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Antibacterial and Antifungal Activities of Green Alga *Cladophora Crispata*

KEYWORDS

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ABSTRACT *Algae contains antimicrobial substances, these substsances have inhibitory effects on pathogenic microbes. In present study extracts of green alga Cladophora crispata (Roth.) Kuetzing were prepared in different solvent and tested against bacterial & fungal strains. Antibacterial and antifungal activity of algal extracts were assayed by agar well diffusion method and by agar plug method respectively. Bacterial strains such as Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhi were selected. Fungal strains which were selected for antifungal assay were Metarhizium anisopliae , Curvularia lunata, Rhizoctonia solani, Penicillium oxalicum, Aspergillus niger, Trichoderma viridae and Fusarium oxysporum. Extracts prepared in acetone ,chloroform, petroleum ether and toluene shows notable antibacterial activity against selected bacterial strains. The zone of inhibition ranges from 4mm to 15mm. In antifungal assay algal extracts prepared in ethanol, Methanol, acetone and chloroform shows antifungal activity. In antifungal assay overall the zone of inhibition ranges from 4mm to 15mm. Cold water and hot water extracts also shows antifungal activity. In antifungal assay overall the zone of inhibition ranges from 4mm to19mm..Algal extract with toluene shows very high antimicrobial activities as compared with other.*

INTRODUCTION

Algae a diverse group of plant kingdom, contains different bioactive compounds . The bioactive substances produced by actively growing cells of algae includes proteins , fats, lipids, carbohydrates, vitamins, free amino acids, enzymes, growth regulators, pigments, toxins and antibiotics. Algae are admirable sources of antibiotics. There are several reports dealing with antimicrobial activities of solvent extracts attained from algae. Partt et. al.(1944) reported the occurrence of the growth inhibiting substance chlorellin in cultures of *Chlorella vulgaris* and *Chlorella pyrenoidosa.* Similarly Lefevre Marcel and Maud Nisbet (1948) observed presence of an extracellular growth inhibiting substance in liquid cultures of *Phormidium uncinatum* and *Scenedesmus quadricauda.* Species of *Spirogyra* are also reported to reduce the number of pathogenic bacteria. Spoeher et. al.(1949) obtained an extracellular antibacterial substance effective against *Staphylococcus aureus* from *Chlorella pyrenoidosa.* Gupta and Shrivastava (1963) studied antibiotic properties of fresh water algae by using different solvents.

Patterson (1991) isolated antifungal agent from blue green algae *Tolypothrix tjipanasensis.* Kulik (1995) reviewed potential of cyanobacteria and algae in biological control of phytopathogenic bacteria and fungi. Jaki et. al.(2001) studied antifungal activity of cyanobacterium *Tolypothrix byssoidea.* Kim (2006) screened blue green algae of paddy field for antifungal activity. Kulandaivel et. al, (2007) worked on antibacterial activity of *Spirulina platensis* and *Oscillatoria sp.* Kamble (2008) studied antimicrobial effect of some fresh water algae. Senthilkumar et. al. studied antimicrobial activities of extracts of *Phormidium fragile.* Hence in present study an attempt has been made to explore antibacterial and antifungal activities of fresh water green alga *Cladophora crispata* (Roth) Kuetzing.

MATERIALS AND METHODS

Collection of algal material and preparation of fine powder : The green alga *Cladophora crispata* (Roth) Kuetzing is a fresh water alga, found abundantly in Ujani reservoir of Pune district of Maharashtra. The alga was collected in large quantity from Malwadi and Kalthan backwater area of Ujani reservoir in April 2013 and identified. After identification, algal material washed carefully and thoroughly with fresh water to remove unwanted impurities, epiphytes, adhering sand particles and mud. On filter paper algal material shed dried at room temperature for 4 days. Shade drying of algal material is followed by oven drying at 40^0 C for 8 hours. After drying fine powder was prepared in grinder and stored in acid washed air tight bottles.

Preparation of algal extracts : Algal extracts in different solvents such as cold water, hot water, acetone, chloroform, petroleum ether, ethanol, methanol, toluene were prepared. 5 gm of algal powder was mixed and homogenized with 100ml of the respective solvents. The crude preparations were left overnight on shaker at room temperature and then centrifuged at 4000 rpm for 20 minutes.The respective supernatants were transferred separately to pre weighed beakers and extracts were concentrated by evaporating the solvent at 60^0 C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 20mg/5_ul.

