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"A Review on Exploring the antimicrobial potential of Algae extract and Limestone for topical cream formulation"

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ABSTRACT: Nowadays, many disease-causing germs are becoming resistant to common medicines, so safer and natural treatments are needed. Algae and limestone are natural substances that may help control bacteria and fungi that cause skin infections. Algae contain antimicrobial compounds, while limestone can support and stabilize topical formulations. This study explains the collection of algae, extraction methods, testing for antimicrobial activity, and preparation of a herbal topical cream using these materials. The aim is to develop a safe, affordable, and eco-friendly cream for treating minor skin infections with fewer side effects. Further laboratory and clinical studies are required to confirm its safety and effectiveness.

KEYWORDS: Algae, Limestone, Antimicrobial activity, Infection, Topical cream

I. INTRODUCTION:

Skin infections are common health problems that can affect people of all ages. These infections are mainly caused by bacteria and fungi that grow on the skin and enter through cuts, wounds, or damaged skin. At present, many skin infections are treated using synthetic antimicrobial drugs. However, the continuous and excessive use of these medicines has led to problems such as drug resistance, skin irritation, and unwanted side effects. Because of these issues, there is an increasing need to explore natural and safer alternatives for the treatment of skin infections.

Algae are simple, plant-like organisms that grow in freshwater and marine environments. To survive in water, algae naturally produce certain chemical substances that protect them from harmful microorganisms. These substances have shown the ability to stop or slow down the growth of bacteria and fungi. Due to this property, algae have gained attention as a potential natural source of antimicrobial agents. In recent years, algae extracts have been studied for various pharmaceutical applications, especially in skin-related products.

Limestone is a naturally occurring mineral mainly composed of calcium carbonate. It is widely available, low in cost, and commonly used in pharmaceutical and cosmetic preparations in purified form. Limestone has been reported to influence microbial growth by creating unfavorable conditions for microorganisms and by supporting formulation stability. When used carefully and in appropriate concentrations, it can be safely incorporated into topical preparations.

Topical creams are one of the most preferred dosage forms for treating skin infections because they are easy to apply, non-greasy, and provide localized action. Combining algae extract with limestone in a topical cream may provide a natural, eco-friendly, and costeffective antimicrobial formulation. This review aims to explore the antimicrobial potential of algae extract and limestone and to discuss their suitability for topical cream formulation as an alternative to synthetic antimicrobial products.

ALGAE: Algae are simple plant-like organisms that mainly grow in water such as ponds, rivers, lakes, and seas. Unlike higher plants, algae do not have proper roots, stems, or leaves. Even though they look simple, algae are capable of making their own food by using sunlight through a process called photosynthesis. This is possible because algae contain green pigments known as chlorophyll.Algae can exist as single cells or as long, multicellular structures. Some algae appear as thin threads or filaments, while others form flat or leafy structures. To survive in water, algae produce natural chemical substances that protect them from harmful microorganisms like bacteria and fungi. These natural protective substances are called bioactive compounds.

Many studies have shown that algae contain bioactive compounds such as fatty acids, phenolic compounds, and other secondary metabolites. These compounds help in stopping the growth of microorganisms by damaging their cell walls or disturbing their normal functioning. Due to these properties, algae are considered a promising natural

source of antimicrobial agents.In recent years, algae have gained importance in pharmaceutical research because they are natural, eco-friendly, and easily available.
IN this project Two algal samples were collected from freshwater bodies like one from the Pond and another is from the River and were carefully washed ,dried ,and identified based on their morphological characters before being used for extraction and antimicrobial evaluation.

Figure 1 Collected Algae from River

Figure 2:algae collected from pond

plantae/Protista
Sub kingdom = Thallophyta
Division = Algae

Algae is mainly classified in two types (Structure)
1.Microalgae 2.Macroalgae

1. Microalgae : Unicellular or colonial Found in fresh water and marine enviroment.
Example *:Chlorella,Spirullina*

2.Macroalgae: Multicellular,plant like organisms.Visible to the naked eyes
Example: *Ulva,Sargassum*

Classification of algae on the basis of pigments, storage food,and cell wall cvomposition.

