Phytochemistry And Antibacterial Activity Of Chlorosarcinopsis Species

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Abstract: The concept of biological control for health maintenance has received widespread attention during the last few years. The present work was focused on identifying the active substances that could be used as antibacterial agents. To achieve this target, five different extracts (petroleum ether, chloroform, acetone, methanol and ethanol) of Chlorosarcinopsis sp were examined. The extracts were tested in vitro for their antibacterial effects against the bacterial sp namely Escherichia coli, Klebsiella sp, Pseudomonas sp, Salmonella, Staphylococcus aureus, Proteus sp, Methicillin-Resistant Staphylococcus aureus, Serratia sp and Bacillus sp. using paper disc diffusion method. The methanol extract were observed to inhibit the growth of all the bacteria tested. A preliminary phytochemical test revealed the presence of Alkaloids, Anthraquinones, Cardiac glycosides, Flavonoids, reducing sugars, Saponins and Terpenoids.. The present study concluded that the active metabolites present in methanol extract of the microalgae were associated with their antibacterial property.

Index Terms: Antibacterial Activity, Chlorosarcinopsis sp, Phytochemical Analysis

1 INTRODUCTION

Microalgae have a significant attraction as natural source of bioactive molecules [8], [17], [21]. Secondary or primary metabolites produced by these organisms are potentially bioactive compounds of interests in the pharmaceutical industry [15], [16], [39]. Algae produce a number of secondary metabolites as a chemical defence against predation, herbivores and competition for space [13]. The first investigation on antibiotic activity of algae Chlorella sp was carried out by [29]. Since then microalgae have been used in traditional medicine for a long time and as some algae also proved to have Bacteriostatic, Bactericidal, Antifungal, Antiviral and Antitumor activity [20]. The present study is aimed at investigations of the Phytochemicals and Antibacterial properties of the Petroleum ether, Chloroform, Acetone, Ethanolic and Methanolic extracts of fresh water green micro algae, *Chlorosarcinopsis* sp. against eight bacterial isolates in order to validate it as an antimicrobial remedy. This study will also hopefully expose new frontiers on the current applications of the algal extract.

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2. MATERIALS AND METHODS:

2. 1. Preparation of Sample Extracts

The microalgae species *Chlorosarcinopsis* sp was collected from Kovai Kutralam, the water fall originating on the Siruvani hill ranges. The algal sample was cleaned and necrotic parts were removed. Then the sample was rinsed with sterile water to remove any associated debris. The pure culture of the sample was incubated in Bold's Basal Medium at 18°C [6], [7]. Pure cultures prior to the stationary phase of growth (5-6 days) were harvested and collected by centrifuging at 10,000 rpm for 3 min. The Collected micro algal pellets were dried under shade and made into a coarse powder with mechanical grinder for further use. The algal dried powders (20gm) were successively extracted using serial Exhaustive Extraction Method **[12]** with Petroleum ether, Chloroform, Acetone, Ethanol and Methanol solvents. The dry powders of each extract were resuspended in the respective organic solvents at a concentration of 100 mg/ml for further phytochemical screening and antimicrobial activity.

2.2. Antimicrobial Activity:

2.2.1. Microorganisms and Growth Conditions

Nine bacterial species were employed as test organisms which included Gram positive bacteria such as *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus,* and *Bacillus sp.* and Gram negative bacteria such as *Escherichia coli, Klebsiella sp, Pseudomonas sp, Salmonella, Proteus sp* and *Serratia sp.* The bacterial cultures were maintained in nutrient Agar medium. Inoculums were prepared by adding an overnight culture of the organism in Nutrient broth.

2.2.2. Antimicrobial Susceptibility Test

The Disc diffusion method [3] was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Nutrient Agar plates inoculated with 0.1% of test bacterial samples. The disc loaded with extracts (10 mg / 100 µl) was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes. The plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre.

These studies were performed in triplicate. AM 1000 was used as standard antibiotic drug.

2.3. Preliminary Phytochemical Screening

Phytochemical analysis of the extract was carried out using chemical method and tested for the presence of several phytochemicals like Alkaloids, Anthraquinone, Cardiac glycosides, flavonoids, Reducing sugars, Saponins, Tannins and Terpenoids.

