



Development and Evaluation of Polyherbal Antiacne Gel

Dr. Archana Gorle Ingle^{*1}, Dr. Deepak Pokharkar², Dr. Sonali Sonulakar³, Shreelekha Desai⁴, Darpan Falak⁵

NCRD'S Sterling Institute of Pharmacy Navi Mumbai-400706, Maharashtra, India

(Received: 14 April 2024

Revised: 1 May 2024

Accepted: 18 June 2024)

KEYWORDS

Antimicrobial, staphylococcus aureus, Amla, Manjishta, Green coffee, Anti-acne

ABSTRACT:

Objective: This study was aimed at selection of medicinal plants possessing antimicrobial activity against the staphylococcus aureus bacteria in the skin responsible for causing acne. Preparation of gel formulation containing extracts of the plants Amla, Manjishta, and Green Coffee which is effective against stephylococuss aureus bacteria.

Method: Three identical formulations were made. Pre- and post-formulation results led to the creation of a gel formulation with concentrations of 10 mg, 20 mg, and 40 mg of Amla, Manjishta, and green coffee. The anti-aging property of F3 was determined to be optimal. Utilizing a Franz diffusion apparatus, diffusion studies are carried out.

Result: A gel composition with favorable qualities that is stable, effective, and aesthetically pleasing was achieved for the objectives of protecting, hydrating, and treating acne on the skin.

Conclusion: An extract of green coffee, amla, and manjishta was combined to create a gel that showed potential for treating acne

1. Introduction

As the expense of contemporary drugs is too exorbitant, herbal plants are essential to the healthcare systems of large segments of the world's population. Medicinal plants are the source of herbal compositions. According to WHO estimates, 80% of people worldwide get their primary medical care from herbal remedies. 25% of the medications in the modern pharmacopoeia are derived from plants. Both national and international markets are embracing herbal medications. Approximately 25000 formulations based on medicinal plants are used in India customarily to treat, prevent, and cure illnesses. Herbal medications show promise in the treatment of a number of illnesses[1].

Acne vulgaris, a common inflammatory dermatological condition affecting the sebaceous unit, has a protracted course. Although it can also affect the upper arms, trunk, and back, the disease usually manifests as papules, pustules, or nodules on the face. Acne vulgaris' major lesion, known as a "comedo," is caused by the interaction of many factors during the disease's evolution. Although teenagers are the most prone to develop acne vulgaris, persons of all ages can be afflicted. Other acne kinds

include pustules, nodules, cysts, blackheads, and whiteheads. Acne develops because the oil glands are vulnerable to normal levels of androgens in the blood. The presence of the bacterial species Cutibacterium acnes (C acnes) and the resulting inflammation exacerbate the process. Other species include *Propionibacterium acne*, *Klebsiella Pneumoniae*, *Staphylococcus epidermidis*, *Staphylococcus pyogenes*, *Streptococcus agalactiae*.

2. Objectives

Antibiotics like Clindamycin and erythromycin as monotherapy has limited effectiveness because of the development of resistant strains of Acnes. Combined Oral contraceptive causes Nausea, vomiting at beginning of therapy. Spironolactone leads to Diuresis, irregular menstrual cycle, breast tenderness, hypotension, dryness of skin, irritation, hyperkalaemia (pre-existing cardiac condition or taking concurrent drugs). A sulphur-based antiseborrheic therapy causes odour problems and stains on clothing. For those with darker skin tones, salicylic acid causes hyperpigmentation, stinging, and drying of the skin. Side effects of topical retinol include flushing and skin inflammation. Increased liver enzymes,



cholesterol levels in the blood, nosebleeds, muscle aches, and dry skin are all brought on by oral retinol. Benzoyl Peroxide causes Irritation and dryness of skin. It can bleach hair, causes irritation to eyes, mouth, lips, etc. Topical Dapsone is well tolerated but it causes methemoglobinemia. Topical Azelaic Acid causes skin irritation, hypo pigmentation, skin dryness[2,3]. The recent work on the polyherbal anti-acne gel has proved to possess antimicrobial activity against the microorganisms in the skin responsible for causing acne using Amla, Green Coffee and Manjishtha.

The motto of the study was to formulate and assess a polyherbal Anti-acne gel with extracts from the chosen plants that is more visually pleasing, stable, and effective than synthetic formulations while also having fewer adverse effects.

3. Methods

Crude drugs were procured from local market. Extract of Amla was prepared from Fruit of *Embllica officinalis* (Amla). Extract of Green Coffee was prepared from Beans of coffee arabica (Green coffee) and extract of Manjishtha was prepared from Roots of *Rubia cordifolia* (Manjishtha). Excipients (carbopol, Propylene glycol, methyl paraben, propyl paraben, glycerine, triethanolamine, rose water) was procured for the college.

It refers to the study of physiochemical properties of drugs whether single or combined, in order to develop a safe and efficacious medicine for use.

Macroscopic Study: This type of study involves using the human eye to describe the morphology of plant parts. Organoleptic characteristics were assessed, including size, colour, odour, taste, and form. Based on eye inspection, the morphological characteristics of the *Rubia cordifolia* root, *Embllica officinalis* fruit, and Coffee arabica seeds were examined.

