

**Formulation and Evaluation of Anti-Acne Cream Using the Extracts of
Oscillatoria sp: Investigating its Biocompatibility and Toxicity by Skin
irritation test and Brine Shrimp Lethality assay**

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ABSTRACT:

Acne through definition is a multifactorial continual inflammatory ailment of pilosebaceous units. Although antibiotics play an essential role in acne control, the development of drug resistance among acne causing bacteria (*Propionibacterium acne* and *Streptococcus* sp) had created a need for development of novel antimicrobial agents. Therefore, the present study focuses on development of anti-acne cream using extracts of *Oscillatoria* sp. *Oscillatoria* extracts was used as a major constituent for acne cream with paraffin wax as cream base. The *Oscillatoria* extracts and developed cream were investigated for antibacterial activity against acne causing bacteria. The biocompatibility of the acne cream was evaluated by patch test and Brine shrimp lethality assay was performed to determine the toxicity of the developed cream. The extract and acne-cream showed 14mm and 15mm; and 13mm and 11mm against *Propionibacterium acnes* and *Streptococcus* sp. The cream showed no irritation end points and it was found to be non-toxic. Thus the developed anti acne cream can be developed as product for treatment of acne.

Keywords: Anti acne cream, Skin care products, *Oscillatoria* sp, Brine shrimp lethality assay, skin irritation test

INTRODUCTION

Acne through definition is a multifactorial continual inflammatory ailment of pilosebaceous units. Various medical presentations consist of seborrhoea, comedones, erythematous papules and pustules, much less regularly nodules, deep pustules or pseudocysts, and last scarring in few of them (Paxk man *et al.*, 2001) Propionibacterium acnes is an anerobicmicro-organism implicated withinside the pathogenesis of acne. The ultimate 30 years have witnessed an alarming boom in resistance to antibiotics typically hired to deal with pimples Acne does now no longer constitute a normal bacterial infection,wherein antibiotic resistance at once correlates to remedy failure,due to the fact antibiotics exhibit each antibacterial and anti -inflammatory consequences.(Leyden JJ *et al.*, 2007)

Although antibiotics play an essential position in acne control, the growth in *P. acnes* resistance ought to be reason for situation and function the impetus for extradewithinside the prescribing styles and remedy algorithms(Webster, 2002). There are unique techniques to be had for the remedy of acne. Many topical antibiotics formulations are to be had, both by myself or in aggregate. They inhibit the increase of *P.acnes* and decrease inflammation. Topical antibiotics consisting of erythromycin and clindamycin are the most popular withinside the control of acne and to be had in plenty of vehicles and packaging.Clindamycin and erythromycin have been each powerful towards inflammatory pimples in topical shape in aggregate of 1-4% without or with the addition of zinc. An addition of topical 2% zinc sulfate and nicotinamide become no one of a kind than placebo for the remedy of acne(Plewig *et al.*, 2000)

The present drugs have numerous facet consequences and diverse negative aspects. Side effects aleven though minor consists of erythema, peeling, itching, dryness, and burning .pseudomembranous colitis that is rare, however has been pronounced with clindamycin. A maximum vital aspect impact of topical antibiotics is the improvement of bacterial resistance and go resistance; therefore, it ought to now no longer be used as monotherapy.(Yang ,2007) Gastrointestinal disfunctions and vaginal candidiasis are maximum not unusualplace facet results. Doxycycline may be related to photosensitivity. Minocycline might also additionally produce pigment deposition withinside the pores and skin, mucous membrane, and teeth. Autoimmune hepatitis, systemic lupus erythematosus-like syndrome, and serum sickness-like reactions arise not often with minocycline (Leyden JJ *et al.*, 2011)

