Formulation of herbal moisturizer of *Senna alata, hibiscus rosa-sinensis, Azadirachta indica, Psidium guajava, a*gainst and evaluate or evaluation of antimicrobial activity against Escherichia coli, *Staphylococcus aureus*

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ABSTRACT

Biocosmetic products face challenges like standardization, certification, and product stability, which are significant issues for the sector. With the market's ongoing growth, regulatory frameworks and technological advancements will be vital in maintaining the effectiveness and trustworthiness of biocosmetic products."Plants are beneficial for the skin and also exhibit antimicrobial activity against numerous bacteria. The aim of this study is to formulate a biocosmetic moisturizer and evaluate its antimicrobial effectiveness."

Plants possess beneficial properties for the skin and exhibit antimicrobial activity against various bacteria. This study aims to formulate a biocosmetic moisturizer incorporating extracts of *Azadirachta indica,Psidium guajava, Hibiscus rosa-sinensis,* and *Senna alata,* and to evaluate its antimicrobial effects. The antimicrobial activity was tested against *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Escherichia coli.* The results demonstrated that the plant extracts exhibited varying degrees of inhibition against these bacterial strains, with significant activity observed in certain formulations. This suggests the potential of these botanical ingredients in developing natural antimicrobial skincare products.

1. INTRODUCTION

Biocosmetics are cosmetic products made from 100% natural ingredients derived from plants, animals, microbes, enzymes, insects, and organic crops that are free of pesticides and chemical fertilizers and used for topical skin, hair, face, and oral care[1]. Most of the conventional skin-care cosmetic formulations use petroleum or mineral oil-derived ingredients, which are harmful and non-biodegradable. To achieve a circular economy while satisfying customer demand for green cosmetics and addressing environmental concerns, many cosmetic giants have diverted their attention from fossil-based ingredients to bio-based ingredients[2]. The current market for sustainable, natural, and greener cosmetics is massive because such products garner trust and respect by fairly treating nature. Additionally, government support and faster product approval for biocosmetics are creating a favorable climate for big business players such as The Estée Lauder Companies, Inc. (USA), LOréal SA (France), Bare Escentuals, Inc. (USA), Nature's Gate (USA), Aubrey Organics, Inc. (USA), and FANCL Corp. (Japan). This market has seen a sharp rise over the period 2018–2021 [3]. As per market analysis reports related to skincare products, the global organic and natural cosmetic market is expected to reach USD25.11 billion by 2024 [3]. Biocosmetic products with standard organic certification labels such as Ecocert, Cosmébio, NaTrue, USDA Organic, BDIH, and Soil

Association allow their commercialization worldwide[4-9]. The distribution channel for such products includes drugstores, pharmacies, organic food shops, health food retailers, department stores, beauty retailers, and online shopping sites.

2.MATERIAL AND METHOD

Fig. 1 *Psidium guajava*

Fig. 2 *Senna alata*

Fig.3 *Azadirachta indica*

Fig .4 *hibiscus rosa - sinesis*

2.1 COLLECTION OF SAMPLE(PLANTS)

Plant material *psidium guajava* was collected from the FRI (forest research institute), dehradun uttrakhand, in month of January 2025.

Plant material *Senna alata* was collected from the FRI (forest research institute) dehradun uttrakhand, in the month of January 2025.

Plant material was collected *azadirachta indica* was collected from the FRI (forest research institute) Dehradun Uttrakhand, in month of January 2025.

Plant material *hibiscus rosa-sinensis* was collected from the FRI (forest research institute) dehradun uttrakhand in the month of January 2025.

2.2 PREPARATION OF EXTRACT

Senna alata, Azadirachta indica , hibiscus rosa-sinensis, psidium Guajava

The freshly collected leaves were then properly washed with tap water and clean with 70% Ethanol and dried in incubator at temprature 70°C for 7 days .

Thoroughly wash the plant material with clean water.

Air dry the plant material in a well-ventilated area until complete

Coarsely chop the dried plant material.

Grind the dried plant material into a fine powder using a grinder or mortar and pestle.