Microscopic Analysis: Using various staining agents, powder microscopy is an assessment and quality control technique used for medicinal herbs to examine particular microscopic characteristics. The process of employing an authenticated sample in a comparative analysis to find adulterants in a sample.

Physicochemical Study: This type of investigation establishes the identity and caliber of the raw drug

samples. "The Ayurvedic Pharmacopoeia of India" and "Indian Pharmacopoeia" were used as guides during the testing process. **Phytochemical Screening:** This study includes Chemical Evaluation by Comprehensive Phytochemical Screening.

Antimicrobial study: This study focuses to evaluate the antibacterial properties of herbal extracts -*Rubia cordifolia*, *Embllica officinalis*, and coffee arabica against the skin pathogen *Staphylococcus aureus* in vitro.

Formulation Studies

Table 1 :Formula For Cream

SR.NO	INGREDIENTS	QUANTITY TAKEN (FOR 100GM)		
		F1	F2	F3
1	Carbopol	1gm	1gm	1gm
2	Propylene glycol	10ml	10ml	10ml
3	Methyl paraben(MP)	0.2gm	0.2gm	0.2gm
4	Propyl paraben(PP)	0.08gm	0.08gm	0.08gm
5	Glycerine	5ml	5ml	5ml
6	Extract of amla	1gm	2gm	4gm
7	Extract of green coffee	1gm	2gm	4gm
8	Extract of manjishtha	1gm	2gm	4gm
9	Triethanolamine	q. s	q. s	q. s
10	Water	q. s	q. s	q. s
11	Rose water	q. s	q. s	q. s

Preparation of extract of *Embllica officinalis*, *Coffee arabica* and *Rubia cordifolia*:

Extraction process: Soxhlet apparatus

Solvent for extraction: Hydroalcoholic solvent (30% ethanol and 70% water) is used for the extraction process.

Procedure: A Soxhlet device is loaded with 50g of the crude medication that has been coarsely powdered. After the 150 ml extracting solvent is added to the flask and heated, its vapours eventually condense in the condenser. When concentrated extractant descends into the thimble holding it, contact extracts the crude medication[4,5]. When the liquid level in the chamber reaches the syphon tube's top, it syphons into the flask. This process is continued until the last drop of solvent



from the syphon tube evaporates without leaving a trace. After the extraction method is completed, the extract in the flask is filtered, and solid extract is created by evaporating it over a water bath[6,7,8].

Method Of Preparation:

Preparation of Polyherbal anti- acne gel: Gel was prepared as per table

Procedure: A magnetic stirring device was utilised to mix 1 g of carbopol 940 with 50 ml of distilled water, and the beaker was left for 30 minutes to allow the carbopol 940 to swell before being swirled to create gel. Melt the necessary amount of methyl and propyl paraben in 5 millilitres of distilled water by boiling it in a water bath. Propylene glycol (PG) was added after the solution had cooled. The aforesaid mixture was supplemented with the necessary amount of extract from *Emblica officinalis*, coffee arabica, and *Rubia cordifolia*. The remaining amount distilled water was then added to bring the volume up to 100 mL. At last, the fully combined chemicals were continuously stirred into the Carbopol 940 gel, and triethanolamine was incorporated drop by drop to the mixture to reach the appropriate skin pH (6.5-7) and gel consistency[9,10].

Thin layer Chromatography :

TLC of the Hydroalcoholic extracts of *Emblica officinalis*, Coffee arabica, and *Rubia cordifolia*. Heat it for 10 minutes at 110°C. Examine the plate in daylight.

TLC for *Emblica officinalis*, Coffee arabica and *Rubia cordifolia* respectively

Mobile phase: 6 mL toluene, 6 mL ethyl acetate, 1.8 mL formic acid, and 0.25 mL methanol

Mobile Phase: Ethyl acetate (10 mL), dichloromethane (2.5 mL), formic acid (1 mL), acetic acid (1 mL), and water (1.1 mL).

Mobile Phase

Toluene:7ml;ethylacetate:3ml;formicacid:1ml.

The HPTLC operates on the same fundamentals as TLC, with the primary method of separation being adsorption. The mobile component or solvent flows via capillary action. Analytes travel towards the stationary part (adsorbent) based on their affinity. The greater affinity

component moves more slowly towards the stationary phase. A low-affinity component moves quickly towards the stationary phase. The components are subsequently separated using a chromatography plate.

Evaluation Of Polyherbal Anti-acne Gel

- pH: pH of formulation should be in the range of 6.5 to 7.
- Spreadability test: The formulation was spread on the skin surface using hands.
- Stability study:

Stability testing of cosmetic items guarantees that an invention or modification meets the desired physical, chemical, and microbiological quality criteria, as well as functioning and aesthetics, when stored under proper conditions. The stability study's goal is to offer information on how the formulation's quality changes when exposed to various environmental elements such as temperature, humidity, or light over time. This would help determine the formula's shelf life and the recommended storage conditions for the product. Changes in colour, aroma, viscosity, pH, and ingredient precipitation. The changes seen in several organoleptic parameters over time while held at room temperature were studied for one month[11,12].