Since there are numerous risks in the use of the drugs and remedy techniques, there may be necessity in upgrading with the options with the usage of herbal assets like algal forms, etc. Along with plants and algae, cyanobacteria have additionally come into attention as a supply of compounds for pores and skin care techniques. Cyanobacteria are one of the richest supply of recognized and novel bioactive compounds with extensive pharmaceutical applications (Rasheed *et al.*, 2012). Cyanobacteria produce a lot of excellent compounds which have proven ability treatments in primary ailment control including cancer, asthma, diabetes, etc (Chapman, 2006) cyanobacteria have resulted withinside the manufacturingskin care and beauty products for UV protection. Some cyanobacterial extracts comprise peptides and proteins which can be broadly utilized in hair care merchandise including lotions, shampoos, answers for everlasting hair wave and hair colouring merchandise . . *Oscillatoria sp* and its extracts confirmed more importance in developing lotions which may be used withinside the remedy of pimples and confirmed more antimicrobial interest. *Oscillatoria acuminata*, *Oscillatoria phigranulata* and *Spirulina platensis* methanolic extracts confirmed the very best inhibition zones. The methanolic extracts of those microalgae show off extra cytotoxicity for all mobileular lines. *Oscillatoria spp.* has many species showing its biodiversity, with maximum of the species actively owning bioactive compounds (Singh *et al.*, 2017).

The present study makes a speciality of extraction of bioactive compounds from *Oscillatoria sp* and improvement of anti-acne cream. Antibacterial hobby of the extracts and cream become decided in opposition to pimples inflicting *Propionibacterium acnes* and *Streptococcus sp.* The biocompatibility of the advanced cream became evaluated through the use of patch test and brine shrimp lethality assay.

MATERIALS AND METHODS

Procurement of Algal Culture and microscopic examination

The *Oscillatoria sp* was maintained at BG 11 media under the suitable light source of 2000 – 4000 lux, growth was observed at the period of 4-15 days at room temperature (Figure-1). The microalgae culture was examined under light microscope for the morphological identification.

Extraction of bioactive metabolites

The isolated samples were cultivated and dried biomass was sonicated with liquid nitrogen and then was extracted with 95 % methanol and 95% acetone. The extracts were centrifuged at 4000 rpm for 10 min, and were further concentrated in vacuum under reduced pressure, the stock solutions of extract were prepared in DMSO at 50 mg ml⁻¹ for the evaluation of antimicrobial activity.

Development of Anti-acne cream

2ml of petroleum jelly was placed into an evaporating dish and melted over a water bath. The dish was removed from heat and the algae extract was added in the melting point. The mixture was continuously stirred to ensure homogeneity, but at the same time gently to avoid incorporation of excess air. After stirring the developed cream was left to cool and stored at a sterile container.

Antibacterial activity of the extract and developed cream

The antibacterial efficacy of the *Oscillatoria* sp., extract and anti-acne cream was evaluated against the two major acne causing *Propionibacterium acne* and *Streptococcus* sp. by well diffusion method (Abhijith et al., 2021). Nutrient Agar was prepared and sterilized, and poured into plates. Overnight cultures of test pathogens were cultured and 0.1% of culture solution of each test organisms was streaked throughout the petri plate with the sterile cotton swab by rotating the plate at 60° angle for each streaking. 6mm well borer was used to bore wells on the agar surface of each NA plates. About 100µl of the samples were loaded into the well and the plates were incubated in an incubator at 37°C for 48h. The antibacterial activity was determined in terms of inhibitory zones around the wells loaded with natural dyes in all the Nutrient Agar plates containing test pathogens. The obtained clear zones were observed and measured in millimetre (mm).

Biocompatibility assay: Patch test

Biocompatibility of the developed anti-acne cream was assessed by skin-irritation test (contact allergy test). The acne cream was tested separately by applying on the skin surface of selected human subjects. The samples in contact with the skin surface were observed for the specified period upto 6h for the development of the symptoms related to contact dermatitis allergy like erythema and oedema.

Brine shrimp lethality assay

Brine shrimp lethality assay is an easy method to determine the toxicity of the compounds. 30g of NaCl is dissolved in 1L of distilled water. Shrimp eggs were added into container and a lamp was placed above to the hatched shrimp. Continuous aeration was given using aquarium air pump. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 5 mL of the water was added to each test tube and 10 brine shrimps were introduced into each tube. The test tubes were left uncovered under the lamp. Plant extract at different concentration were added to the tubes. The number of surviving shrimps were counted and recorded after 24 hours. As mentioned by Meyer and others, LC50 value of less than 1000 µg/mL is toxic while LC50 value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. (Meyer *et al.*, 1982)

RESULTS AND DISCUSSION

Microscopic observation of *Oscillatoriasp*

The cultivated *Oscillatoria* sp was found to be Blue-green in colour, straight, irregularly flexuous, cylindrical, 7-11 µm broad, slightly narrow towards ends, slightly constricted, apical cell conical to widely rounded with slightly thickened outer cell wall (Figure-1).