Antibacterial assay: The effect of algal extracts on the selected strains were assayed by agar well diffusion method. In present study bacterial strains such as Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhi were selected. Sterile Muller Hinton agar plates were prepared and seeded with 24 hours old culture of bacterial strains. Wells were cut and 20_ul of algal extracts were added separately. The antimicrobials present in the algal extracts were allowed to diffuse in to the medium and interacts with freshly seeded test organisms. The plates were then incubated at 370 C for 24 hours and observed for clear zone of inhibition. The antibacterial activity was assayed by measuring the diameter of zone of inhibition in mm. Streptomycin was used as a positive control.

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Antifungal assay: The antifungal activity of algal extracts against various fungal strains were assayed by agar plug method . Fungal strains which were selected for study are Metarhizium anisopliae, Curvularia lunata, Rhizoctonia solani, Penicillium oxalicum, Aspergillus niger, Trichoderma viridae and Fusarium oxysporum. Sterilized Potato Dextrose Agar plates were prepared and a fungal plug of inoculum was placed in the centre of plate. Sterile discs were immersed separately in algal extracts and were placed in the plates. In agar plug method the fungicidal effect of the algal extracts can be assessed by the inhibition of mycelial growth of fungus. This is observed as a zone of inhibition in mm near the disc.

RESULTS AND DISCUSSION

Antibacterial assay : Extracts prepared in cold water, hot water, acetone, chloroform, petroleum ether, ethanol, methanol and toluene were tested against *Escherichia coli ,Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhi*

(Table 1). Cold water, hot water, ethanol and methanol extracts does not show any inhibitory effect against all bacteria. Acetone, chloroform, petroleum ether, and toluene shows notable antibacterial activity against selected bacterial strains. All algal extracts does not show any antibacterial activity against *Pseudomonas aeruginosa*.The zone of inhibition ranges from 4mm to 15mm. Acetone extract shows minimum inhibition zone against *Bacillus subtilis.* while extract in toluene shows maximum zone of inhibition against *Escherichia coli.* Petroleum ether extract shows antibacterial activity against *Escherichia coli* (10mm) and *Salmonella typhi* (8mm) All algal extracts does not show any antibacterial activity against *Pseudomonas aeruginosa.*

Jorgensen (1962) reported that, the antibacterial activity of fresh water algae was due to pigments. Gupta and Shrivastav (1963) studied antibacterial activity of fresh water algae and found effective against bacteria. Kulik (1995) studied potential of blue green algae against phytopathogenic bacteria. Kulandaivel et. al. observed antibacterial activity of *Spirulina platensis* and *Oscillatoria sp.* They found that diethylether extract was effective against bacteria. Kamble (2008) observed that extract of *Cladophora callicoma* inhibits the growth of *Salmonella typhi* and *Bacillus megaterium.* Patil et. al . (2011) found that acetone extract of *Cladophora sp.* Shows highest antibacterial activity against *Escherichia coli* .

Antifungal assay: Extracts of alga *Cladophora crispata* in different solvents were tested for antifungal activity against plant pathogenic fungi such as *Metarhizium anisopliae , Curvularia lunata, Rhizoctonia solani, Penicillium oxalicum, Aspergillus niger, Trichoderma viridae* and *Fusarium oxysporum* (Table 2). Petroleum ether extract did not shown antifungal activity. All algal extract does not show any antifungal activity against *Fusarium oxysporum.* Ethanol and methanol extracts shows antifungal activity only against *Aspergillus* niger. Chloroform extracts shows antifungal activity against *Aspergillus niger* (12mm) and *Trichoderma viridae* (6mm). Acetone extract shows activity against *Metarhizium anisopliae* (10mm), *Penicillium oxalicum* (4mm) and *Aspergillus niger* (9mm). Cold water and hot water extract also shows antifungal activity. Extract in cold water show activity against *Metarhizium anisopliae* (12mm), *Curvularia lunata* (8mm) and *Rhizoctonia solani* (6mm), whereas hot water extract shows antifungal activity against *Curvularia lunata* (11mm) and *Trichoderma viridae* (9mm). Among all extracts Toluene shows highest antifungal activity. It shows activity against *Metarhizium anisopliae* (18mm), *Curvularia lunata* (18mm), *Aspergillus niger* (19mm) and *Trichoderma viridae* (17mm). Overall the zone of inhibition ranges from 4mm to 19mm. Acetone extract shows minimum zone of inhibition against *Penicillium oxalicum* while extract in toluene shows maximum zone of inhibition against *Aspergillus niger.*

Kulkarni (1993) studied antifungal activity of algae against *Aspergillus flavus , Aspergillus niger* and *Alternaria brasica.* Kamble (2008) found that hot water extract of algae proved inhibitory for *Fusarium roseum, Alternaria alternata* and *Trichoderma harzianum.* It is evident from the results of present study that green alga *Cladophora crispata* possess antimicrobial properties.