Aging Studies :

Ageing examinations on the formulation were carried out at three distinct temperatures, namely 4°C and room temperature, for a time frame of one month. In the ageing experiments, the formulation is subjected to a range of temperatures for one month, and the product is monitored for physical changes such as colour, aroma, texture, flow, viscosity, component precipitation, amalgamation and phase separation.

Sun Exposure evaluation :

The product's photo stability is evaluated using a sun exposure test. In this test, herbal cream was subjected to sunlight from 9 a.m. to 6 p.m., and a change in physical properties was detected.

Diffusion Study:

It was performed using franz diffusion assembly to evaluate in vitro drug permeation providing key insights into the relationships between skin, drug and formulation.



Result and discussion:

(1) Macroscopic studies:

Morphological descriptions of plant components were made with the naked eye. Form, dimensions, colour, odour, and taste were all rated as organoleptic criteria. Morphological characters of root of *Rubia cordifolia* (Manjishta), Fruit of *Emblica officinalis* (Amla) and

Beans of coffee arabica (Green coffee) were studied as per visual observation.

(2) Microscopical studies:

Microscopy of *Rubia cordifolia*, *Emblica officinalis* and Coffee arabica is carried out by using various staining reagent.

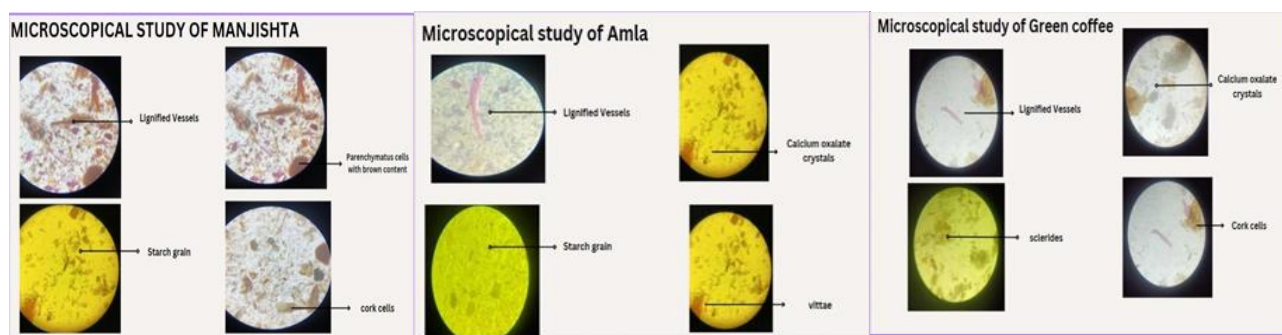


Figure 1: Microscopical Studies.

Table 2 :Microscopical Evaluations using Staining Reagent :*Rubia cordifolia* (Manjishta)

SR NO	Reagents	Observation	Characteristics
1)	Phloroglucinol + con. HCl	Pink	Lignified vessels
2)	Dil. H ₂ SO ₄	White	Calcium oxalate crystals
3)	Alc. Picric acid	Yellow	Starch grains
4)	Phloroglucinol + conc. HCL	Brown	Cork cells

(3) Physicochemical studies:

Physicochemical experiments were performed on the three herbs.

Following are the physicochemical test which are carried out on *Rubia cordifolia*, *Emblica officinalis* and *Coffee arabica* [13,14].

Emblica officinalis (Amla)

Table 3 : Physicochemical Test For *Emblica officinalis*.

SR. NO	TEST	OBSERVATION	LIMIT
1)	Foreign organic material	1.2%	Not more than 2%
2)	Ash value	11.5%	Not more than 15%
3)	Loss on drying	3.9%	Not more than 5%

Table 4 : Physicochemical Test For *Rubia cordifolia*.

SR. NO	TEST	OBSERVATION	LIMIT
1)	Foreign organic material	1.4%	Not more than 2%



2)	Ash value	6%	Not more than 10%
3)	Loss on drying	4.5%	Not more than 5%

Coffee arabica(Green Coffee)

Table 5 : Physicochemical Test For Coffee arabica.

SR. NO	TEST	OBSERVATION	LIMIT
1)	Foreign organic material	1.8%	Not more than 2%
2)	Ash value	1%	Not more than 4%
3)	Loss on drying	2.5%	Not more than 6%

Table 6: Physicochemical Test For Emblicaofficinalis, Rubia cordifolia, Coffee arabica.