Extraction and development of Anti acne cream

After the microscopic observation was done, the extract was obtained by using methanol as a solvent. The colonies maintained at BG 11 media were subjected to extraction procedure and the extracts were obtained. The anti-acne cream was developed by using paraffin wax as a cream base. By the use of petroleum jelly, coconut oil and the extracts obtained, the cream was prepared and stored in a sterile container at 4°C for further purposes (Figure-2)

Antibacterial activity against acne causing bacteria

The anti-bacterial activity of the cream and the extracts were determined against acne causing bacteria and the results were reported on the basis of the inhibition zones (mm). The drug Ciprofloxacin was used as a standard (Table-1). The test organisms used were

Propionibacterium acnes and *Streptococcus sp*. The extract and the cream were used for testing the antibacterial activity and the inhibition zones were formed. The extract showed 14mm and 15mm against *Propionibacterium acnes* and *Streptococcus sp*. Whereas, acne cream showed 13mm and 11mm against *Propionibacterium acnes* and *Streptococcus sp* (Figure-4 and 5).

Brine shrimps lethality assay:

The results of Brine Shrimp lethality assay was shown on (Table-2). The concentration of the plant extract was increased sequentially and the death rate of the Nauplii was calculated. The lethality rate is directly proportional to the concentration of the substance. Hence increase in the concentration, there was increase in the count of dead nauplii. The number of surviving shrimps were counted and recorded after 24 hours. As mentioned by Meyer and others, LC50 value of less than 1000 µg/mL is toxic while LC50 value of greater than 1000 µg/mL is non-toxic. From the obtained results, the extracts were found to be non toxic.

Skin irritation test:

Biocompatibility of the developed anti acne cream was assessed by skin-irritation test (contact allergy test). The human subjects showed no symptoms of dermatitis like erythema and oedema (Figure-6).

DISCUSSION

The procured culture turned into tested under light microscope for the affirmation of *Oscillatoria sp*. The colonies regarded as lengthy filamentous, unbranched and discovered to be in oscillatory movement. The look at on Cyanobacteria extraction concluded that the energetic dealers within the crude extract own antibacterial interest towards pimples inflicting bacteria. The Collected Cyanobacteria became observed to be *Oscillatoria sp*. and grown in BG eleven medium for mass cultivation. Extract centrifuged become quantitatively analysed and used for bioactive compounds extraction. (Prakash, Johnson, Jeeva, 2011). Under the light microscopy, the microalgae became located as Filamentous unbranched algae with mucilaginous sheath, taking place singly or in tangled mats; named for its oscillating motion. (Sriram and Seenivasan, 2012).

Oscillatoria sp accommodates with a row of cells forming trichomes or un-branched filaments with a totally skinny gelatinous sheath. The cells are broader than longer however cylindrical. All cells of a filament are comparable aside from apical cell that is convex on the top. In a few species it can result in a subacute factor or be extra or much less dilated, or capitated having a thickening called a cap or calyptra on the tip. Species with the slim trichome but have cylindrical cells wherein the period can be same to or, more than the breadth e.g. *O. splendid*. If clean cloth is found beneath the microscope, particular oscillating motion is determined. (ToreAune, Takeshi Yasumoto, Else Engeland, 1991). From the above studies, the accrued pattern turned into showed as *Oscillatoria sp*.

The increase of the bacterial lines examined have been inhibited through the lifestyle extracts organized through the usage of exceptional solvents Chloroform, Methanol and Water wherein Chloroform extracts of Cyanobacteria have proven most inhibition zones below investigation. Antimicrobial results of Cyanobacterial aqueous and natural solvent extracts are visualized in bioassays the usage of decided on microorganisms as test organisms (Frankmollet *al*, 2008). In some cases, species like *Oscillatoria angusta*. FS 19 (Mazandaran Province) confirmed antibacterial results towards *B. subtilis*. PTCC 1204 and *S. epidermidis*. (Ghasemi *et al*, 2003). Crude extract of *Oscillatoria sp*. confirmed most antibacterial interest towards gram fine bacterial pathogens in comparison with Streptomycin. The phycocyanin pigment confirmed excessive pastime towards *Klebsiella sp*. in comparison to Streptomycin The phycocyanin pigment confirmed excessive interest towards *Klebsiella sp*. in comparison to the opposite pathogens. (Johnson, 2011) Bacterial bioassay incorporate specific check bacteria, *Bacillus subtilis* and *Pseudomonas aeruginosa* which are typically used to discover antibiotic residues in food. The antibacterial interest turned into decided towards *Propionibacterium acnes* and *Streptococcus sp*. and it suggests that the cream and extracts can inhibit the acne inflicting bacteria. The effects have been in comparison with diverse studies works and the acquired effects properly correlates with the interpretations.

