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Antibacterial activity of freshwater green- algae, *Chara vulgaris*

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Abstract : The *Chara vulgaris* is a macro-algae found in fresh water bodies and it is rich with calcium carbonate deposits in the cell wall. This alga was studied for its antibacterial activity against chosen pathogenic bacteria. In the present experiment various solvents were used to find out the antibacterial activity against different pathogens. The highest antibacterial activity was found in the Acetone crude extract (15.33 ± 0.40 mm) against *Micrococcus luteus* when compared with other solvents under study. The present investigation under study clearly shows that the chosen member of green-alga, *Chara vulgaris* proved to be a potential for antibacterial properties.

Key words : *Chara vulgaris*, Organic solvents, Antibacterial activity, Bioactive compounds.

Introduction:

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. Algae have a significant attraction as a natural source of bioactive molecules with a broad range of biological activities. The first investigation on antibiotic activity of algae was carried out by Pratt [14]. Now a days algae have become very important constituents, for their bioactive compounds. Algal compounds are great interest as a source of safer or more effective substitutes than the synthetically produce antimicrobial agents.

Since algae have been used in traditional medicine for a long time and also some algae have bacteriostatic, bactericidal activity, they have been extensively studied by several researchers [8, 17, 10, 20]. Many authors had found antibacterial activities of microalgae due to fatty acids [3, 5, 21]. Recently many workers have published papers in various journals with reference to the algal members and their antimicrobial activities, the various workers like [16] on *Amphora* sp. and *Calothrix* sp.; [19] on *Synechococcus elongates*; [11] on certain members of cyanobacterial natural products; [4] on Cyanotoxins and their potential applications.

Components of micro-algal origin with antimicrobial, antiviral, anticoagulant, antienzymatic, antioxidant, antifungal, anti-inflammatory and anticancer activity among others were identified (15, 1, 6, 13, 18,2). The study of the extraction of bioactive compounds from various micro-algae, such as *Arthrospira* (*Spirulina*), *Botryococcus braunii*, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pulvialis* and *Nostoc* has been investigated (12, 9,7).

Material & Methods:

The macroscopic fresh water algae, *Chara vulgaris* belongs to Characeae family under the Class of Chlorophyceae, they normally grows in freshwater bodies with submerged manner or attached mud bottom and it was collected from Karimnagar district, Telangana State, India. The collected sample was thoroughly washed with sterilized water to remove all the extraneous matter such as epiphytes, sand particles and other plant debris, sample was brought to the laboratory in plastic bag then again sample was cleaned with the fresh and distilled water. Crushed the dried up algal material with the help of mortar and pestle, the fine grinded powder 500mg extracted with 15 ml of Acetone, Chloroform, Methanol, Petroleum Ether and Water, to get extract compounds soaked for 24 hours. The extracts was filtered and the filtrates were evaporated under reduced pressure at room temperature (37°C), dried crude extracts were weighed and dissolved in 2 ml of DMSO (Di Methyl Sulfoxide) and they were stored at 4°C for further studies.

Test organisms:

The extracts were tested on the three gram positive (*Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*), and two gram negative bacteria (*Klebsiella aerogenes*, *Salmonella typhi*), all the strains were obtained from Department of Microbiology, Kakatiya University, Warangal. T.S. The Muller Hinton Media (Hi-Media), ingredients were mixed with distilled water and then sterilized at 15 lb pressure for 45 minutes. The sterilized media were poured in to petri-dishes. Five bacterial strains *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Salmonella typhi*, and *Klebsiella aerogenes*, were tested by using agar well diffusion method. Bacterial strains were grown overnight in nutrient broth at 37°C. Muller Hinton Agar medium, were poured into Petri dishes were allowed to cool and solidify and then 100µl of bacterial suspension were spread on MHA plates with a sterile cotton swab. Made 5 wells in each plate with equal distance with the help of 6 mm borer in the plates. The wells filled with 50µl of the crude extracts with sterile pipette on Muller Hinton Agar Plates were incubated with different strains of bacteria at 37 °C for a period of 24 hrs. In the present study Gentamycin(10µg/ml) was used as standard control.