Alkaloids	Observation	Manjishtha	Amla	Greencoffee
Dragendorff's Test:	Orange brown ppt	+	+	+
Mayer's Test	Precipitation	-	+	+
Hager's Test	Yellow ppt	-	+	+
Wagner's Test	Reddish brown ppt	+	+	+
Glycoside				
Brontragerstest :	Ammonium layer aquire pink color	+	+	-
Legal' test	Pink to red colour	+	+	-
Carbohydrates				
Feeling test	Brick red ppt	+	+	+
Molish test	Violet purple color	+	+	+
Saponins				
Foam test		+	+	+
Tannins and Phenols				
Ferric chloride Test	Deep Blue black color	+	+	+
Lead acetate Test	White ppt	+	+	+
Bromine water Test	Discoloration of bromine water	+	+	-
Acetic acid Test	Red colour	+	-	-
Dilute Iodine Test	Transient red colour	+	+	-



Flavanoids				
Shinoda Test	Green to Blue Color	+	+	+
Lead Acetate Test	Yellow ppt	+	+	+

5)Formulation Studies

- All topical gel formulations had a pH between 6.5-7.
- Spreadability Test: The formulation was tested by distributing it on the skin using hands.
- Thin Layer Chromatography:

TLC of the hydro-alcoholic extracts of *Emblica officinalis*, *Coffea arabica*, and *Rubia cordifolia*

Note: Heat for 10 minutes at 110°C. Examine the plate under daylight.

(1) TLC of *Emblica officinalis*

Mobile phase : 6 ml of Toluene , 6 ml of Ethyl acetate, 1.8ml formic acid, 0.25ml methanol.

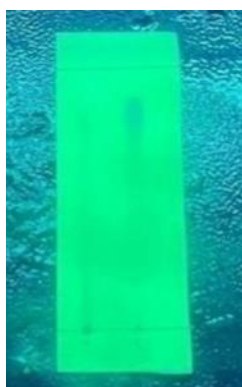


Figure 2: TLC Of *Emblica officinalis*

Rf value of Amla	Distance travel by solute	2.3	0.44
	Distance travel by solvent	5.2	
Rf value of Gallic acid (standard)	Distance travel by solute	2.5	0.48
	Distance travel by solvent	5.2	

Table 7: Rf value of *Emblica officinalis*.

(2)TLC of *Coffea arabica*

Mobile phase : Ethyl Acetate:10 ml, Dichloromethane:2.5ml, Formic Acid : 1ml , Acetic acid :1ml , water : 1.1 ml

Standard Rf value of chlorogenic acid is 0.74



Figure 3: TLC Of *Coffea arabica*.

Rf value of Green coffee	Distance travel by solute	3.7	0.71
	Distance travel by solvent	5.2	

Table 8: Rf value of *Coffea arabica*

(3)TLC of *Rubia cordifolia*

Mobile phase: Toluene: 7ml, Ethyl acetate: 3ml, Formic acid: 1ml

Standard Rf value of Alizarin is 0.57.

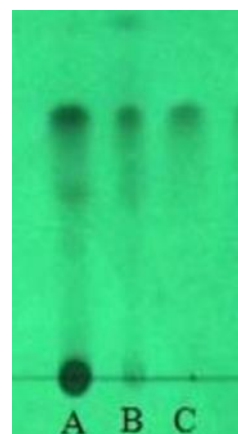


Figure 4: TLC Of *Rubia cordifolia*.



Rf value of manjishta B	Distance travel by solute	$\frac{2.9}{5.2}$	0.55
	Distance travel by solvent		

Table 9: Rf value of Rubia cordifolia.

(4) Combined TLC of *Embllica officinalis*, *Coffee arabica* and *Rubia cordifolia*:

Mobile phase composition: Toluene: 7ml, Ethyl Acetate: 3ml, Acetic acid: 1ml, Dichloromethane: 2.5ml

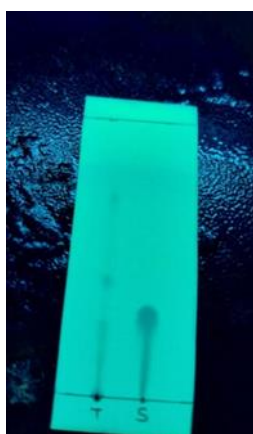


Figure 5: Combined TLC of *Embllica officinalis*, *Coffee arabica* and *Rubia cordifolia*.

Rf value of 1st compound	Distance travel by solute	$\frac{1.3}{5}$	0.26 cm
	Distance travel by solvent		
Rf value of 2nd compound	Distance travel by solute	$\frac{2.2}{5}$	0.44
	Distance travel by solvent		
Rf value of 3rd compound	Distance travel by solute	$\frac{3.8}{5}$	0.76
	Distance travel by solvent		

Table 10: Rf value of Combined TLC of *Embllica officinalis*, *Coffee arabica* and *Rubia cordifolia*.

(6) ANTIMICROBIAL STUDIES:

The Aim of this evaluation was to perform the invitro anti-acne effects of hydroalcoholic extract of *Rubia cordifolia*, *Embllica officinalis* and *coffee arabica* against skin pathogen *Staphylococcus aureus*. Three different concentration were used (10mg, 20mg, 40mg).

