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FORMULATION OPTIMIZATION AND *IN-VITRO* ANTIMICROBIAL ASSESSMENT OF A SYMPLOCOS RACEMOSA ETHNOBOTANICAL GEL

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Abstract: In Ayurveda, acne is considered to be caused by an imbalance of the tri doshas - Vata, Pitta, and Kapha. Following a balanced Ayurvedic diet that is suitable for your dosha type can also help in preventing and treating acne. Human skin is the most susceptible body part, which is highly vulnerable to exposure to microorganisms and pathogens, leading to a variety of skin-related diseases. One of the causes of acne vulgaris at puberty is hormonal changes in the body. E. Coil species & Staphylococcus are mainly responsible for the formation of acne and cutaneous infections on the skin. Ethanolic extract of Symplocos bark was investigated extensively for its antiacne activity. Formulation of topical gel was prepared by using Carbopol 934, Sodium alginate, Symplocos bark extract, methyl paraben, sufficient quantity of distilled water. The prepared formulation was evaluated for physical appearance, pH, spreadability, and viscosity for antiacne activity. In vitro antibacterial activity was performed against S. aureus and E. coli, using Muller-Hinton agar by the well diffusion method. The results from the Muller-Hinton agar well diffusion method confirmed that Symplocos racemosa Roxb. would inhibit the growth of S. aureus and E. coli, and the prepared herbal gel showed antibacterial activity against these bacteria.

KEYWORDS:

Sagging, Follicles, Melanocytes, Vulgaris, Pustule.

INTRODUCTION:

Physiology of skin:

The skin is the largest organ of the body, with an entire area of about 20 square feet. The skin protects us from germs and the elements, helps regulate body temperature, and permits the sensations of touch, heat and cold.

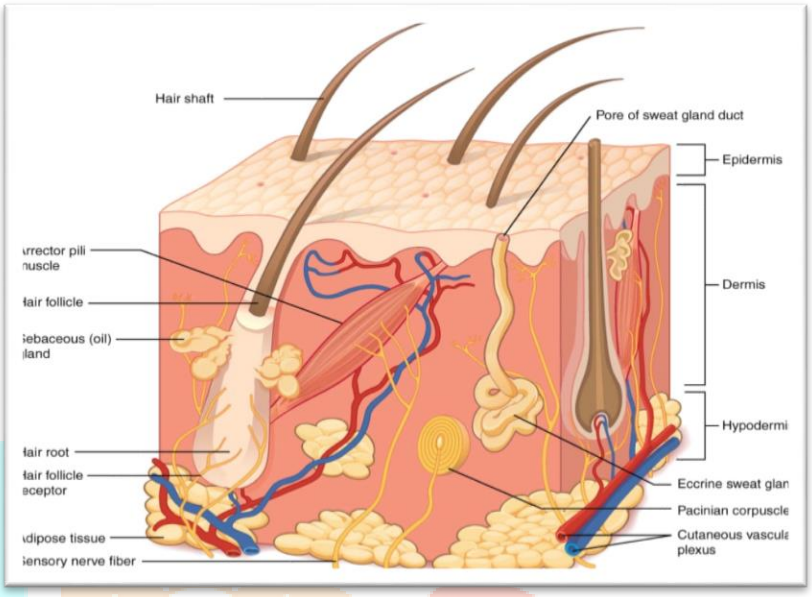


Fig. 1. Structure of Skin

Skin has three layers:

The **epidermis**, the outermost layer of skin, provides a waterproof barrier and creates our skin tone. The **dermis**, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands. The deeper subcutaneous tissue **(hypodermis**) is made of fat and connective tissue. The skin colour is created by special cells called melanocytes, which produce the pigment melanin. Melanocytes are located in the epidermis.

Advantages:

- Protection of skin.
- Regulating body temperature.
- Sensation of touch, pressure, temperature, and pain.
- Immunity serves as a defence mechanism against infections.

Disadvantages:

- Hyperpigmentation: Uneven pigmentation or dark spots on the skin, known as hyperpigmentation, can be caused by sun exposure, hormonal changes, inflammation, or genetics.
- Environmental damage: Environmental factors such as pollution, smoke, and harsh weather conditions can damage the skin's barrier function and accelerate ageing.
- Skin cancer risk: Prolonged exposure to UV radiation from the sun or tanning beds increases the risk of developing skin cancer.
- Ageing: As we age, the skin undergoes natural changes such as decreased collagen production, loss of elasticity, and thinning, which can result in wrinkles, sagging, and age spots.