CONCLUSION

Results of present study indicates that green alga *Cladophora crispata* contains antimicrobial compounds which prove inhibitory for bacterial and fungal growth. The effect of extracts varying with solvent type which is used for extraction purpose. Out of all, some algal extracts showing promising activity against pathogenic microorganisms. Specially extract with toluene shows very high antimicrobial activities as compared with other.

Table1 : Antibacterial activity of different extracts of green alga *Cladophora crispata* against various bacteria.

Bacterial strain	Cold water	Hot water	Acetone	Chloroform	Petroleum ether	Ethanol	Methanol	Toluene	Standard (streptomycin)
Escherichia coli	--	--	--	8 ± 0.1	10 ± 0.5	--	--	15 ± 1.2	15 ± 1.3
Bacillus subtilis	--	--	4 ± 0.1	--	--	--	--	--	12 ± 0.2
Pseudomonas aeruginosa	--	--	--	--	--	--	--	--	14 ± 0.4
Salmonella typhi	--	--	--	--	8 ± 0.1	--	--	--	11 ± 0.8

Zone of inhibition in mm

-- = No activity

Table2 : Antifungal activity of different extracts of green alga *Cladophora crispata* against various fungi .

Fungal strain	Cold water	Hot water	Acetone	Chloroform	Petroleum ether	Ethanol	Methanol	Toluene	Standard (streptomycin)
Metarhizium anisopliae	12 ± 1.2	--	10 ± 1.4	--	--	--	--	18 ± 1.5	18 ± 0.2
Curvularia lunata	8 ± 1.3	11 ± 1.6 --	--	--	--	--	--	18 ± 0.6	16 ± 0.1
Rhizoctonia solani	6 ± 1.1	--	--	--	--	--	--	--	0.8 ± 0.0
Penicillium oxalicum	--	--	4 ± 1.2	--	--	--	--	--	15 ± 1.1
Aspergillus niger	--	--	9 ± 0.6	12 ± 1.0	--	11 ± 1.1	8 ± 0. 5	19 ± 1.1	18 ± 1.2
Trichoderma viridae	--	9 ± 1.6	--	6 ± 1.5	--	--	--	17 ± 0.1	18 ± 0.3
Fusarium oxysporum	--	--	--	--	--	--	--	--	7 ± 0.6

Zone of inhibition in mm

-- = No activity

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REFERENCE

Gupta A. B. and Shrivastava G. C. (1963) On antibiotic properties of some fresh water algae Hydrobiologia 25: 285-288 | Jaki B., Zerba o., Heilmann J. Sticher o. (2001) Two novel cyclic peptides with antifungal activity from the Cyanobacterium Tolypothrix byssoidea J. Nat. prod. 64 : 154-158. | Jorgensen,E. G.(1962) Antibiotic substances from cells and culture solutions of unicellular algae with special reference to some chlorophyll derivatives. Physiol. Plant, 15: 530-545. | Kamble Suhas M. (2008) Studies on effect of bioactive compounds of algae on some fungi. Ph.D. thesis. Dr Babasaheb Ambedkar Marathwada University, Aurangabad (M. S.) | Kim J. D. (2006) Screening of cyanobacteria from Paddy soil for antifungal activity against plant pathogenic fungi Mycobiology 34: 138-142. | Kulandaivel S., R. Prakash, R. Anita and N. Arunnagendran (2007) Antibacterial activity of Spirulina platensis and Oscillatoria sp. Journal of Pure and applied microbiology 1(1) :127-129. | Kulik, M. M.(1995) The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Pathol. 101: 585-599. | Kulkarni, M. K. (1993) A study of biotoxins of algal origin . Ph. D. thesis. Dr. Babasahrb Ambedkar Marathwada University, Aurangabad (M. S.). | Lefevere Marcel and Maud Nisbet (1948) (C. R. Acad. Sci (paris) 226 (1), 107-109) Biol. Abst.30520, 1950. | Patil. K. J., Patil V. A., Mahajan S. R. and Mahajan R. T.(2011) Bio-activity of algae belonging to Bhusawal region, Maharashtra. Curr. Bot. 2 (1) :29-31. | Patterson G. M. L.(1991) Tjipanazoles, new antifungal agents from the blue green algae Tolypothrix tjipanasensis, Tetranendron 47 :7739-7750. | Pratt R.,Mauther H.,Gardner, G. M., Yi-Hsiensna and J.Dufrenoy (1944) Science 99: 351-352. | Senthilkumar N. S.,V. Sivsubramanian and S. Mukund (2009) Antimicrobial activities of extracts of Phormidium fragile Gomont. Journal of algal biomass Utilization.2229-2235 | Spoeher H., Smith H. C. and Strain H. H.(1949) Carnegie. Ins. Washington Publ. 586.