• Antimicrobial investigations of Extract with the agar cup plate method:

The antibacterial activity of the anti-acne gel formulation was determined using the agar cup plate method (10 mg, 20 mg, and 40 mg). To conduct the antibacterial experiment, bacterial stock cultures were revived by inoculating them in broth media and incubating them at 37°C for 24 hours. Muller Hilton's agar medium plates were set up. Each plate was inoculated with a bacterial suspension that was evenly dispersed under aseptic conditions. The suspension in each plate was allowed to dry for 20-25 minutes [15, 16]. Then, with a sterilised cork borer, 6 mm size wells were drilled in the solid media and filled with 50 ml of test samples. The wells were filled with different doses of anti-acne gel formulations (10 mg, 20 mg, and 40 mg). All plates were incubated at 37 degrees Celsius for 24 hours. Each extract's antibacterial activity was tested by determining the diameter of the zone of inhibition surrounding each well. Three replications of each extract were carried out against each test organism. **Coffee arabica (green coffee)**: Three wells on each plate were filled with coffee extract solutions at varied concentrations (10mg, 20mg, and 40mg). After 24 hours of incubation at 37°C, to eliminate inaccuracy, the inhibitory zones' diameters (including the well diameter) were measured in three different directions.. The largest zone of inhibition was reported at a dosage of 40 mg.

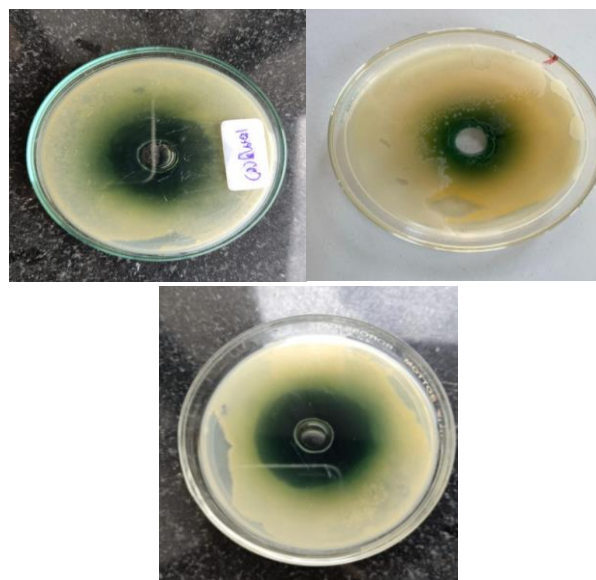


Figure 6 : Zone of Inhibition for Coffee arabica.



Emblica officinalis (Amla) : *Emblica officinalis* has demonstrated effective antibacterial activities and utility in the production of efficient anti-acne gel solution. Three different concentrations were generated, and the highest zone of inhibition was found at 40 mg.

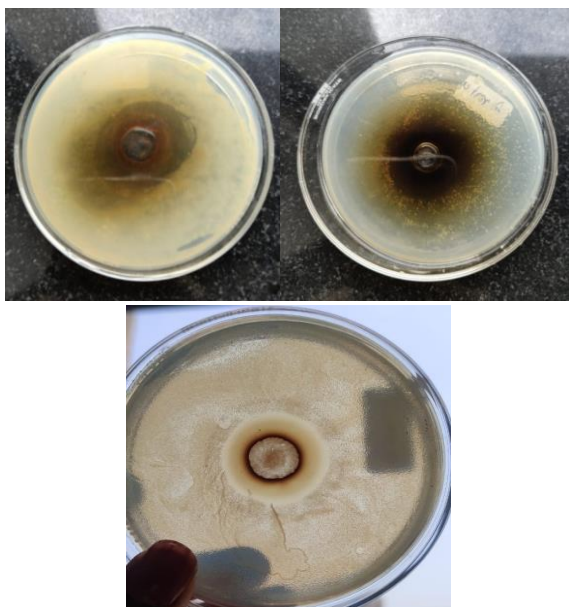


Figure 7: Zone of Inhibition for *Emblica officinalis*.

Rubia cordifolia (Manjishtha) : *Rubia cordifolia* (Indian Manjishtha) was discovered to have antibacterial action against *Staphylococcus aureus*. Three different concentrations were placed in the wells, with the highest concentration being 40 mg, which had the greatest zone of inhibition.

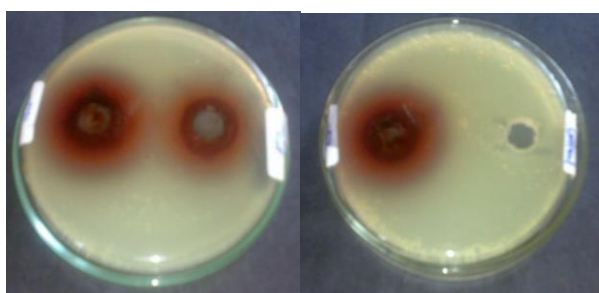


Figure 8: Zone of Inhibition for *Rubia cordifolia*

The antimicrobial activity on the formulation was evaluated and the zone of inhibition was observed the

well was filled with the gel formulation made from the extracts mention. The wells were filled with the broth prepared by addition the beef extract, peptone and water, the bacteria was collected and was placed in the tubes. Then it was filled in the wells and the result was observed[17]. It had the properties of anti-oxidant, anti-inflammatory and anti-bacterial which has a good effect on face to clear the acne. It is very similar to the marketed product used such as clindamycin gel which was observed by the differential in their zone of inhibition to kill the bacteria.