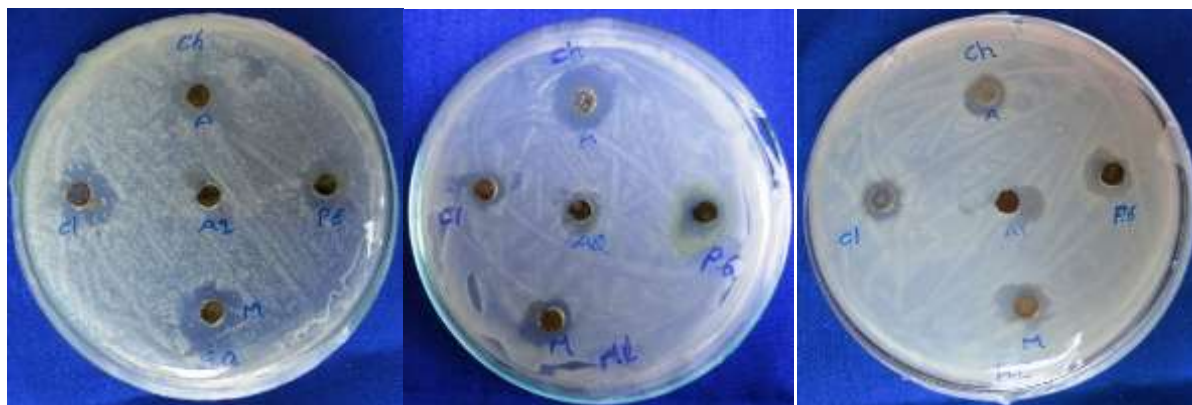
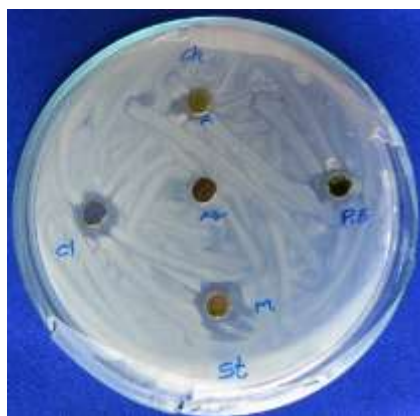
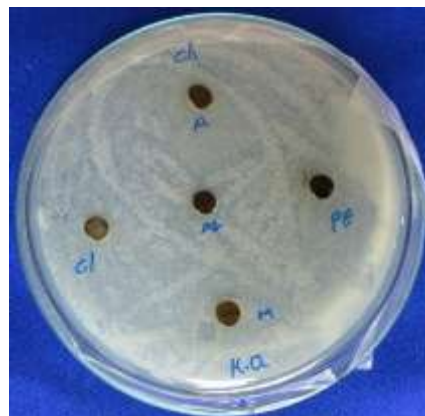
Results and Discussion:

The different solvents of *Chara vulgaris* was tested for the antibacterial activity against five strains of gram positive and gram negative pathogenic bacterial strains using agar well diffusion method. The results of antimicrobial activity against tested pathogenic bacteria were tabulated in the Table-1. The degree of activity was varied with reference to algal species, type of the solvent used and the tested bacterial strains under study. The present work gives a clear picture of high antibacterial activity in the extract of Acetone, the highest activity was recorded for the Acetone crude extract (15.33±0.40mm) against *Micrococcus luteus* and it was followed by 11.50±0.35mm against *Staphylococcus aureus*, 10.33±0.40mm against *Bacillus cereus*, 8.33±0.40mm against *Klebsiella aerogenes* and no zone of inhibition was observed against *Salmonella typhi* in Acetone extract, respectively. In the solvent of Methanol extract the maximum zone of inhibition was observed (13.33±0.40mm) against *S. aureus* and it was found 12.66±0.40mm against *S. typhi*, 11.50±0.35mm against *M. luteus*, 11.33±0.40 against *B. cereus* and 8.66±0.20mm against *K. aerogenes*. The Chloroform extract was exhibited with 12.66±0.20mm against *S. aureus*, followed by 11.66±0.40 against *S. typhi*, 9.66±0.20mm *K. aerogenes*, 9.50±0.35mm against *B. cereus* and 8.33±0.40mm against *M. luteus*. In the solvent extract of Petroleum Ether the inhibition zones were found quite significantly in all strains of pathogens (11.66±0.20mm against *K. aerogenes*, 10.66±0.20 against *S.aureus*, 10.33±0.40 against *M.luteus* 9.50±0.35 against *B.cereus* and 9.33±0.54 were against *S. typhi*). The water extract shows *only one zone of inhibition it was observed as 8.83±0.20 against M. luteus*. The remaining pathogens were not expressed any kind of zone of inhibitions. All the results in various extracts used to be found with less zone of inhibitions in the results when compared to the positive control (Zentamycin, 10µg/ml) Finally, it is concluded from this study that crude extracts of *Chara vulgaris* proved to be worthy by showing antimicrobial character in different solvents used against the different pathogens in the present investigation. It indicates that the Chlorophyceae members also have some medicinal properties against tested pathogens, in view of this in future the research is required further to identify the bioactive compounds and their purification and identification to know the chemical properties this alga.

Table-1 Antibacterial activity of *Chara vulgaris* extracts on various pathogens.

Test sample	Zone of inhibition diameter(mm)				
	<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	<i>Bacillus cereus</i>	<i>Salmonella typhi</i>	<i>Klebsiella aerogenes</i>
Acetone extract	11.50±0.35	15.33±0.40	10.33±0.40	-	8.33±0.40
Chloroform extract	12.66±0.20	8.33±0.40	9.50±0.35	11.66±0.40	9.66±0.20
Methanol extract	13.33±0.40	11.50±0.35	11.33±0.40	12.66±0.40	8.66±0.20
Petroleum ether extract	10.66±0.20	10.33±0.40	9.50±0.35	9.33±0.54	11.66±0.20
Water extract	-	8.83±0.20	-	-	-
Control (Gentamycin 10µg/ml)	25.33±0.20	28.00±0.35	24.16±0.40	26.16±0.40	24.16±0.75

Results including diameter of the well (mean ± standard error), (-) = not found.

**1. *Staphylococcus aureus*****2. *Micrococcus luteus*****3. *Bacillus cereus*****4. *Salmonella typhi*****5. *Klebsiella aerogenes***

Conclusion:

The present data indicates that the different solvent extracts of *Chara vulgaris* have strongest antibacterial properties and may be considered as alternative source for synthetic substances, these substances are potential source of bioactive compounds against various pathogens which can be used as natural non-toxic preservative and may be more acceptable to consumers. Further work is needed to identify the active compounds of this alga.

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