Acne:

The most prevalent long-term inflammatory skin condition of the pilosebaceous unit, acne vulgaris affects the face and back, which have the most oil glands. 95% of males and 83% of females, suffer from this nearly universal illness, which affects people of all colours. Seborrhoea, comedone, inflammatory lesions, the presence of Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus in the follicular canal, and sebum production are the main characteristics of acne vulgaris. According to certain descriptions, P. acnes is an obligatory anaerobic microbe.

Pathophysiology of Acne:

The development of acne involves a combination of the following factors:

1. Excess Sebum Production: Triggered by increased androgen levels during puberty
2. Follicular Hyperkeratinization: Abnormal shedding of dead skin cells leads to blockage of hair follicles.
3. Bacterial Proliferation: Especially cutibacterium acnes, which thrives in the sebum-rich environment.
4. Inflammation: Triggered by bacterial colonisation & immune response.

Types of acne:

Whiteheads: These little bumps that remain under the skin's surface.
Blackhead: Blackheads are a type of acne (acne vulgaris). They're open bumps on the skin that fill with excess oil and dead skin.
Papules: These little, pink pimples on the skin are visible and painful to the touch.
Pustules: Pimples or zits can be seen on the surface of the skin. They are red at the lowest level and contain pus at their top.
Nodules: Prominent growths on the skin's surface. These are painful, huge, solid pimples that are visible on the skin's surface as well as deep into the skin.
Cysts: Clearly discernible growths on the skin's surface. They are firmly embedded, painful, packed with pus, and extremely susceptible to scarring.

Causes of Acne:

- Hyperactive sebaceous glands (overactive lipid secretion).
- Hyperkeratosis (accelerated keratinisation) at the hair infundibulum.
- Activity of bacteria (Propionibacterium acnes) promoting comedogenesis.
- Cyclic hormonal levels in women.

Anti-Acne:
A skin condition characterized by red pimples on the skin, especially on the face, due to an infection.

Anti-Acne Drugs:

- Clarina cream
- Pimpewin gel
- Salicylic acid gel
- Aloe vera gel

Plan of Work
Material and Equipments

Table No. 1: List of Materials Used

Sr.No	MATERIALS	USES
1.	Bark extract of Symplocos	Used in skin issues and shows anti-acne activity.
2.	Sodium alginate	Used as a polymer
3.	Carbopol 934	Used as a polymer.
4.	Triethanolamine	Emulsifying agents and pH modifiers are employed to stabilize
5.	Propyl paraben	Preservative
6.	Methyl paraben	Preservative
7.	Glycerol	Moisturizer
8.	Distilled Water	Vehicle

Plant & Excipient profile:

Fig. 8. Lodhra

One of the significant therapeutic herbs is Lodhra. It belongs to the Symplocaceae family and is known by its botanical name, Symplocos racemosa Roxb. It is a little, 10 to 15 m tall evergreen tree. They are mostly found in the Himalayan regions of north and east India.

Taxonomical classification:	
Kingdom	**:** Plantae
Division	**:** Magnoliophyta
Class	**:** Magnoliopsida
Order	**:** Ericales
Family	**:** Symplocaceae
Genus	**:** Symplocos

Vernacular names:
Sanskrit: Rodhra, Paittki Lodhra
English: Symplocos bark
Hindi: Lodha
Kannada: Lodhra
Marathi: Lodha, Lodhra

Part used: Bark

Bark: Bark greyish lenticellate; blaze cream

Fig. 9: Bark

Chemical constituents:

The plants of the genus Symplocos contain a variety of constituents, viz., terpenoids, flavonoids, lignans, phenols, steroids, alkaloids, iridoids, and many others. Ursolic acid, corosolic acid and 2α, 3α,19α, 23-tetrahydroxyurs-12-ene-28-oic acid tannins are reported to be present in S. paniculata. Besides some triterpenoids, triterpenoid saponins, and flavanol glycosides.

Medicinal uses:

- Used in Antiacne treatment.
- Lodhra is used to improve women's health in gynaecological disorders.
- Lodhra paste is used to treat skin diseases.
- Treatment of eye diseases
- Snake bite and detoxification

Excipient Profile:

Table 2: Excipient profile

Sr.No	Excipient	Uses
1.	Sodium alginate	Used as a polymer.
2.	Carbopol 934	Used as a polymer.
3.	Triethanolamine	Emulsifying agent and pH modifier are employed to stabilize.
4.	Methyl paraben	Preservative
5.	Propyl paraben	Preservative
6.	Glycerol	Moisturizer

Experimental Work:

Material Method:

The bark of Symplocos Racemosa Roxb. was collected from **APURVA MEDICAL (near laxmi mandir), GADHINGLAJ**. Its authenticity was confirmed by the Department of Botany at Dr. Ghali College, Gadhinglaj.