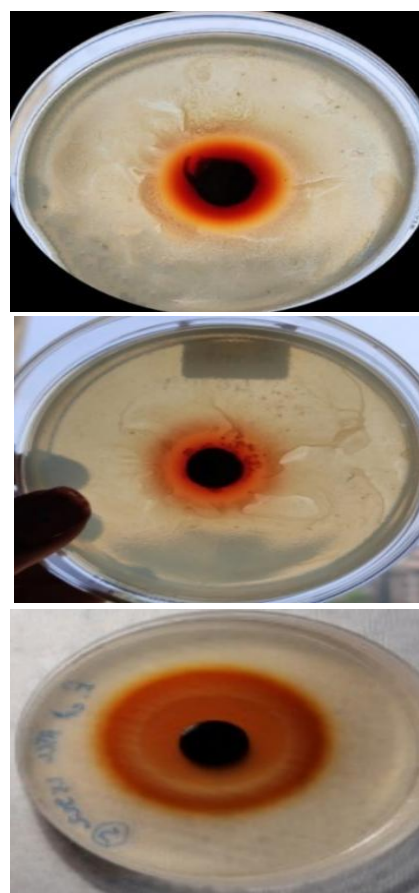


Figure 9: Zone of Inhibition for Formulated Gel

Antimicrobial activity of standard used in formulation using Cup Plate Method: Topical clindamycin is used to help control acne. It may be used alone or with one or more other medicines that are used on the skin. Clindamycin Phosphate Gel is an antibiotic that fights bacteria. Topical clindamycin can be used to treat severe acne. The anti-bacterial activity of clindamycin gel was



compared with the prepared polyherbal gel and the zone of inhibition was observed[18].

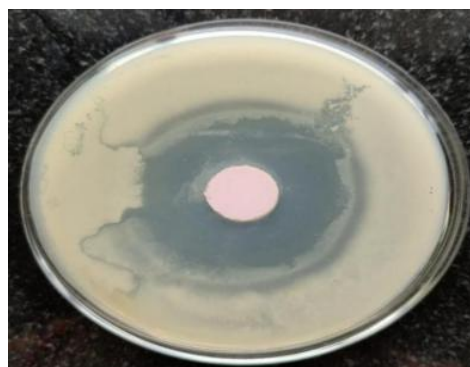


Figure 10: Zone of Inhibition for Clindamycin

Table 11: Zone of Inhibition.

HERBAL EXTRACT	DIAMETER OF ZONE OF INHIBITION (mm)	DIAMETER OF ZONE OF INHIBITION (mm)
	10mg	20 mg
	40 mg	
Embilica officinalis (Ala)	15 mm	19mm
	22mm	
Rubia cordifolia (Manjistha)	17mm	20 mm
	25mm	

Coffea Arabica (green coffee)	14mm	17 mm
	21mm	

Anti microbial activity of the formulated gel (F1,F2, F3) using Cup Plate Method:

Table 12 : Zone of Inhibition For Clindamycin and Formulated Gel.

CONCENTRATION	DIAMETER OF ZONE OF INHIBITION
Clindamycin gel	28mm
Formulated gel	
F1	14mm
F2	19mm
F3	25mm

(7) Accelerated stability study:

- Aging studies:

N stands for No change

(1) Aging Studies at Temperature: 4°

Table 13: Aging Studies at 4°C.

PARAMETERS	DAY1	DAYS	DAY10	DAY15	DAY20	DAY25	DAY30
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Fragrance	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Flow	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous
Precipitation of ingredients	N	N	N	N	N	N	N
Phase separation	N	N	N	N	N	N	N

(2) Aging Studies at Temperature: Ambient °C

**Table 14: Aging Studies at Ambient Temperature.**

PARAMETERS	DAY1	DAY5	DAY10	DAY15	DAY20	DAY25	DAY30
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Fragrance	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Flow	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous
Precipitation of ingredients	N	N	N	N	N	N	N
Phase separation	N	N	N	N	N	N	N

(3)Sun exposure examination (photosensitivity):

Beginning at 9:00 a.m., the herbal gel was exposed to light. The change in physical qualities was observed till 6 p.m., as shown in the table below.

Table 15: Photosensitivity (Sun Exposure Examination).

PARAMETERS	DAY1	DAY5	DAY10	DAY15	DAY20	DAY25	DAY30
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Fragrance	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour	Herbal/Rose flavour
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Flow	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous	Highly viscous
Precipitation of ingredients	N	N	N	N	N	N	N
Phase separation	N	N	N	N	N	N	N



From accelerated stability study it was found that, the present formulation is stable for one month when

(8) Diffusion Study of Topical Gels:

The diffusion study of gels was performed using the dialysis membrane, which was dry washed, open ended, and pre-cut. The flat width was 35mm, the inflated diameter was 21mm, and the length was 30mm. The membrane was immersed in a buffering agent pH 7.4 (PB), for 6-8 hours. The receptor compartment consisted

exposed to room temperature and 4 °C also passed the test for photosensitivity.

of a beaker filled with 100 cc of phosphate buffer. The membrane was uniformly covered with 0.5 g gel. The initial sample was collected at 11:45, followed by another at 12:45, and so on until 4:45 pm. The solutions which were collected were taken for the UV analysis and the absorbance were noted and the graph was prepared on the basis of the absorbance reading [19].

