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STUDIES ON ANTIBACTERIAL POTENTIALS OF MICROALGAE *OSCILLATORIA LUTEA* AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Oscillatoria lutea is a blue green thylakoids coiled usually irregular throughout the cells. In the present study *O. lutea* was collected from fresh water lakes in and around Chennai, BG-11 culture media was used for cultivation under suitable lab conditions. Organic solvents used for extraction was cold ethanol, cold petroleum ether and cold ammonia solution. The present study aims to test its effectiveness against four strains of bacteria. Two gram (+) ve bacteria namely *E. coli*, *K. pneumoniae* and two gram (-)ve bacteria namely *S. aureus*, *S. pyogenes* by well diffusion method. Results showed the sensitivity in all bacteria. In cold ethanol extract, *K. pneumonia* and *E. coli* showed zone of inhibition with 17 and 20mm. In cold petroleum ether extract *K. pneumoniae*, *E. coli* showed zone of inhibition with rate of 15 and 24mm while in cold ammonia solution extract *S. aureus* showed inhibition of 19mm respectively.

KEY WORDS: Blue green, algal extracts, antimicrobial assay, well diffusion.

INTRODUCTION

Antibiotic resistance in bacteria and fungi is one of the major emerging health care related problems in the world; it became a greater problem of giving treatment against resistant pathogenic bacteria.[1] One approach to antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application.[2-3] Algae are organisms with rich source of structurally novel and biologically active secondary and primary metabolites which may be potential bioactive compounds of interest in the pharmaceutical industry.[4-5] Microalgae

and cyanobacteria offer numerous advantages for antimicrobial investigations because of their enormous biodiversity and fast growth rate.[6]

The cell extracts and active constituents of various algae shown to have antibacterial activity *in vitro* against Gram positive and Gram negative bacteria.[7-9] A wide range of *in vitro* antifungal activities have also been reported from extracts of green algae, diatoms and dinoflagellates.[10] and from *Nostoc* sp.[11,12] explored bioactive compounds of a group of microalgae which showed antibacterial effect against *Staphylococcus* sp.[13] tested antibacterial activity of *Scenedesmus* sp. isolated from a natural pond against three pathogenic bacteria with different solvents, the aqueous and methanol extracts gave better results.[12] explored microalgae for their bioactive compounds and affirmed promising applications encompassing antibacterial, antiviral, and antifungal activities; also he stated that the application of bioactive compounds derived from algae will prove beneficial much more effectiveness as compared with traditional treatment methods.

Antimicrobial activity depends on both algal species and the solvents used for their extraction.[14-15] The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always provide a higher efficiency in extracting compounds for antimicrobial activity.[16-17] Analytical methods play important roles in the discovery, development and manufacture of bioactive molecules.[18]

MATERIALS AND METHODS

Collection of Sample

Water samples containing cyanobacteria were collected from fresh water lake in and around Chennai. To study the antibacterial activity of *O.lutea* micro organisms namely *E. coli*, *K. peumoniae*, *S. aureus* and *S. pyogenes* were used.

Preparation of algal extract

Three different extracts (cold ethanol, cold petroleum ether and cold ammonia solution) were made from the above prepared powder samples. The dried powder of the algae was extracted by organic solvents and allow it to stand for three days. The process was continued until the colour is obtained. The extracts were filtered using whatmann filter paper no.1 and concentrated under reduced pressure using rotary evaporator and stored at 4°C until further use.

IN-VITRO ANTIBACTERIAL SCREENING

In vitro antibacterial activity of the cold ethanol, cold petroleum ether and cold ammonia solution extracts of *O.lutea* was screened against a total of the four bacterial strains.

Microbial strains and inoculum preparation

The microorganisms used in this study were human pathogens namely *Escherichia coli* , *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pyogenes.*

Bacterial strains stock cultures were maintained at 4°C on Nutrient Agar medium. Active cultures were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures at 37°C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were subcultured in fresh Nutrient broth at 37°C.

Well diffusion method

The well diffusion test (Bennet *et al.*,1966; Janssen *et al.*,1987; Magaldi *et al.*,2004) was performed using NA medium. The medium was prepared and autoclaved at 15 lbs pressure (121ºC) for 15 minutes immediately cooled in a 50-55 ºC water bath after removed from the autoclave. The cooled medium was poured into sterile petriplates to a uniform depth of 4 mm; this is equivalent to approximately 25 mL in a 90 mm plate. Once the medium was solidified, then the culture was inoculated on the medium. Within 15 minutes of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacterial suspension. The sterile swab was used to streak on the surface of the NA containing plates. The plates were allowed undisturbed for 3 to 5 minutes to absorb the excess moisture. Sterilized 9 mm cork borer was used to make agar wells, 25µg, 50µg, 75µg of extract from the stock solutions were placed into each wells. Positive control was made by adding ampicillin 30 µg which were suspended in 100% DMSO solvent. Zone of inhibition (ZI) were measured by 1mm accuracy scale prescribed method and the zone of inhibition percentage was calculated by the following formula.