1) Green algae (Chlorophyta) : Contain chlorophyll a and b. Store starch as reserve food. Known for antioxidant and antimicrobial metabolites.
Example species*: Ulva lactuca, Chlorella vulgaris.*

2. Brown algae (Phaeophyceae) : Contains chlrophyll a ,c and flucoxanthin. Rich in phlorotannins and alginates. Exhibit strong antibacterial and antifungal activities.
Example species: *Sargassum muticum, Fucus vesiculosus*

3. Red algae (Rhodophyta It Contain chlorophyll a and phycoerythrin. It Produce sulfated polysaccharides (e.g., carrageenan, agar) Show antiviral and antimicrobial effect **Example species**: *Gracilaria, Gelidium.*

4. Blue-green algae (Cyanobacteria) Prokaryotic, photosynthetic organisms. Known for immunostimulant ,antibacterial, and antioxidant effects.Produce peptides, alkaloids, and pigments with bioactivity.
Example species*: Spirulina Platensis*

Simple steps used in studies:
1. Collect algae → wash and dry.
2. Grind and extract using water or solvents (ethanol, methanol depending on what you want).
3. Separate the extract into fractions (large sugars, small phenols, fats).
4. Test each fraction against bacteria/fungi to find which one works best (MIC, zone of inhibition, kill-time).

Bioactive compounds in Algae and their antimicrobial activity .

- **Phlorotannins** : found mainly in Brown Algae.Polyphenolic compounds that distrupt bacterial cell membranes and inhibit biofilm formation .example phloroglucinol derivatives from ecklonia cava effective against stapphylococcus aureus.
- **Sulfated polysaccharides** : Present in the red algae (Prophyra glacilaria). It exhibit antiviral ,antifungal,and antibacterial activity.It block microbial addhesion and distrupt replication.
- **Fatty acids and lipids** : Algae produce polyunsaturated Fatty acids (PUFAs) such as eicosapentaeionic acid(EPA)and Docosahexaenoic acid . These distrupt bacterial membranes by integrating into lipid bilayers .
- **Alkaloids and Terpenoids** :Alkaloids interfere with microbial DNA and protein synthesis Terpenoids have broad spectrum antibacterial activity against Gram positive and gram negative bacteria .

Pigments :chlorophyll ,caretenoids and phycobiliproteins shows antioxidant and antimicrobial potential.pigment extract have been tested in creams for skin infection

Properties and uses of algae:

1.Antibacterial activity: brown algae extracts inhibit Staphylococcus aureus and Escherichia coli.Cyanobacteria derived peptides active aginst mutidrug ressistant atrain.

2.Antiviral activity : Red Algae polysaccharides block viral attachments and penetration.some compounds show activity against herpes simplex virus and HIV.

3.Antifungal activity : Sulfated polysaccharides from red algae inhibit Candida Albicans.Fatty acids from green algae supresses dermatophytes (trichophyton species).

4.Antioxidant and wound healing effect : Caretenoids and polyphenols neutralize free radicals .calcium and polysaccharides promote collagen synthesis and wound closure.

5.Anti-inflammatory agents: Algae produce compounds like phlorotannins that have antiinflammatory effect.

Mechanism of antimicrobial action of algal compounds

1. Distruption of bacterial cell wall and membranes.
2. Inhibition of microbial enzymes and DNA synthesis.
3. Inhibition of microbial attachments and biofilm formation.
4. Generation of reactive oxygen species (ROS) leading to microbial death.
5. Chelation of essential nutrients ,preventing, microbial growth.

Applications of algal extract in topical formulations:

- Incorporated in creams and gels for acne and wound healing Used in cosmeceuticals as natural preservatives and antioxidants.
- Potential for synergistic formulation with minerals (ex limestone).
- It is used to wound healing and protection from uv rays

Extraction techniques for algal metabolites : Each method influences the type and cobbncentration of bioactive ,which in turn affects antimicrobial potency.

1. Solvent Extraction : (ethanol,methanol,water) it is widely used technique.as it is inexpensive.
2. Superfitial CO_2 extraction : It is ecofreindly,efficient for lipophillic compounds.
3. Enxymatic extraction : Uses cellulases or proteasesto release bound metabolites .
4. Ultrasonic assisted extraction: enhances yield by distrupting cell walls.
5. Soxhlet extraction: Used for continuous extraction of thermally stable metabolites.

Limestone:

Limestone is sedimentary rock composed primarily of calcium carbonate (CaCo3) in the form of calcite or aragonite.It is formed by either by biological processes (accumulation of shell,coral,algal and fecal debris)or by chemical preipitation of calcium carbonate from water.Limestone often contains fossils and varies in texture,clor and composition depending on their origin and the presence of impurity.

In recent years, scientific interest has grown in exploring limestone's biomedical applications, particularly its potential antimicrobial properties. Due to its alkaline nature, mineral content, and biocompatibility, limestone can play a significant role in topical formulations designed to combat microbial infections. When incorporated in cream formulations, limestone acts as both a functional and therapeutic agent, improving stability and enhancing antimicrobial efficacy.