Preparation of Plant Material:

Preparation of Ethanolic Extract of Symplocos Bark.

1. In the present study, the bark was carefully selected & washed to remove impurities, and shade drying of the bark was done.
2. The dried material was reduced to fine powder in the mechanical grinder.
3. The fine powder was passed through sieve no. 43 and stored in an airtight container for further processing.
4. About 100 gm of powdered material was extracted with ethanol as a solvent by the hot extraction method using a Soxhlet apparatus.
5. The extraction was continued until the solvent in the thimble became clear; then a few drops of solvent were collected in the test tube during the completion of the cycle, and a chemical test of the solvent was performed.
6. After each extraction, the extract was evaporated to obtain a solid extract from the thermal water bath.
7. Moreover, some part of the extract was stored for preliminary phytochemical screening for the detection of various plant constituents, and the rest of the extract was used for the formulation of gel batches.

Fig 11: Soxhlet Apparatus

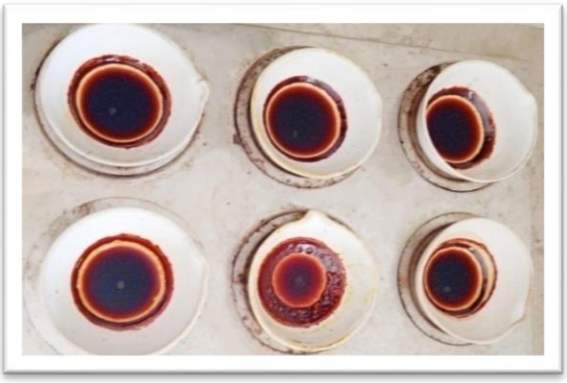


Fig 12: Evaporation of Solvent

Fig 13: Crude Drug

Phytochemical Screening of Extract

Standard analytical techniques were used to undertake a qualitative screening of Symplocos root bark for the presence of secondary metabolites such as tannins, carbohydrates, proteins, fats and oils, sterols, glycosides, alkaloids, phenolic compounds, flavonoids, etc.

Table 3: Phytochemical screening

Name of tests	Observation	Inference	Images
Test for Saponin: **1) Foam Test:** 1ml drug + 5ml distilled water shake vigorously	Soaking appearance	Present	
Test for Tannin: **1) $FeCl_3$ Test:** Ferric chloride - 1 ml drug + Ferric chloride solution	Brownish green colour observed	Present	
Test for Alkaloids: **1) Dragendroff's test:** 1 ml drug + 2ml Dragendroff's reagent	Reddish brown ppt	Present	
Test for Carbohydrates: **1) Fehling's Test:** Add 1 ml Fehling's solution A and 1ml Fehling's solution B, boil for one min. Add equal volume of test solution. Heat in boiling water bath for 5-19 minutes.	First yellow and then brick red precipitation is observed.	Present	

Test for Flavonoids: **1) Lead acetate:** 1 ml drug + 5ml ammonia solution + Conc. H_2SO_4	Yellow color	Absent	

Experimental Design:
During formulation, gelling agents were used at **3** different concentrations, resulting in **6** different batches of gels of bark extract being prepared. In this case, **Carbopol 934** & **Sodium alginate** gelling agents were taken. Gelling agents were used as follows:
a. Carbopol 934 & Sodium alginate **(at concentrations of 0.5%, 1% and 1.5%)**
All the batches were prepared according to the experimental design.

Preparation of Gel:
Carbopol 934, sodium alginate, and purified water were taken in a beaker and allowed to stand for 24 hrs.
The required amount of 1 gm of the drug was dispersed in Carbopol gel & Sodium alginate.
Carbopol gel & Sodium alginate gel were neutralized with a sufficient quantity of triethanolamine.
Glycerol was added as a moisturizing agent.
Methyl paraben and propyl paraben as preservatives were added slowly with continuous gentle stirring.