Store the powder in airtight containers in a cool, dark place [10-14].

Fig. 5 Extract of *psidium Guajava, Azadirachta indica, hibiscus rosa-sinensis, Senna alata.*

2.3 ISOLATION OF BACTERIA

The microorganisms were isolated and cultured on NAM (Nutrient agar medium) plates using the streaking method. The cultures were then incubated at 37°C for 24 hours.

2.4 GRAM STAINING

The selected colony gram staining to different between gram positive and gram negative bacteria.

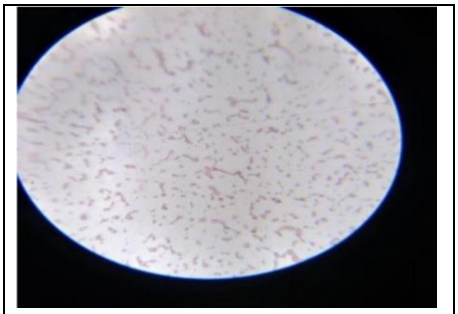

Fig.6 Showing gram staining *klebsiella*

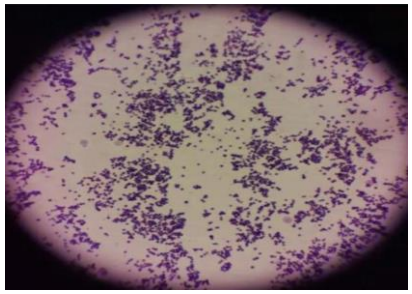

Fig. 7 Showing gram staining *staphylococcus aureus.*

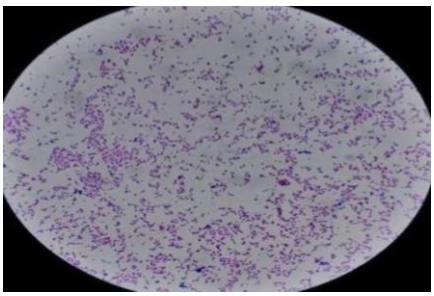

Fig.8 Showing gram staining *Escherichia coli.*

Biochemical Testing: Standard biochemical tests were performed on the isolated bacteria to Identify their metabolic and enzymatic properties. [15,16,17,18,19]

Table 1 Biochemical test describe the characterization isolated bacteria.

S.no	Biochemical test	Klebsiella	S.aureous	*E.coli*

1.	Sucrose	Positive	Positive	Positive
2.	Dextrose	Positive	Positive	Positive
3.	Maltose	Negative	Positive	Positive
4.	D-manitol	Positive	Positive	Positive
5.	Indole	Positive	Positive	Positive
6.	Citrate	Negative	Negative	Positive
7.	Urease	Negative	Positive	Positive
8.	H2S	Positive	Positive	Positive
9.	Catalase	Positive	Negative	Positive
10.	Nitrate	Positive	Positive	Positive
11.	MR	Positive	Positive	Positive
12.	MR-VP	Positive	Positive	Positive

Phytochemical Testing: All the phytochemical done by standard method [20,21,22,23,24,25,26]

Table 2 Phytochemical test describing the characterization of isolated bacteria.

S.no	Phytochemical test	*Sena alata*	*Hibiscus rosa-sinensis*	*Psidium guajava*	*Azadirachta indica*
1.	**Saponin**	Negative	Negative	Positive	Positive
2.	**Tanin**	Positive	Positive	Positive	Positive
3.	**Phenolic flavonoids**	Negative	Positive	Positive	Positive
4.	**Alkaloid or wagner**	Positive	Positive	Positive	Positive
5.	**Terpenoid**	Negative	Positive	Negative	Positive
6.	**Phenolic**	Positive	Positive	Positive	Positive
7.	**Alkaloid (Mayers reagent)**	Positive	Positive	Positive	Negative
8.	**Dragendroff reagent**	Negative	Negative	Negative	Negative
9.	**Ammonia**	Positive	Positive	Positive	Positive
10.	**Carbohydrate**	Positive	Positive	Negative	Negative

4. ANTIMICROBIAL ACTIVITY

The Minimum Inhibitory Concentration (MIC) is a critical parameter in microbiology used to determine the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism.