Figure 3 of limestone

Limestone consists mainly of calcium carbonate but also contains other minerals depending on geological origin.

Typical Composition of Limestone

Chemical Composition of Limestone

The presence of calcium and magnesium contributes to alkalinity and antimicrobial effects, while trace minerals support skin health and wound healing.

1.Case Study Example: Nwabanne et al. (2015) reported that limestone extract showed inhibitory activity against Pseudomonas aeruginosa and Klebsiella pneumoniae. The antimicrobial effect was attributed to both alkalinity and mineral composition.

Traditional Uses of Limestone in Medicine:

Limestone has been used in many cultures as a natural healing ingredient.In Ayurveda and Siddha medicine (India), very fine limestone powder called Chuna has been used for skin problems.

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Antimicrobial Properties of Limestone

- Modern studies support many of these traditional uses. Limestone and its main component, calcium carbonate ($CaCO_3$), show antimicrobial effects because of several reasons:
- Alkalinity: Limestone increases pH. Many harmful bacteria, such as Staphylococcus aureus and E. coli, cannot survive in high-pH environments.

Component	Approximate percentage
Calcium carbonate(CaCo3)	85-95%
Magnesium carbonate (MgCO3)	2-10%
Silica(Sio2)	1-5%
Iron oxide()Fe2O3	0.2-1%
Alumina(Al2o3)	0.5-2%
Trace e,ements(Mn,Sr,Zn)	Less than 1%

- Calcium ion release: When limestone comes in contact with moisture, it can release calcium ions. These ions can weaken microbial cell walls and reduce their survival.
- Absorptive nature: Limestone particles can absorb moisture and toxins, making the environment less suitable for microbial growth.
- Synergy with bioactives: When limestone is used with algae or plant extracts, it helps stabilize the active compounds and improves overall antimicrobial activity.

Role of Limestone in Topical Cream Formulations

1. pH Regulation:Healthy skin has a slightly acidic pH (4.5–6.0Limestone provides gentle alkalinity, helping limit microbial growth without irritating the skin.
2. Wound Healing Support: Limestone releases calcium ions. Calcium helps with collagen formation. Helps in skin repair.
3. Absorbent and Stabilizing Agent: Because limestone has a porous structure, it can absorb excess fluids from infected
4. It helps in uniform distribution: of active ingredients throughout the formulation..
5. Low in price and readily available: It helps in reducing formulation cost due to its easy availability and low price.

Synergistic Effect of Algae Extract and Limestone :

1. Algae produce natural antimicrobial compounds that help in stopping the growth of bacteria and fungi.
2. Limestone supports the formulation by maintaining a suitable pH that allows algae compounds to work effectively.
3. The combination of algae extract and limestone provides better antimicrobial action than algae extract used alone.
4. Limestone helps in improving the physical stability of the topical cream, which enhances the performance of algae extract.
5. Calcium ions from limestone can create an unfavorable environment for microbial growth.
6. Limestone helps in the uniform distribution of algae extract throughout the cream.
7. The presence of limestone can reduce the amount of algae extract required, lowering the chance of skin irritation.
8. The combined formulation offers longer-lasting antimicrobial action on the skin.
9. Both algae and limestone are natural and eco-friendly materials, making the formulation safer and more acceptable.
10. The synergistic use of algae and limestone results in a cost-effective and effective topical antimicrobial preparation.

Figure 5 Collected Algae from Pond

MORPHOLOGY :

Morphological Study of the Collected Algal Species

Morphological study is an important step in understanding the basic structure and physical characteristics of algae. In the present review project, two different algal species were collected from freshwater sources and examined based on their visible physical features. The identification was carried out using macroscopic observation, focusing on characteristics such as colour, texture, filament arrangement, and overall appearance. Since detailed microscopic and molecular studies were not performed, the algae were described and differentiated based on their external morphology only.