The brine shrimp lethality bioassay become used to expect the cytotoxic interest of the n-hexane, carbon tetrachloride, chloroform, and aqueous fractions from methanolic crude extracts. The mortality endpoint of the bioassay become described because the absence of managed ahead movement at some point of 30 s of observation. From the determined data, the percentage of lethality of the brine shrimp nauplii for every awareness and manage become calculated. (McLaughlin, Rogers, 2009). The received outcomes indicates that the cream is non-poisonous and studied in evaluation with the alternative study works. The

acne cream turned into examined one at a time through making use of skincream of decided on human volunteers. The samples in touch with the pores and skin cream have been determined for the required duration upto 6h for the improvement of the signs and symptoms associated with touch dermatitis (allergy)like erythema and oedema. The human topics proven no signs of dermatitis like erythema and oedema and subsequently it's miles taken into consideration as secure for human use.

CONCLUSION:

The present study focuses on development of anti-acne cream using extracts of *Oscillatoria* sp. *Oscillatoria* extracts was used as a major constituent for acne cream with paraffin wax as cream base. The *Oscillatoria* extracts and developed cream were investigated for antibacterial activity against acne causing bacteria. The biocompatibility of the acne cream was evaluated by patch test and Brine shrimp lethality assay was performed to determine the toxicity of the developed cream. The extract and acne-cream showed 14mm and 15mm; and 13mm and 11mm against *Propionibacterium acnes* and *Streptococcus* sp. The cream showed no irritation end points and it was found to be non-toxic. Thus the developed anti acne cream can be developed as product for treatment of acne.

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Table 1: Antibacterial activity analysis

S. No	Samples	Inhibitory zones (mm)	
		<i>Propionibacterium acnes</i>	<i>Streptococcus sp</i>
1	methanol	0	0
2	Extract	14	15
3	cream	13	11
4	Drug (ciprofloxacin)	27	25

Table-2: Brine shrimp lethality assay

S. No	Concentration of plant extract	Naupli count		Death rate (%)	Inference
		Nauplis alive initially	Nauplis alive after 24hrs		
1	1mg	10	10	0	Non-toxic
2	2.5mg	10	8	20	Non-toxic
3	5mg	10	5	50	Non-toxic
4	7.5mg	10	4	60	Non-toxic

