Original Research Paper

Volume-10 | Issue-2 | February - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

SCREENING OF ALGAE FOR ITS ANTIBACTERIAL AND ANTIACNE POTENTIAL

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ABSTRACT Acne is chronic, inflammatory skin condition which is generally observed on face, shoulders, back, neck, chest, and upper arms. They appear in the form of whiteheads, blackheads, pimples, cysts, and nodules. People are more cautious about acnes as sometimes they develop skin scars. *Propionibacterium acnes* and *Staphylococcus* spp. is the main causative agent for the acne formation. There are many anti acne agents available in market. Salicylic acid, Triclosan and benzyl peroxide are the anti acne drugs are incorporated in to the soaps, cream and gel for treatment purpose. Naturally derived anti acne agent are the potential agents for treatment without side effects. Algae produce various metabolites of therapeutic potential. The objective of present study is to assess anti acne potential of various algae which were isolated from different ecological niche.

KEYWORDS : Algae, Anti Acne Agent, Propionibacterium Acnes

INTRODUCTION:

Acne is a chronic inflammatory disorder of the skin especially in adolescent stages. It is characterized by the plugged pores or the pilosebaceous units. Pilosebaceous units are made up of oil gland or sebaceous glands and hair follicles in the dermis layer. Sometimes the sebum combines with dead, sticky skin cells and bacteria. [1] *Propionibacterium acnes* and *Staphylococcus epidermidis* are the main causative agents of acnes. [9] These are normal inhabitants of skin. [9] Bacteria lives on the sebum and oil which is secreted by hair follicle and cause infection. Acne is caused by the overproduction of sebum, during puberty mainly due to high levels of the androgen hormone. [4] The inflammation of glands is also caused by stress, hereditary factors.

Acnes is common problem in teenagers. Moreover, acnes leaves scar or spots on skin hence people are more cautious. There is great demand for anti-acne drugs. Anti acne drugs, are the medicines that help clear up the pimples, blackheads, whiteheads, and more severe forms of lesions. Lotions, soaps, gels, face wash and creams containing substances like benzoyl peroxide, salicylic acid, glycolic acid and Triclosan are mainly used for acne treatment. These anti acne compounds limit the growth of bacterium through cleansing activity. Natural plant extracts like neem extract, tulsi extract, and tea tree oil possess anti acne property because of phytochemicals.

Algae produce various phytochemicals like tannins, polyphenols, flavonoids which have therapeutic potential and acts as protectants [6]. The present study reveals the potential of algae with respect to its antimicrobial efficacy in control of acne. [2][10] Antimicrobial efficacy was studied against Propionibacterium *acnes MTCC 1951* and opportunistic pathogens of skin like *Staphylococcus aureus ATCC 6538* and *Candida albicans ATCC 10231*. [1]

MATERIAL AND METHODS:

SAMPLE COLLECTION:

Soil and water samples were collected from various ecosystems like rivers, gardens, farms and extreme environment such as Lonar Lake, salt pans, tree barks from the state of Maharashtra. The samples were collected in sterile container and stored at 4^0C till further use.

ISOLATION AND ENRICHMENT

5 gm sample was enriched in 100 ml Bold Basal (BB) medium. Tree bark samples were thoroughly washed with sterile distilled water. 3-4 gm of bark pieces having algal growth were inoculated in 100 ml sterile BB medium neutral pH. Incubation was done at 25^0C $\pm$ 2^0Cfor 15 days for 12 hrs. photo period &12 hrs. dark. In case of Lonar isolates pH of medium was maintained to 11-11.5. For salt pan isolates BB medium with 1.5% salt was used. Repeated sub culturing was done for isolation of algal strains.

