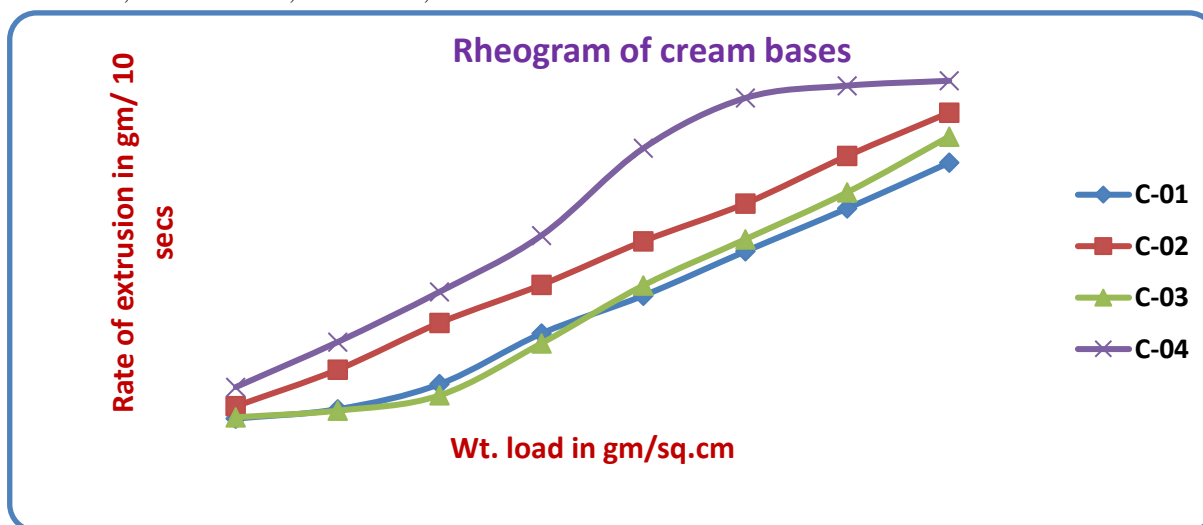
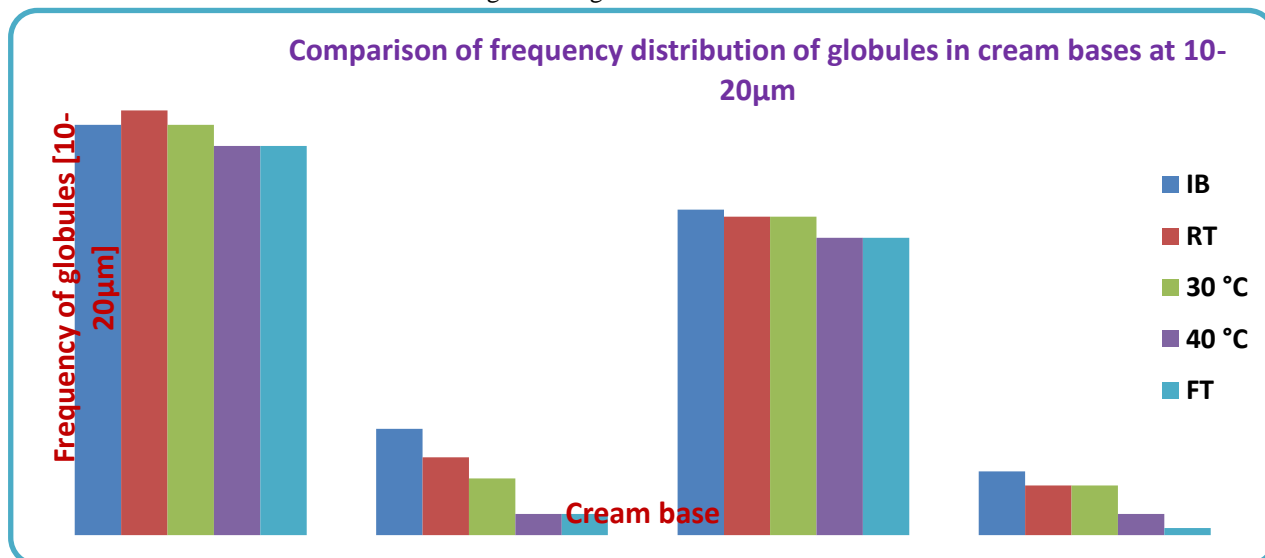