2.3.1. Test for Alkaloids

Solvent free extract, 50mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with Mayer's reagent. To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicated the test as positive [18].

2.3.2. Test for Anthraquinones (Borntrager's Test):

About 0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet colour in the Ammoniacal layer (lower layer) indicates the presence of Anthraquinone [38].

2.3.3. Test for Cardiac Glycosides (Keller-Killiani Test)

About 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 mL of concentrated Sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of Cardenolides [38].

2.3.4. Test for Flavonoids

Three methods were used to test for Flavonoids. In the first method, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract followed by concentrated sulphuric acid (1 ml). The disappearance of yellow colouration on standing indicates the presence of Flavonoids. Secondly, a few drops of 1% aluminium solution were added to a portion of the filtrate. Formation of yellow colour indicates the presence of flavonoids. In the third method, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was then filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Appearance of yellow colouration indicates the presence of flavonoids [18], [35].

2.3.5. Test For Reducing Sugars (Fehling's Test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The mixture was shaken and heated in a water bath for 10 min. A brick-red precipitate indicates a reducing sugar [35].

2.3.6. Test for Saponins

To 0.5 g of extract 5 ml of distilled water was added and shaken vigorously. The solution was then observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion [18].

2.3.7. Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration [35].

2.3.8. Test for Terpenoids (Salkowski Test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of Terpenoids [18].

3. RESULTS

The Petroleum ether, Chloroform, Acetone, Ethanol and Methanol extracts of *Chlorosarcinopsis* sp tested for antibacterial activity against nine human bacterial pathogens *Bacillus sp., Escherichia coli, Klebsiella,* Methicillin – Resistant *Staphylococcus aureus, Proteus sp, Pseudomonas sp, Salmonella sp* and *Serratia sp* were presented in the Table 1 . The degree of activity was varied with reference to different solvent extracts of the algae. From the Table, the Methanol extracts was found to the show the maximum zone of inhibition against *Escherichia coli, Klebsiella sp* and *Salmonella sp* followed by *Proteus sp, Bacillus sp, Pseudomonas sp, Serratia sp* and Methicilin –resistant *Staphylococcus aureus*. It also revealed that the inhibition of Methanol extract was comparatively similar to that of the standard AM1000. The Ethanol extract showed the maximum zone of inhibition against *Proteus sp* followed by *Serratia sp*, *Bacillus sp*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Salmonella sp* and *Proteus sp*,. The Acetone extract showed the maximum zone of inhibition against *Serratia sp* followed by *Salmonella sp*, *Proteus sp*, *Bacillus sp*, *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* and *Escherichia coli*. The Petroleum ether extract showed the maximum zone of inhibition against *Klebsiella sp* followed by *Serratia sp*, *Proteus sp*, Methicillin resistant *Staphylococcus aureus* and then *Pseudomonas sp*. The Chloroform extract was found to be ineffective against the organisms used except Methicillin-resistant *Staphylococcus aureus*. From the above result it was also observed that the low polar extracts showed less inhibition compared to that of the high polar extracts. Phytochemical screening of *Chlorosarcinopsis sp* extracts showed the presence of most important Phytoconstituents. The medicinal value of the title algae can be correlated due to the presence of various bioactive chemical constituents (Table 2). The petroleum ether extracts confirms the presence of Flavonoids and negative to rest of the phytoconstituents. The chloroform extract gave positive results for reducing sugars and negative to the rest of the Phytoconstituents. Acetone extract showed the presence of alkaloid and Terpenoids and negative to the rest. Methanol extract of *Chlorosarcinopsis* sp gave positive result to Alkaloids, Cardiac glycosides, Saponins and Terpenoids and negative to the rest. From the above test the methanol extract was found to possess the most important phytoconstituents. Similarly the ethanol extract showed the presence of Alkaloid, Anthraquinone, Saponins and Terpenoid and negative to the rest of the phytoconstituents.