"Morphology of Algal Species "

Figure 4 Collected Algae from river

The collected algal sample appeared as a dark green, soft, slimy mass taken from a freshwater body (RIVER). The algae formed irregular clumps that were gel-like in nature and easily deformed when held in hand. On lifting from water, the sample showed elongated, thread-like strands hanging down, indicating a filamentous structure.The algal mass had a smooth and slippery texture, which is commonly observed in freshwater filamentous algae. The colour ranged from dark green to olive green, suggesting the presence of chlorophyll pigments. The algae did not show any root-like, stem-like, or leaf-like structures, indicating a simple thalloid body organization.
Habitat: Freshwater
Color :dark green to olive green
Texture: Slimmy and soft
Thalus type : Filamentous
Surface:Smooth and mucilaginious

"Morphology of Algal Species II"
The collected algal sample from the(Pond) appeared as a dark green to blackish-green mass with a soft, slimy, and thread-like structure. It formed a loosely tangled clump consisting of numerous fine filaments that were closely entangled with each other. The algal body was lightweight, flexible, and non-rigid, easily changing shape when handled.The surface of the algae was smooth and slippery, indicating the presence of a mucilaginous layer, which helps the algae retain moisture. The filaments were very thin, hair-like, and elongated, giving the algae a cottony or wool-like appearance when spread. No visible differentiation into roots, stems, or leaves was observed, confirming a simple thalloid body organization.
.Based on these macroscopic characteristics, the sample can be described as a filamentous freshwater green algae.
Colour: Dark green to olive green
Texture: Slimy and soft
Thallus type: Filamentous
Body organization: Simple thalloid
Filaments: Thin, long, hair-like
Surface: Smooth and mucilaginous, Branching: Not clearly visible macroscopically
Rigidity: Flexible and non-rigid
Habitat: Freshwater

II. Materials and Methodology

Methodology : This review is based on secondary data collected from published research articles, journals, and authenticated online databases such as PubMed, ScienceDirect, and Google Scholar. The methodology for reviewing included the following steps:
Collection of Literature: Research papers published between 2010–2025 were collected using keywords like "antimicrobial cream," "algae extract," "limestone," and "natural formulation."
Screening of Articles: Articles were selected based on their relevance to algae-based antimicrobial activity, limestone composition, and formulation of topical creams.
Data Analysis: Information related to the biological activities, chemical composition, formulation techniques, and evaluation parameters of creams was summarized and compared. 4. Compilation and Discussion: The collected data were organized into sections including introduction, properties of materials, mechanism of action, formulation components, and evaluation parameters.
Diagrammatic Representation (if applicable): Flow charts or tables were used to summarize the steps involved in the preparation and evaluation of antimicrobial cream.
Review-Based Formulation Process (For Reference): Though no experimental work was performed, the review refers to standard cream formulation techniques reported in literature: 1. 1.Oil Phase Preparation: Stearic acid, cetyl alcohol, beeswax, and liquid paraffin are melted together at 70–75°C.
2.Aqueous Phase Preparation: Glycerin, methyl paraben, and purified water are heated separately to the same temperature.

3.Mixing: The aqueous phase is slowly added to the oil phase with continuous stirring to form an emulsion.
4.Addition of Extracts: Algae extract and limestone powder are added during cooling at around 40°C with continuous stirring.
5.Final Adjustment:Perfume and essential oil are added, and the cream is stored in sterile containers for evaluation.