Fig 3. Rheograms of cream bases



IB – Initial cream base, RT- Room temperature, 30 °C - Storage at 30 °C,  
40 °C - Storage at 40 °C, FT – Freeze - thaw cycling

Fig 4. Histogram depicting the comparison of frequency distribution of globules of cream bases at 10-20 µm.

From the overall observation it was seen out of O/W base that the cream base C-01 showed better results towards satisfactory observations by students. C-01 showed better spreadability with good flow and satisfactory yield value. It was more stable at higher temperature without much change in distributed globule size. This might be due to widely used emulsifying agent, cetomacrogol 1000 which is incorporated in many pharmaceutical creams. The Cetyl alcohol helped in softening the base giving proper consistency and better spreadability. The C-02 base showed poor result in comparison to C-01. Similarly out of W/O base C-03 showed satisfactory result with proper yield value and good flow due to ingredients which were incorporated in oil phase. Increase in spreadability may be due to isopropyl myristate. C-03 cream was more stable than C-04. There was bleeding in case of C-04. Considering all the parameter, C-01 and C-03 cream bases were considered for further study.

Results of antimicrobial potential of medicated creams:

[ML-70] and [ET-70] extracts at 5,10,15, & 20% were incorporated in the selected base C-01 and C-03 and medicated creams were subjected to antimicrobial study by determining ZOI.

C-01 cream base containing 15% w/w of [ML70%] extracts showed ZOI value as 26.35-28.65 mm against tested bacterial strains. Similar results were obtained in case of creams containing 20%w/w (26.40-28.55 mm). C-01 cream base containing 15% w/w of [ET-70%] extracts showed ZOI as 21.98- 24.12 mm against tested bacterial strains. Similar results were obtained in case of creams containing 20%w/w (22.00- 24.14mm). Other creams with less drug content showed proportionally lower ZOI against tested bacterial strains. Similarly 15% w/w of [ML70%] extracts showed ZOI value as 23.33-26.74 mm against tested fungal strains, 20% w/w of [ML70%] extracts showed ZOI value as 23.30-26.74 mm. 15% w/w of [ET-70%] extracts showed ZOI value as 20.36-21.14 mm against tested fungal strains, 20% w/w of [ET-70%] extracts showed ZOI value as 20.41-2.18 mm. Other creams showed lesser ZOI against tested fungal strains. From the antimicrobial study it was concluded that the Methanol(70%) extract showed the maximum effect at 15%w/w concentration and there was no

more effect on increase in drug content further. Methanol(70%) extract showed better result than Ethanol(70%) extract.

C-03 cream base containing 15% w/w of [ML70%] extracts showed ZOI value as 16.72-25.37 mm against tested bacterial strains. Similar results were obtained in case of creams containing 20%w/w (16.70-25.42 mm). C-03 cream base containing 15% w/w of [ET-70%] extracts showed ZOI, 20.36- 21.87 mm against tested bacterial strains. Similar results were obtained in case of creams containing 20%w/w (20.40-21.90 mm). Other creams with less drug content showed proportionally lower ZOI against tested bacterial strains. Similarly 15% w/w of [ML70%] extracts showed ZOI value as 22.80-26.75 mm against tested fungal strains, 20% w/w of [ML70%] extracts showed ZOI value as 22.87-26.80 mm. 15% w/w of [ET-70%] extracts showed ZOI value as 21.04-21.56 mm against tested fungal strains, 20% w/w of [ET-70%] extracts showed ZOI value as 21.00-21.60 mm. Other creams showed lesser ZOI against tested fungal strains. From the antimicrobial study it was concluded that the Methanol(70%) extract showed the maximum effect at 15%w/w concentration and there was no more effect on increase in drug content further. Methanol(70%) extract showed better result than Ethanol(70%) extract.