Formulation Table:

Table 4: Formulation Table

	Ingredients	F1	F2	F3	F4	F5	F6
1.	Drug (gm)	1	1	1	1	1	1
2.	Sodium alginate (gm)	0.5	1	1.5	-	-	-
3.	Carbopol 934 (gm)	-	-	-	0.5	1	1.5
4.	Ethanol (ml)	4	4	4	4	4	4
5.	Triethanolamine (ml)	1	1	1	1	1	1
6.	Methyl paraben (gm)	0.5	0.5	0.5	0.5	0.5	0.5
7.	Propyl paraben (gm)	0.5	0.5	0.5	0.5	0.5	0.5
8.	Glycerol (ml)	2.5	2.5	2.5	2.5	2.5	2.5
9.	Distilled Water (ml)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

Table 14: Preparation of Batches

Evaluation of Topical Herbal Gel:

1. Physical appearance
2. Spreadability test
3. Viscosity
4. pH measurement
5. Washability test

Physical appearance: Physical parameters such as color, odor, appearance was observed.

Spreadability test: By placing 1 gm of the gel in a 1 cm circle on a petri dish, the spreadability of the herbal gels was assessed. A 1.0 gm weight was then resting on the top most petri dish which was then placed on top of another petri dish. The diameter then expanded as a result of the gel spreading, and this was recorded. This parameter contributed in determining the spreadability properties of the gel.

Viscosity: The viscosity of the produced gel was measured using a Brookfield Viscometer. (Brookfield viscometer DV III ULTRA) Viscosity was measured. Spindle no. 64 was used to rotate the gels at 30 and 40 rpm, 25+ 1°C The equivalent dial reading was taken at each speed.

pH measurements: A digital pH meter was used to determine the pH of gel formulations. 1gm of gel was dissolved 100 ml of distilled water and kept for two hours. The pH of each formulation was measured & results was recorded.

Washability test: Washability test was carried out by applying a small amount of gel on the hand and then washing it with help of tap water, formulation was easily washable.

Introduction of bacteria: -

E-Coli:

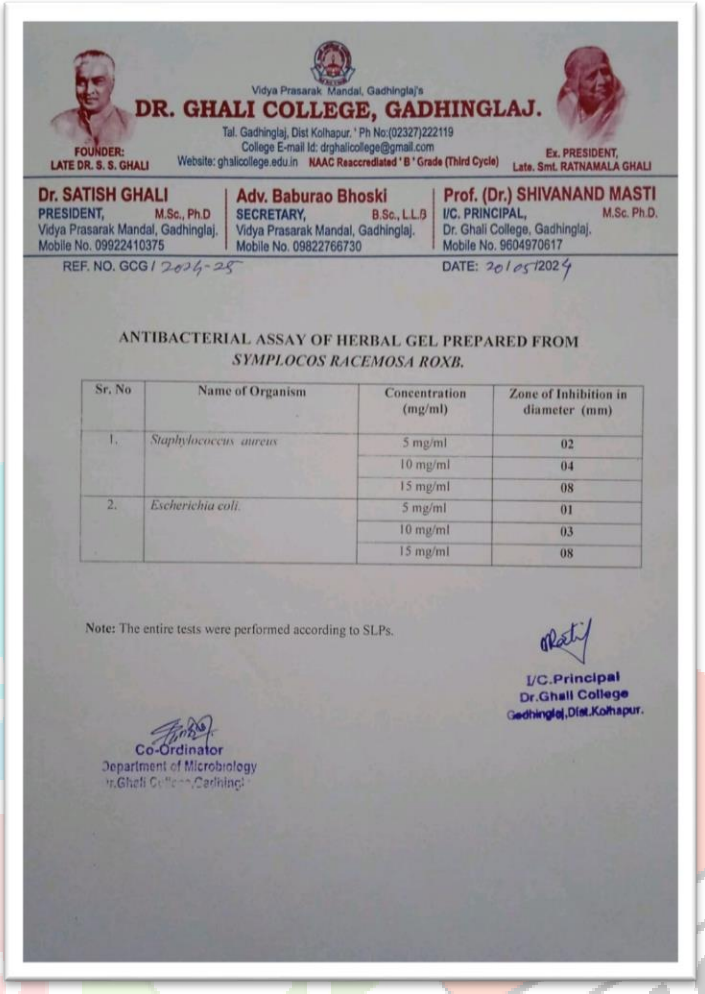
The bacteria were first described by Theodor Escherich in 1985. Escherichia coli is a Gram-negative, rod-shaped, non-spore-forming, motile with peritrichous flagella or non-motile. This bacteri causes superficial pustules with relatively few papules and comedones E. coli bacteria mostly found around the area of the upper lip under the nose, to the chin and cheeks.