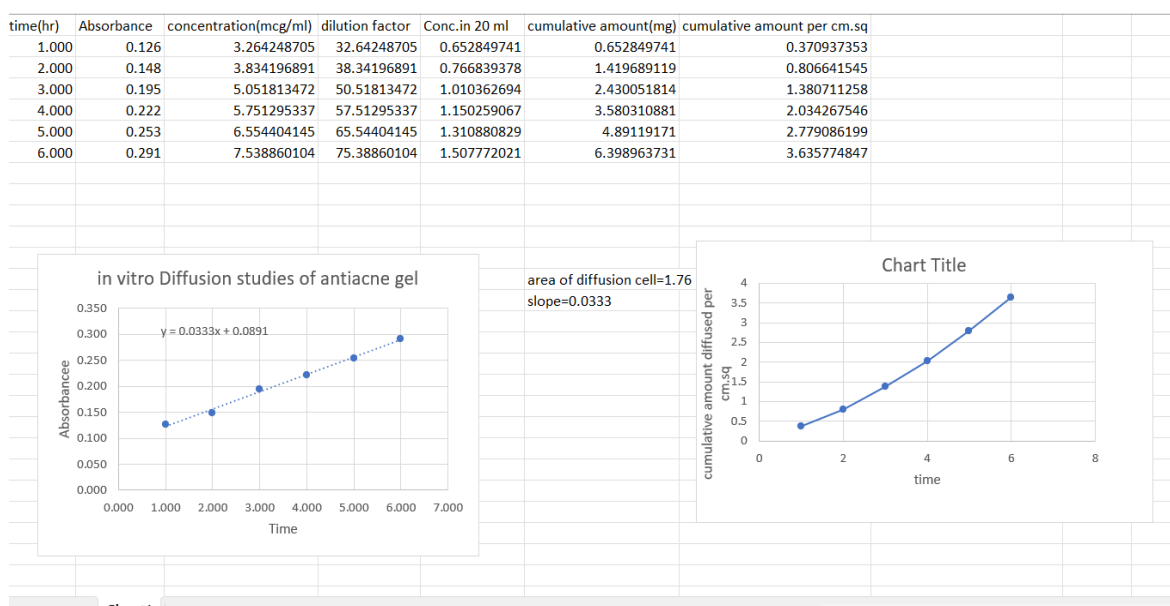


Figure 11: Diffusion Study Readings And Graphs.

(9) HPTLC Fingerprinting :

The HPTLC works on the concept of separation, which is adsorption. The capillary action causes a mobile phase or solvent to flow. Analytes travel to the stationary phase, which is the adsorbent, based on their affinity. Higher affinity components go slowly towards the stationary phase. The components with low affinity move quickly towards the stationary phase. The constituents are separated on a chromatographic plate [20].

Mobile phase composition: Toluene: 7ml, Ethyl Acetate: 3ml, Acetic acid: 1ml, Dichloromethane: 2.5.

The first five bands are for the formulation and the next three are the drugs and the last band is of the standard Gallic acid.

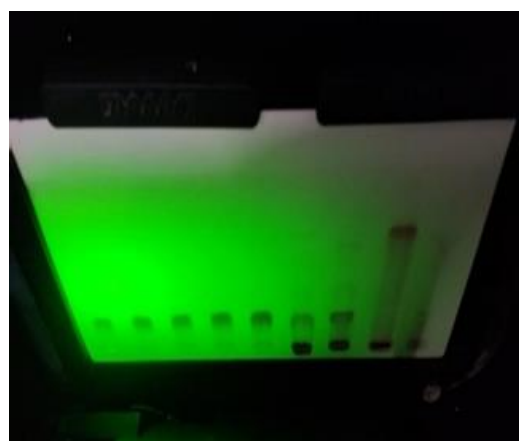


Figure 12: HPTLC Fingerprinting For Three Drugs And Standard

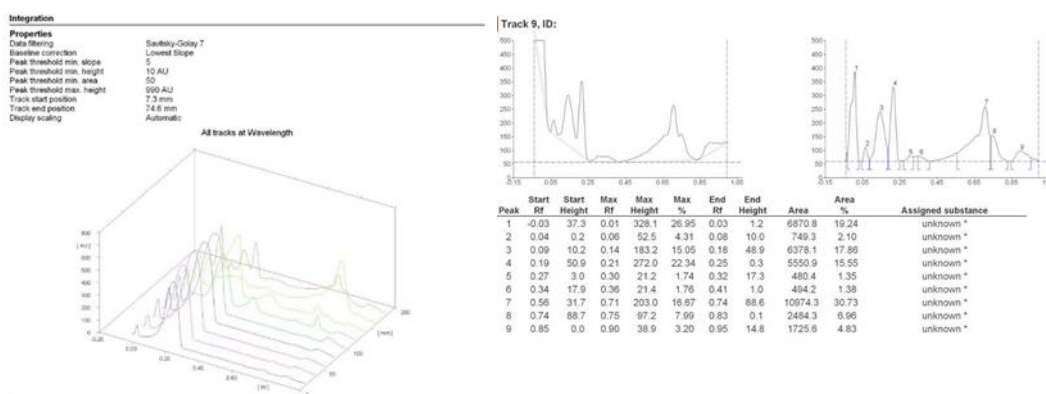


Figure 13: Graphical Plots Of HPTLC Fingerprinting.