EXTRACTION:

20 mg dry algae was used for extraction using THF and methanol (2:8 proportion). The tube was incubated at 4^0C for 24 hrs. [10] The procedure was repeated till colorless pellet was obtained. The supernatants were combined and stored at 4^0C till further use. The extracts were dried and suspended in DMSO to get final concentration of 1 mg/ml for further testing. [5][8]

SCREENING OF ANTIMICROBIAL ACTIVITY OF ALGAE EXTRACTS USING AGAR CUP METHOD

The test extracts in DMSO were screened for their antimicrobial activity against 3 indicator organisms i.e. *Propionibacterium acnes* MTCC 1951, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231 by agar cup method. [10] 1ml of 24 hr. old test culture was seeded in Muller Hinton agar plates.100 µl of test sample was added in the well. Incubation was done at 32^0C for 24 hrs. In case of *P acnes*, the plates were incubated under anaerobic condition. Salicylic acid was used as standard.

CREAM PREPARATION:

The extracts with anti acne potential were formulated with a cream base. The cream was prepared using 10% Stearic acid, 6% Cetyl alcohol, 6.6% liquid paraffin, 5% Glycerin, 30% polyethylene glycol. 1% algal extract in DMSO [11].Oily Phase i.e. Stearic acid 10% Cetyl alcohol 6% Liquid paraffin 6.6% and aqueous phase Glycerin 5%, Propylene glycol 30% ,distilled water 20 ml were heated at 70^0C using homogenizer. With constant mixing remaining distilled water (to make the volume 100ml) was added. The mixture was cooled to 40^0C and then extract was added. Mixing was continued till the consistency was achieved.

STUDY OF ANTIMICROBIAL POTENTIAL ALGAE EXTRAC T IN CREAM FORMULATION

Same set of algae extracts was studied for its antimicrobial efficacy in cream formulation. Cream with 1% salicylic acid was used as standard. Cream being non miscible with water was diluted 1 in 10 times in DMSO. Cream without extract was used as control.

In this technique, the test sample disperses in agar forming concentration gradient. As the distance increases from well concentration decreases. If the organism is sensitive to the test sample, its growth is inhibited. Growth appears from the point where concentration is tolerable. Thus, inhibition of organism is indicated by clear zone around the well.

MINIMUM INHIBITORY CONCENTRATION OF ALGAL EXTRACTS USING TUBE DILUTION METHOD

The isolates having higher zone of inhibition for all the three test organisms were further screened for MIC. The extracts were diluted in 10% DMSO. Various dilutions of test extracts were prepared in nutrient broth for bacteria to get final concentration of 100 µl, 250 µl, 500 µl, 750 µl and 1000µl. For *Candida albicans*. Sabouraud's dextrose agar was used. 100 µl of 24 hour old test culture was added to 5 ml of diluted test samples. Salicylic acid was used as standard. Test

samples with *S. aureus* were incubated at 37^{0}C for 24 hrs. Test samples with *candida albicans* were incubated at 25 ^{0}C for 48 hrs. Incubation was done anaerobically at 37^{0}C for 24 hr for test samples with *P. acnes*. Minimum concentration of algae extract which inhibits growth of test organism was considered as MIC value.

TIME KILL STUDY:

The efficacy study of potential extracts in cream was performed by time kill study. [7] 10 gm of test sample of cream was inoculated with 50 µl of test organism. The aliquots were removed at intervals of 0 min, 5min, 10 min, 20 min and 30 min in neutraliser. The microbial count was determined by serial dilution method using Modified Leethen Agar for bacteria and Sabarauds dextrose agar for Candida sp. Cream with salicylic acid was used as standard. Incubation was done at 37^{0}C for 48 hrs for bacteria and 25^{0}C for C. albicans. Anaerobic conditions were maintained for P. acnes. Per cent reduction in initial microbial count with respect to time was determined till 30 min.

RESULTS:

SAMPLE COLLECTION:

Samples were collected from different ecosystems of Maharashtra. One sample from alkaline soda lake Lonar, Two samples from Fish breeding pond and three samples of salt pans were collected to study extreme environment. Tree barks are exposed to sunlight and desiccated environment which is extreme environment. Six samples were collected from barks of various trees. Five samples of soil from various fields, five samples from various rivers and 2 samples from lakes. Thus, out of 24 samples, 12 samples were collected from extreme environment and 12 from fresh environment.