Staphylococcus aureus: -

Skin infections are most frequently caused by Staphylococcus aureus germs. Boils, blisters, and skin redness may result from this. These infections can occur anywhere on the body, including the face, frequently in the vicinity of the mouth and nose.

In-vitro Antibacterial study of herbal gel: -

To check antibacterial activity against E. coli and S. aureus of the prepared herbal gel, the sample of formulation was submitted to **Dr GHALI COLLEGE, GADHINGLAJ.** This activity was carried out by well diffusion method. The results are obtained as below;



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ANTIBACTERIAL ASSAY OF HERBAL GEL PREPARED FROM *SYMPLOCOS RACEMOSA ROXB.*

Sr. No	Name of Organism	Concentration (mg/ml)	Zone of Inhibition in diameter (mm)
1.	*Staphylococcus aureus*	5 mg/ml	02
		10 mg/ml	04
		15 mg/ml	08
2.	*Escherichia coli.*	5 mg/ml	01
		10 mg/ml	03
		15 mg/ml	08

Note: The entire tests were performed according to SLPs.

I/C.Principal
Dr.Ghali College
Gadhinglaj,Dist.Kolhapur.

Co-Ordinator
Department of Microbiology

Fig 15: Certificate of Analysis

Results And Discussion

Organoleptic properties of Symplocos racemose:

Table 5: Organoleptic properties of Symplocos racemosa

Color	**Greyish Brownish**
Smell	Odorless
Taste	Slightly bitter
Texture	Rough

Solubility determination:

The solubility of Symplocos bark was found to be in water as well as solubility profile in different media like ethanol.

Table 6: Solubility determination

Sr. No.	Solvent	Solubility Profile
1.	Water	Highly Soluble
2.	Methanol	Slightly Soluble
3.	Ethanol	Soluble

Phytochemical screening:

Table 7: Phytochemical screening

Sr. No	Phytoconstituents	Chemical test	Observations
1.	Saponin	Foam Test	Present
2.	Tannin	Ferric Chloride Test	Present
3.	Alkaloids	Dragendroffs Test	Present
4.	Carbohydrates	Fehling's Test	Present
5.	Flavonoids	Lead Acetate Test	Absent

Evaluation result of Viscosity, Spreadability & pH:

Table 8: Evaluation results of viscosity, spreadability, and pH

Batches	Viscosity (pas)	Spreadability (g.cm/sec)	pH
F1	0.0286	2	6.45
F2	0.0298	2.3	6.39
F3	0.0226	1.5	6.57
F4	0.0542	2.5	6.58
F5	0.0396	1.5	6.45
F6	0.0423	2.1	6.5

Antibacterial Activity of samples against E. coli, S. aureus:

Table 9: Antibacterial activity of samples against E. coli, S. aureus

Sr. No.	Name of Organism	Concentration (mg/ml)	Zone of Inhibition in Diameter (mm)
1.	Staphylococcus aureus	5 mg/ml	02
		10 mg/ml	04
		15 mg/ml	08
2.	Escherichia coli	5 mg/ml	01
		10 mg/ml	03
		15 mg/ml	08

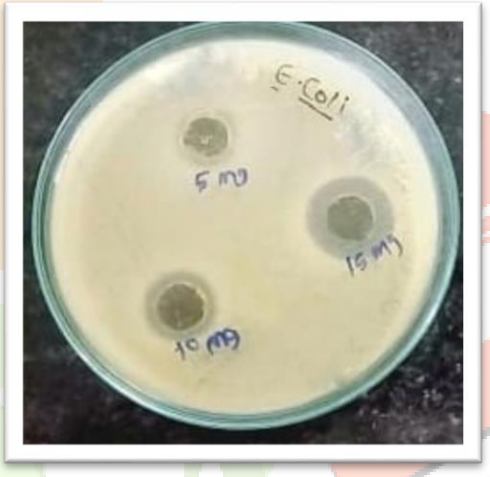

Fig 16: E. coli

Fig 17: S. aureus

Conclusion:

The findings of the present study suggest antiacne activity of Symplocos racemosa bark.
In conclusion, it can be said that the ethanolic extract of Symplocos racemosa bark can be considered as having potential antiacne activity. The formulation of evaluation studies shows good properties like spreading, viscosity, and physical appearance.
In vitro studies are shown by antibacterial activity against E. coli & Staphylococcus aureus bacteria.

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