CONCLUSION:

Based on the findings, it is reasonable to conclude that the polyherbal antiacne gel containing extracts of *Rubia cordifolia* (Manjishta) root, *Emblica officinalis* (Amla) fruit, and coffee arabica (Green coffee) beans was ideal for application and stability. It exhibited anti-acne properties on human skin. Microbial investigations conducted yielded a beneficial result, assisting in the treatment of acne on the affected skin area. Based on all of the factors, it was determined that the F3 formulation was the most effective among the other formulations. No such detrimental effect was reported following the evaluation.

REFERENCES:

- Martins Ekor, the Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety, Researchgate, 1-6 January 2014.
- Sutaria AH, Masood S, Saleh HM, et al. Acne Vulgaris. [Updated 2023 Aug 17]. In: StatPearls [Internet]. Treasure Island (FL): Jan 2023 .
- Motosko CC, Zakhem GA, Pomeranz MK, Hazen A. Acne: a side-effect of masculinizing hormonal therapy in transgender patients. *Br J Dermatol*;180(1):26-30 January 2019.
- Gouri Dixit, Ganesh Misal, Vijay Gulkari and Kanchan Upadhye. International journal of pharmaceutical sciences and research vol. 4(3): 1186-1191.2013.
- G. Misal,G. Dixit,Vijay Gulkari, Formulation and evaluation of herbal gel,Indian Journal of Natural Products and Resources 3(4):501-505,December 2012.
- International Journal of Recent Technology and Engineering (IJRTE) ISSN: 2277-3878, Volume-8 Issue-4, November 2019.
- Prashant L. Kapure, Kalpesh P. Makade, Mahesh D. Sanap, Sanket J. Gandhi, R.A. Ahirrao, S.P. Pawar various extraction method and Standardization parameter of Amla and Durva An International Journal Of Pharmaceutical Sciences *Pharma Science Monitor* 6(2),61-72 ,Apr-Jun 2015.
- Milan Hait, Extraction Techniques of Herbal Drugs,Research Trends in Medicinal Plant Sciences (Volume-5) (pp.17-33),October 2019.
- Prashant L. Kapure, Kalpesh P Makade, Mahesh D. Sanap, Sanket J. Gandhi, R.A. Ahirrao. S.P. Pawar, various extraction method and standardization parameter of Amla and durva,pharma science monitor an international journal of pharmaceutical scienceS,*Pharma Science Monitor* 6(2), Apr-Jun 2015.
- Sachdeva College of Pharmacy, Mohali, Punjab, India. Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi,India. "Aimil Pharmaceuticals Ltd, Nalagarh, Himachal Pradesh, India. Vol 9, Issue 4, 2016
- Mohsin J. Jamadar, Rajmahammadhusen Shaikh. Preparation and Evaluation of herbal gel formulation. *Journal of Pharmaceutical Research and Education* 1(2):201-224 2017.
- Ayurvedic pharmacopiea part 1, volume 1, year 1990 (Amla).
- Ayurvedic pharmacopiea part 1, Volume-3,year 2001(Manjishta).



14. Javale, P. and Sabnis, S. Antimicrobial properties and phytochemical analysis of *Emblica officinalis* Asian J. Exp. Biol. Sci., 91-95 (2010).
15. Purna SK, Babu M. 2000. Collagen based dressings/a review. Burns 26: 54-62. 5. Rao GM, Rao CV, Pushpagandan P. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. J Ethnopharmacology 103: 484-490, 2006.
16. Brandao F, Oliveira L, Landucci L, Koga-Ito C. Antimicrobial activity of coffee-based solutions and their effects on *Streptococcus* adherence. Braz J Oral Sci 6:1274-7 2007.
17. Yang H, Imbert DC. Inventor; Static Diffusion Cell for Diffusion Sampling Systems. U.S. Patent, <https://www.slideshare.net/laboratoryenterprises/diffusion-cell-apparatus> US 8133721B2: 200-212, 2011.
18. Sakthivel lakshmana prabu, Appanoose umamaheswari, Chandran Arun kumar, Murugesan banumuthupriya, Dharumadurai dhanasekaran. Formulation and evaluation of polyherbal gel containing natural antimicrobials for the management of acne vulgaris. International Research Journal of Pharmacy8(5):65-69, 2017.
19. Deepak p pawar, prashant b shamkuwar, formulation and evaluation of herbal gel containing lantana camara leaves extract, Asian journal of pharmaceutical and clinical research, vol 6, issue 3, 2013.
20. Mohammad Ali Shahtalebi, Gholam Reza Asghari, Farideh Rahmani, Fatemeh Shafiee and Ali Jahanian-Najafabadi Formulation of Herbal Gel of *Antirrhinum majus* Extract and Evaluation of its Anti-Propionibacterium acne Effects, Mar2018.