ISOLATION OF ALGAL STRAINS:

Total 40 strains of algae were isolated from various ecosystems of Maharashtra region. (Figure a) Out of 40 isolates, 8 isolates were obtained from salt water environment. Eight algal strains were isolated from tree barks i.e. desiccated environment. One isolate was selected from alkaline soda lake of Lonar. 14 algal strains were isolated from soils of garden and various fields. 11 isolates from rivers of Krishna, Kadhava, Vajreshwari, Kālu and Manda and Sagareshwar Lake were selected for this study. Nature of algal isolates were either unicellular or filamentous strains. Details of algal isolates and sampling locations are as given in figure 1.

Figure 1. Details of algal isolates and sampling locations selected from study

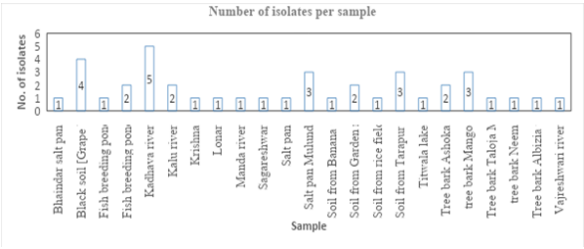

Table 1: Study of antimicrobial property of extracts by agar cup method.

	Zone of inhibition of algal extracts (mm)		
Algal Isolate	*S. aureus* ATCC 6538	*P. acnes* MTCC 1951	*C. albicans* ATCC 10231
Isolate 8	43.33 ± 0.58	43.00 ± 0.0	42.67 ± 0.29
Isolate 12	43.66 ± 1.15	42.67 ± 2.08	42.33 ± 1.15
Isolate 25	42.33 ± 0.58	40.0 ± 0.50	39.8 ± 0.80
Isolate 32	41.17 ± 0.29	41.00 ± 0.00	40.00 ± 0.5
Salicylic acid	41.7 ± 0.6	41.3 ± 0.8	41.3 ± 1.0

STUDY OF ANTIMICROBIAL EFFICACY TESTING OF ALGAE EXTRACTS USING AGAR CUP METHOD:

Experiment was performed in triplicates. Average zone of inhibition was measured in mm. Zone of inhibition was > 38 mm in case of Isolate 8, 12, 25 and 32 for all the three test organisms. In case of remaining 36 strain, zone of inhibition was intermediate between 10 mm to 43 mm for all the three test organisms.The four isolates 8, 12, 25 and 32 found to have higher potency than other strains. The efficacy of all the four extracts was comparable with salicylic acid (Table 1).

STUDY OF ANTIMICROBIAL EFFICACY TESTING OF ALGAE EXTRACTS IN CREAM FORMULATION USING AGAR CUP METHOD:

Same set of extracts were also studied for their antimicrobial activity in cream formulation. Cream without any extract found to have no inhibitory activity (0 mm zone of inhibition).

Table 2: Study of antimicrobial property of cream with algae extracts by agar cup method.

	zone of inhibition in cream 1 in 10 diluted (mm)		
Algal Isolate	*S. aureus* ATCC 6538	*P. acnes* MTCC 1951	*C. albicans* ATCC 10231
Isolate 8	34.5 ± 0.87	34.5 ± 0.87	34.33 ± 0.29
Isolate 12	33.50 ± 0.86	33.83 ± 0.58	33.67 ± 1.15
Isolate 25	33.0 ± 0.0	33.33 ± 0.58	33.67 ± 0.76
Isolate 32	32.67 ± 0.58	33.17 ± 0.29	33.33 ± 0.58
Salicylic acid	22.00 ± 0.00	21.17 ± 0.29	21.67 ± 0.29
Placebo Cream	0.00	0.00	0.00

Zone of inhibition was >30 mm for all test organisms in case of 4 algal isolates viz. isolate 8, 12, 25 and 32. Zone of inhibition was observed in the range of 12 mm to 30 mm for the remaining 36 strains. Zone of inhibition was 21-22 mm in case of cream with salicylic acid. The efficacy of isolates no. 8, 12, 25 and 32 was better in cream compared to salicylic acid. (Table II) These four strains were isolated from extreme environment like saltpan, tree bark and alkaline soda lake. They found to have better potency over isolates from fresh water ecosystem against all the test organisms.