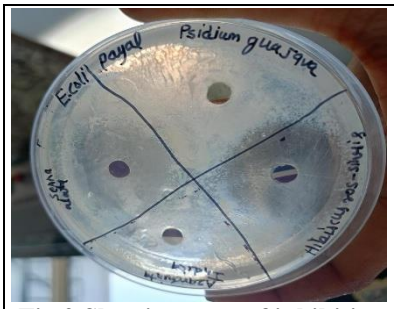

Fig.9 Showing zone of inhibition E.coli

Fig.10 Showing zone of inhibition s.aureous

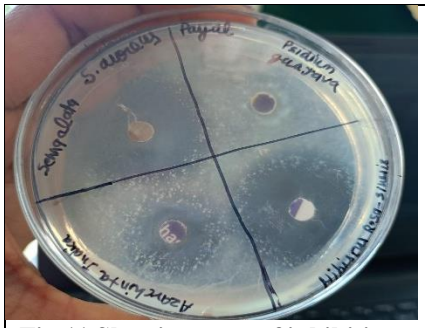

Fig.11 Showing zone of inhibition Klebsiella

Table 3 MIC Results

Strain	**Zone of inhibition (mm) *psidium guajava***	**Zone of inhibition (mm) *Senna alata***	**Zone of inhibition (mm) *hibiscus rosa -sinesis***	**Zone of inhibition (mm) *Azardirachta indica***
E.coli	**1.0 ±.15**	**3.6 ± .20**	**2.1 ± .15**	**1.0 ± .15**
S.aureous	**3.7 ± .25**	**2.1 ± .20**	**3.8 ± .26**	**1.5 ± .10**
Klebsiella	**2.8 ± .18**	**3.1 ± .24**	**3.2 ± .20**	**3.8 ± .24**

MIC values help categorize microorganisms as susceptible, intermediate, or resistant to specific antimicrobial agents. These categories are based on extensive research correlating MIC with achievable serum levels, resistance mechanisms, and therapeutic outcomes.

- A Larger Zone of klebsiella 3.1 ± .25mm, (Senna alata) 2.15 ± .15mm, 2.10 ± .10mm means Inhibition Indicates strong antimicrobial activity. Suggests that S. aureus is highly susceptible to the tested antibiotic. S.aureous Hibiscus rosa-sinesis 3.8 ± 26. A Larger Zone of E.coli3.6 ± .20mm means Inhibition Indicates strong antimicrobial activity. A very good Minimum Inhibitory Concentration (MIC) result corresponds to a low MIC value, meaning a small amount of the antibiotic effectively inhibits bacterial growth. MIC and Zone of Inhibition Correlation. MIC (Minimum Inhibitory Concentration) The lowest concentration of an antibiotic that prevents visible bacterial growth. A low MIC means the antibiotic is effective at lower doses, which is good. A high MIC means the bacteria require more antibiotic to be inhibited. Suggests that guava pulp and seed extracts are effective in inhibiting Bacillus and Salmonella growth.
  Stronger antimicrobial activity guava seed and leaves a very good result are show. A good MIC result that even at a low concentration, guava extracts successfully inhibit bacterial growth.

4.1 RELATIONSHIP WITH ZONE OF INHIBITION

There is an inverse correlation between MIC and the zone of inhibition; lower MIC values indicate higher susceptibility and larger zones of inhibition.

5.CONCLUSION

The formulated herbal moisturizer containing *Senna alata, Hibiscus rosa-sinensis*, *Azadirachta indica*, and *Psidium guajava* demonstrated significant antimicrobial activity against Escherichia coli and Staphylococcus aureus. The combination of these medicinal plant extracts not only provided skin-nourishing benefits but also exhibited potent antibacterial properties, making it a promising natural alternative to synthetic antimicrobial agents. The evaluation confirmed that the formulation effectively inhibited bacterial growth, highlighting its potential use in skincare products for maintaining skin health and preventing infections. Further studies on stability, efficacy, and safety would be beneficial for commercial applications.

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