From the comparison of antimicrobial potential of creams containing 15% of extracts in selected bases(C-01& C-03) against standard(Table 7 & Fig 5) , it was observed that 15% cream of [ML-70] extract in C-01 base showed highest antimicrobial potential than other three creams. The higher activity of [ML-70] extract might be due to presence of higher concentration of phytoconstituents and mainly flavonoids and phenolics which are supposed to be responsible for better antimicrobial potential of plant.

Reports of wound healing activity studies:

Percentage wound contraction of infected wounds(by bacterial strains) was determined and compared to the control and standard. It was observed that the 15% cream of [ML-70] extract in C-01 base had taken about 20.23days (mean time of epithelialization) for wound closer where the standard took 15.33 days [MTE].(Table 7)

Table 7: Comparison of antimicrobial potential of creams containing 15% of extracts in selected base(C-01& C-03) against standard

Medicated creams	% inhibition in terms of ZOI by medicated creams of Plant II against  Microbial strains
Containing 15%	

extract	Bacterial strains				Fungal strains			
	Sa	Pc	Kp	Pa	Ca	Ct	Mg	An
C-01-[ML-70]	86.82	86.91	78.62	93.21	83.51	85.40	83.18	84.94
C-01-[ET-70]	70.97	72.49	66.61	78.61	64.67	74.52	70.90	69.65
C-03-[ML-70]	74.70	76.98	70.89	73.10	76.17	82.28	79.16	74.86
C-03-[ET-70]	59.36	67.15	60.15	44.08	62.05	73.32	68.14	67.74
Standard	100	100	100	100	100	100	100	100

\*Soframycin skin cream (antibacterial) and Surfaz cream(antifungal)

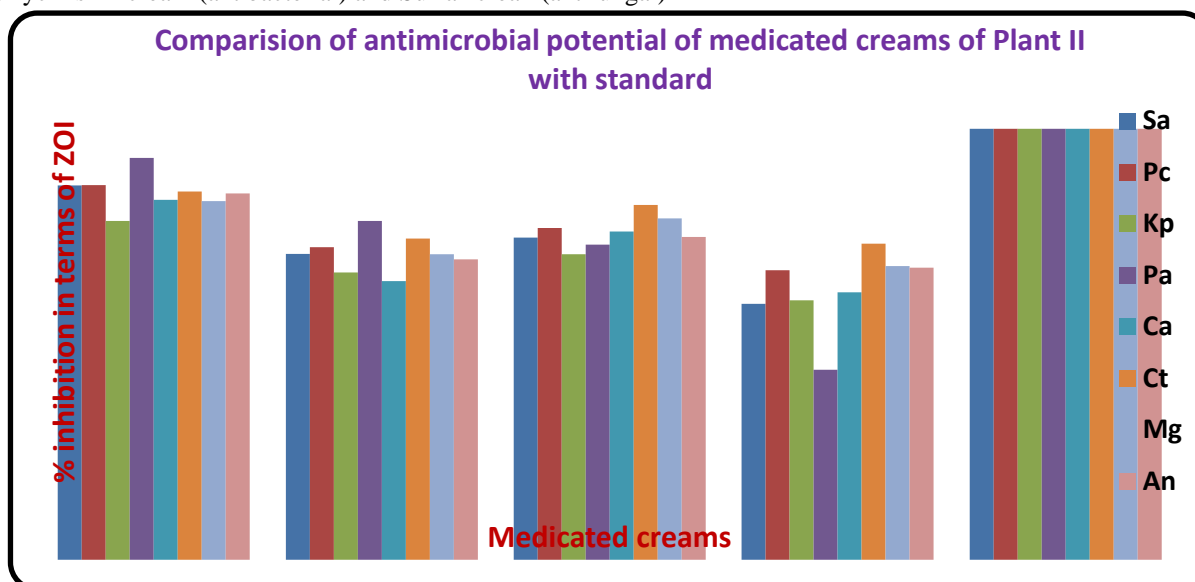


Fig 5. Comparative figure of antimicrobial potential of creams with standard.