Based on antimicrobial efficacy in cream for all the three organisms, the extracts of 4 strains i.e. ISOLATE 8, 25, 12, and 32 were further evaluated for minimum inhibitory concentration.

MINIMUM INHIBITORY CONCENTRATION

MIC value of Isolate 8 and Isolate 25 was found to be 500 µg/ml for all the three test organisms. In case of Isolate 12 and Isolate 32, MIC value was 500 µg/ml for *S. aureus* and *C. albicans* and 750 µg/ml for *P .acnes*.

MIC value of salicylic acid was found to be 250 µg/ml for all the three test organisms. MIC value of test extracts was found to be more than the salicylic acid in case of all 4 isolates. (Table III)

Table no 3: Results of Minimum inhibitory concentration of algae extract

Minimum inhibitory concentration of algal extracts (µg/ml)			
Algal Isolate	*S. aureus* ATCC 6538	*P. acnes* MTCC 1951	*C. albicans* ATCC 10231
Isolate 8	500	500	500
Isolate 12	500	750	500
Isolate 25	500	500	500
Isolate 32	500	750	500
Salicylic acid	250	250	250

TIME KILL STUDY:

The efficacy of algal extracts in DMSO in presence of cream was evaluated using time kill study. Results are presented in tabular manner for different test organisms *Staphylococcus aureus, Propionibac terium acnes* and *Candida albicans* [Table IV]

ISOLATE 32 from Lonar, found to inhibit S. *aureus* by 99.13%, *P. acnes* by 99.35% reduction and *C. albicans* by 99.22 in 30 min. In case ISOLATE 25, 99.02 % inhibition for S. aureus, 99.44% reduction for P. acnes and 99.56% reduction in *C. albicans* was observed. In case isolate from Mango tree bark viz. ISOLATE 8 reduction of 99.19% for S. aureus, 99.38% for of *P. acnes* and 99.21 % of *C. albicans* was observed. In case of salt pan isolate from Bhaindar i.e. ISOLATE 12, 99.34% for *S. aureus*, 99.25% for *P. acnes* and 99.09 % for *C. albicans* was observed. Thus, there was > 2log reduction in 30 min thus all the 4 strains have anti acne potential. ISOLATE 25 from Albizia tree bark was found to have the highest potential as compared to remaining isolates.

Table no 4: Results of time kill study of algae extracts in terms of percentage inhibition

Test Organism	Per cent Inhibition (%)				
	Isolate 32	Isolate 8	Isolate 25	Isolate 12	Salicylic Acid
S. aureus ATCC 6538	99.13	99.19	99.02	99.34	100.00
P. acnes MTCC 1951	99.35	99.38	99.44	99.25	100.00
C. albicans ATCC 10231	99.22	99.21	99.56	99.09	100.00

CONCLUSION:

Algae produce various secondary metabolites as protective measure which have therapeutic potential. 40 algal strains were isolated from these 24 samples collected from different ecosystems of Maharashtra. The 40 isolates were screened for their anti-acne potential against *Propionibacterium acnes, S. aureus and C. albicans*. The environmental conditions have major impact on metabolite production. The isolates from extreme environments were found to have more potential than fresh ecosystem isolates.

The anti-acne potential was further evaluated in cream formulation by agar cup method and time kill study. Four isolates, Isolate 32, Isolate 8, Isolate 25 and Isolate 12 found to have better efficacy as compared to other isolates. All 4 algal extracts have potential as anti-acne agent in cosmetic formulations. The Isolate from Albizia tree bark Isolate no 25 is the most potent strain for anti- acne application. Thus the current study has assessed algae with anti-acne potential which can be an important ingredient in cosmetic formulations.

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