Percentage of inhibition = I/diameter of the petriplate in mm×100.

RESULTS

Antibacterial activities of the crude extracts of *O. lutea* were tested against four pathogenic bacteria and they were compared with standard antibiotic ampicillin by measuring the zone of inhibition diameter and expressed in mm as shown in tables 1,2 and 3. The average zone of inhibition ranges from 15-24mm. Highest inhibitory activity was observed in cold petroleum

ether extract against the growth of *K. pneumoniae*, *E. coli* with the zone of inhibition of 15 and 28mm respectively. (Table -2). The concentration above 50µg/mL showed a moderate effect against all the four bacteria namely *E. coli*, *K. pneumonia*, *S.aureus* and *S. pyogenes*.

Table 1. Antibacterial activity of cold ethanol extracts of *O.lutea* against human pathogens.

Sample	Pathogens	Concentration of cold ethanol extracts (µg)					
		25		50		75	
		ZI	% I	ZI	% I	ZI	% I
O. lutea	*S. aureus*	13.33±0.54	14.81±1.26	15.33±2.01	17.03±1.23	18.33±2.22	20.36±1.3
	K.pneumoniae	14.77±0.86	16.41±1.03	16.33±2.12	18.14±1.21	17.7± 1.23	19.66±1.42
	S. pyogenes	10.12±0.12	11.24±1.08	13.67±1.44	15.18±1.26	15.55±1.08	17.27±1.34
	E. coli	19.19±0.63	21.32±1.08	18.17±0.99	20.18±1.24	20.56±1.34	22.84±1.26

Values are mean ± standard deviation of triplicates.

Table 2. Antibacterial activity of cold petroleum ether extracts of *O.lutea* against human pathogens.

Sample	Pathogens	Concentration of cold Petroleum ether extracts (µg)					
		25		50		75	
		ZI	% I	ZI	% I	ZI	% I
O. lutea	*S. aureus*	11.11±1.04	12.34±1.26	12.22±0.15	13.57±1.23	12.55±1.64	13.94±1.3
	K.pneumoniae	12.16±1.55	13.51±1.03	14.12±0.66	15.68±1.21	13.13± 0.44	14.58±1.42
	S. pyogenes	16.66±1.78	18.51±1.08	15.89±1.14	17.65±1.26	18.12±1.13	20.13±1.34
	E. coli	22.33±0.99	24.81±1.08	18.18±1.38	20.2±1.24	25.55±0.47	28.38±1.26

Values are mean ± standard deviation of triplicates.

Table 3. Antibacterial activity of cold ammonia solution extracts of *O.lutea* against human pathogens.

Sample	Pathogens	Concentration of cold ammonia extracts (µg)					
		25		50		75	
		ZI	% I	ZI	% I	ZI	% I
O. lutea	*S. aureus*	14.44±1.58	16.04±1.26	17.13±1.02	19.03±1.23	15.36±1.77	17.06±1.33
	K.pneumoniae	10.12±0.44	11.24±1.03	11.11±0.12	12.34±1.21	11.89± 0.22	13.21±1.42
	S. pyogenes	13.96±0.89	15.51±1.08	14.36±1.04	15.95±1.26	17.64±0.99	19.6±1.34
	E. coli	11.12±1.34	12.35±1.08	10.00±1.00	11.1±1.24	12.59±0.66	13.98±1.26

Values are mean ± standard deviation of triplicates.

DISCUSSION

In the present study the antibacterial activities of the crude extracts of *O.lutea* were tested against four pathogenic bacteria and they were compared with standard antibiotic ampicillin.

Highest inhibitory activity was observed in cold petroleum ether extract against the growth of *K.pneumoniae*, *E. coli* with the zone of inhibition 15 and 28mm respectively. (Table -2).

According to Prakash *et al.,* 2011 the antimicrobial activity of *O.sancta* of methanol and acetone extracts were tested against *S. aureus* which showed the clear zone of inhibition. According to vijayakumar *et al.,* 2011 the acetone extracts of *O.latevirens* showed highest antimicrobial activity against *S. aureus*, *S. mutans* and had moderate activity against *K.pneumoniae*. According to Ghaidaa H Abd *et al.,* 2015 the intracellular ethanolic extract of *O. tenuis* of *K.pneumoniae* and *Acinetobacter sp* showed better efficacy with inhibition zone of 36 and 30mm respectively.

Antibacterial studies helps us to find out whether the drug is effective against various microbes which are harmful causing diseases. For example *E.coli* causes bloody diarrhea, *S.aureus* causes food poisoning, *K.pneumoniae* causes surgical site infection and meningitis, *S.pyogenes* causes scarlet fever. The overall synergistic effect of drug on various microbes could lead a therapeutic activity of the drug.

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