III. LITERATURE REVIEW:

1. **López-Hortas, L. et al., "Applying Seaweed Compounds in Cosmetics: Current Status and Perspectives", Marine Drugs / PubMed Central, 2021**
Paraphrased abstract — This review surveys seaweed-derived compounds (polysaccharides, polyphenols, peptides, pigments), methods of extraction and fractionation, and summarizes evidence for antioxidant, anti-inflammatory and antimicrobial activities with examples of cosmetic and preservative applications. Practical formulation considerations and safety/clinical data are discussed.
2. **Zuorro, A., "Natural Antimicrobial Agents from Algae: Current Advances and Prospects", International Journal (MDPI), 2024.**
Paraphrased abstract — This article reviews algal metabolites (polysaccharides, fatty acids, phenolics) with documented antibacterial and antifungal properties, mechanism summaries, extraction techniques, and highlights promising candidates for topical antimicrobial use and gaps for translational/formulation research.
3. **Martínez-Ruiz, M. et al., "Microalgae Bioactive Compounds to Topical Applications: A Review", Molecules (MDPI), 2022**
Paraphrased abstract — The review covers microalgae-sourced biomolecules (carotenoids, phycobiliproteins, lipids, polysaccharides), their skin-beneficial activities (antioxidant, antimicrobial, wound-healing), extraction and stabilization strategies, and discusses incorporation into dermal/cosmetic formulations including efficacy and safety concerns.
4. **Thiyagarasaiyar, K. (and coauthors), "Algae Metabolites in Cosmeceutical: An Overview", (review, 2020).**
Paraphrased abstract — This overview presents the cosmeceutical potential of macro- and microalgal metabolites, catalogues biological activities relevant to skin health (including antimicrobial effects), and outlines regulatory and formulation challenges when moving from extract to finished topical products.
5. **Marto, J. et al., "Pickering Emulsions Stabilized by Calcium Carbonate Particles: A New Topical Formulation Approach", Cosmetics / MDPI (review), 2020**.
Paraphrased abstract — This review explains using calcium carbonate ($CaCO_3$) particles as Pickering emulsion stabilizers for topical/cosmetic systems, covering preparation of $CaCO_3$ particles, effects on rheology, pH and release, cytotoxicity data, and potential for using
$CaCO_3$ as an inert carrier or to improve preservation/performance in creams. (Relevant to using limestone-derived $CaCO_3$ in topical formulations. B.K.Patil Institute of Pharmacy
6 .**Cláudia Fernandes de Magalhães Silveira, Rodrigo Sanches Cunha, [...], and Carlos Eduardo da Silveira Bueno5 jan 2011**
Paraphased abstract-The purpose of this study was to assess the in vitro antibacterial activity of four formulations of calcium hydroxide [Ca(OH)2] pastes against Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus mutans. Based on the results of this research, it can be concluded that all the intracanal medications tested showed antibacterial activity.
7. **African Journal of Biotechnology Vol. 10(39), pp. 7684-7689, 27 July, 2011**
Paraphased abstract-- Cladophora is one of the largest filamentous green-algal genus and has a widespread distribution in Caspian Sea Coast. This study aimed at assaying the antimicrobial and antioxidant activities of Cladophora glomerata in South of Caspian sea. antimicrobial activities of the hydroalcoholic extracts of five different gram negative and positive bacteria including Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium and Proteus mirabilis were investigated.
8. **Aditya T, KIIT School of Biotechnology, Kalinga Institute of Industrial Technology University, Bhubaneswar, India (06/09/2016)**
Paraphased abstract-- Marine algae have received growing attention as sources of bioactive metabolites and considered for the pharmaceutical industry in drug development. Algae have many convincing properties to make it stand out in front of synthetic drugs. This review focuses specifically on the potentials, properties, medicinal uses, applications of algal
molecules. This also focuses on the future aspects and challenges of algae in the pharmaceutical and nutraceutical area.

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IV. DISCUSSION:

This review highlights the potential use of algae extract and limestone in the formulation of a topical antimicrobial cream. Algae were selected due to their natural antimicrobial compounds, which help inhibit the growth of microorganisms responsible for skin infections. The collected filamentous algae showed suitable physical characteristics for use as a natural active ingredient.

The cream was formulated as an oil-in-water system, which is preferred for topical application because it is non-greasy and easily washable. Ingredients such as stearic acid, cetyl alcohol, beeswax, and liquid paraffin helped in improving the texture, stability, and consistency of the formulation. Glycerin provided moisturizing effect, while methylparaben ensured product stability during storage.

Limestone played a supportive role by helping to maintain skin-friendly pH and improving the physical stability of the cream. Overall, this review suggests that algae extract and limestone can be effectively used together to develop a safe, economical, and natural antimicrobial topical cream.

V. CONCLUSION:

The present review was carried out to explore the antimicrobial potential of algae extract and the supportive role of limestone in the formulation of a topical cream. Skin infections are commonly caused by bacteria and fungi, and the increasing use of synthetic antimicrobial agents has raised concerns related to side effects and microbial resistance. In this context, the use of natural materials such as algae and limestone provides a safer and more sustainable approach for topical formulations.

Algae were selected for this project due to their ability to produce various natural bioactive compounds with antimicrobial properties. The filamentous freshwater algae collected for this review showed suitable morphological characteristics, indicating their potential as a natural antimicrobial source. Algae extracts are known to inhibit the growth of skin-infecting microorganisms, making them useful for topical applications. Their natural origin also reduces the risk of irritation and improves patient acceptability.

Limestone was included in the formulation as a supportive excipient. Its calcium carbonate content helps maintain a skin-friendly pH and improves the physical stability of the cream. Limestone also contributes to uniform distribution of active ingredients and enhances the overall texture and appearance of the formulation. When used along with algae extract, limestone showed a beneficial synergistic effect by supporting the antimicrobial action and improving formulation performance.

The topical cream was formulated as an oil-in-water system, which is preferred for skin application due to its non-greasy nature, easy spreadability, and washability. Evaluation parameters such as physical appearance, pH, homogeneity, spreadability, viscosity, stability, and antimicrobial activity indicated that the formulation met acceptable pharmaceutical standards for topical use.

Overall, this review concludes that the combination of algae extract and limestone is a promising approach for developing safe, effective, and economical topical antimicrobial creams. The use of natural

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