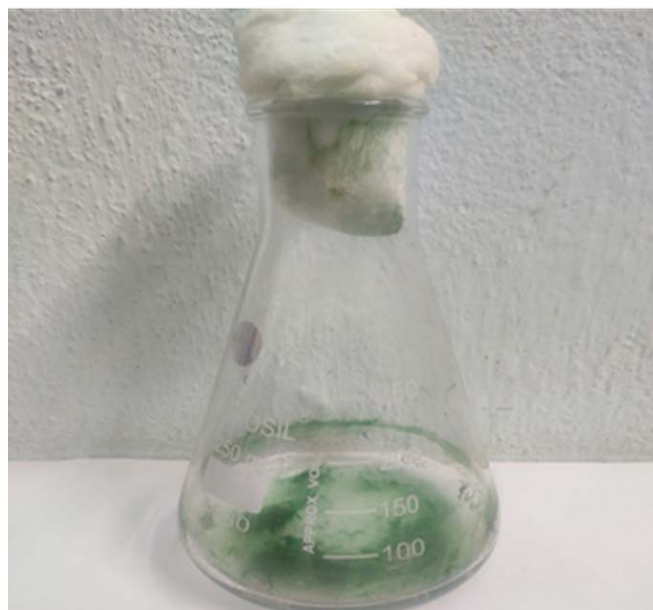


Figure 1: Collection and cultivation of *Oscillatoria* sp

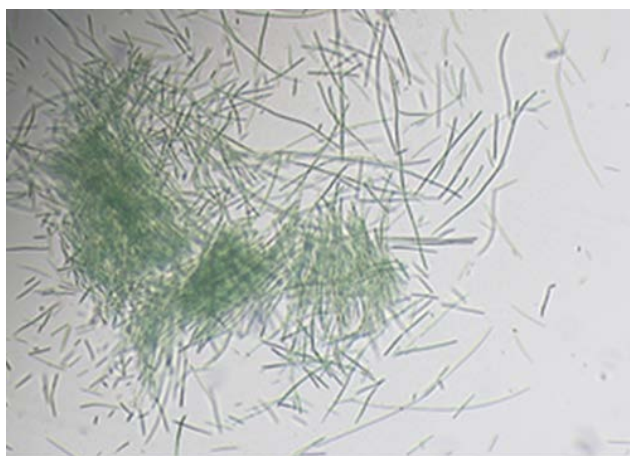


Figure 2: Microscopic observation

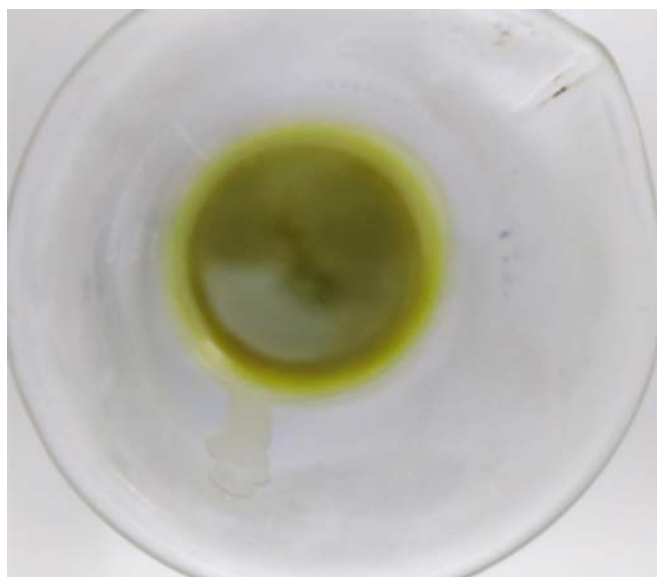


Figure 3: Developed antiacne cream



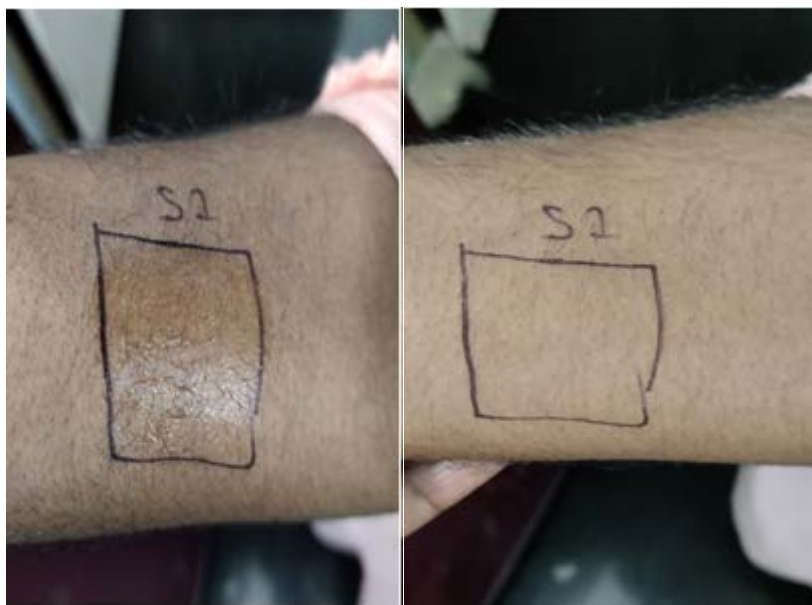
Figure-4: Antibacterial activity against acne causing bacteria (*Propionibacterium acnes*)



Figure-5: Antibacterial activity against acne causing bacteria (*Streptococcus* sp.)

Figure-6: Skin irritation tests

Subject 1:



during

After

Subject 2:



during

After

Subject 3:



during



after