Table 7: Effect of selected herbal cream of leaf extract of Ficus racemosa Linn on % wound contraction:

Treatment	% Wound contraction of excision wounds						
	± SEM on post wounding days						
	0 day	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	18 <sup>th</sup> day	20 <sup>th</sup> day
Control	0	3.34	10.32	26.44	33.67	41.80	57.50
[Cream base]		± 1.84	±3.19	± 2.79	± 1.39	± 1.16	± 0.59
Standard	0	30.06	72.39±	93.10±	100.0*	100.0*	100.0*
[Soframycin]		± 2.98	1.86*	0.38*			
[ML-70]	0	29.97	54.91	79.14	94.96	97.36	99.07

		$\pm 1.96$	$\pm 2.34$	$\pm 1.05$	$\pm 0.78$	$\pm 0.42$	$\pm 0.23^*$
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Values are mean  $\pm$  S.E. (n=6); \* P < 0.01 Vs. respective control by student "t" test.

## CONCLUSION

*Ficus racemosa* Linn is a well known plant. Bark, fruits, leaf, roots, latex and seeds of the plant are medicinally used in different forms, sometimes in combination other herbs by the Indian practitioners. Many researchers evaluated the pharmacological activity of the plant. However, from the literature survey, it was found that no study has been done on the antimicrobial potential on the leaves of the plant available in the collected area considering its biodiversity. The leaves were pharmacognostically characterized and the physical values of leaf were determined which were comparable with the standard record. The extracts were rich in phytoconstituents such as flavanoids, phenols, alkaloids, glycosides, sterols and saponins. Methanol(70%) extract have shown profound antimicrobial activity. The leaves are rich in flavonoids and phenolics for which the methanol(70%) extract showed the higher antimicrobial potential. An attempt was taken to prepare both O/W and W/O type cream bases which were evaluated scientifically. One best formulation of each cream base was selected and the medicated creams were prepared using varied concentration of extracts. The percentage incorporation of extract was finalized as 15%. The O/W cream(C-01) of 15% Methanol(70%) extract[ML-70] was effective in wound healing as compared to the standard. The results conclude that the metanolic(70%) extract of leaves are effective as antimicrobial and can be formulated as cream. If the creams are properly formulated, pharmaceutically and pharmacologically studied in a scientific manner, the formulation can be used therapeutically.[28]

### Statistical analysis

All the experiments were conducted in triplicates and results are represented as the means and standard errors of the means. For antibacterial data, student 't' was performed.

**FUNDING:** The research study was performed by self-funding.

**CONFLICT OF INTEREST:** The authors declare no conflict of interest

**AUTHOR CONTRIBUTIONS:** All authors equally participated in the designing of the study, collection of data, and drafting of the manuscript.

**ACKNOWLEDGMENTS:** I fully Acknowledge the staff of The pharmaceutical college Barpali, my supervisor Dr. B B Panigrahi and co-supervisor Dr. M K Pani and our Laboratory staff for their help and support for this work.

### ABBREVIATIONS

[CL]=Chloroform extract

[AC]=Acetone extract

[ET]= Ethanol extract

[ML]= Methanol extract

[AQ]= Aqueous extract

[ML-70]= Methanol extract in methanol:water(70:30)

[ET-70]= Ethanol extract in ethanol:water(70:30)

ZOI= Zone of inhibition

MTE= Mean time of epithelialization

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