4. DISCUSSION

Literature reveals that antibacterial activity of green algae has been verified on various bacterial strains. In the present work *Chlorosarcinopsis sp*, a chlorophycean unicellular algae, when extracted with different solvents exhibited various range of activity against all bacterial strains tested. The results of the present study agrees with a few earlier reports that the methanol extracts of Chlorophycean members *Desmococcus olivaceous*, *Chlorococcum humicola, Chlorella vulgaris, Scenedesmus* and *Dunaliella* proved to be comparatively more effective in exhibiting antibacterial activity against *Klebsiella pneumonia*, *Pseudomonas*, *Escherichia coli*, methicillin resistant *Staphylococcus aureus* and *Staphylococcus aureus* compared to acetone and ethanol extracts[4], [36], [40], But in a different study, chloroform extract of the Chlorophycean member *Halimeda* sp exhibited the greatest Antibacterial activity against both gram positive and gram negative bacteria compared to the benzene and methanol extract [33]. Chloroform extracts of *Chlorosarcinopsis* sp in this experiment proved to have antibacterial activity only against methicillin resistant *Staphylococcus aureus.* Preliminary phytochemical screening of various organic extracts revealed the presence of phytoconsituents including Alkaloids, Anthraquinones, Cardiac glycosides, flavonoids, Reducing sugars, Saponins and Terpenoids. The methanol extract was found to be the most prominent extract in extracting the major phytoconstituents of the species compared to other organic extracts. The present study was in agreement with the few earlier reports on Chlorophycean members - *Desmococcus olivaceous, chlorococcum humicola* and *Chlorella vulgaris* in showing the presence of alkaloids, cardiac glycoside, terpenoids and saponins [5] , [40]. These phytoconstituents were also earlier reported for their antimicrobial activity [2], [11], [22]. In this context the active metabolites present in methanol extract of the microalgae may be associated with their antibacterial property.

5. CONCLUSION:

The Chlorophycean member, *Chlorosarcinopsis* sp was observed to exhibit a high antibiotic activity against the human pathogens tested. Among the tested extracts the methanolic extract showed as a promising and potential solvent for the extraction of antimicrobial compounds. In this context phycochemical screening of methanol extract of the *Chlorosarcinopsis sp* showed the presence of Alkaloid, Anthraquinone, Saponins and Terpenoid which may be attributed to its antibacterial activity. Further analysis of the active compound from the alga might lead to a potent therapeutic agent.

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Table 1

Antibacterial Activity of *Chlorosarcinopsis Sp* Extracts of Different Solvents.

Micro-organisms	Zone of inhibition in mm					
	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol extract	Ethanol extract	AM 1000
Bacillus sp.	0.0	0.0	7.0	7.7	6.9	8.0
Escherichia coli	0.0	0.0	6.8	8.7	0.0	8.0
Klebsiella sp.	8.5	0.0	0.0	8.7	0.0	8.0
Methicillin-resistant *Staphylococcus aureus.*	7.5	7.3	6.9	7.2	6.8	7.8
Proteus sp.	7.6	0.0	7.2	7.8	7.2	8.0
Pseudomonas sp.	7.3	0.0	0.0	7.4	6.7	7.6
Salmonella sp.	0.0	0.0	7.3	8.7	6.8	8.0
Serratia sp.	7.8	0.0	7.7	7.3	7.0	7.7
Staphylococcus aureus	0.0	0.0	6.0	5.8	7.8	8.0

Table 2

Phytochemical Screening of *Chlorosarcinopsis Sp* Extracts of Different Solvents.

Chemical components	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol extract	Ethanol extract
Alkaloids	-	-	+	+	+
Anthraquinones	-	-	-	-	+
Cardiac glycosides	-	-	-	+	-
Flavonoids	+	-	-	-	-
Reducing sugars	-	+	-	-	-
Saponins	-	-	-	+	+
Tannins	-	-	-	-	-
Terpenoids	-	-	+	+	+

\+ Presence
\- Absence

Fig 1

Antimicrobial Activity of *Chlorosarcinopsis Sp* Extracts of Different Solvents Against Human Pathogen by Disc Diffusion Method.