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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MARINE MICROALGAE *TETRASELMIS* SP.

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ABSTRACT

The aim of the present study was to screen the bioactive chemicals present in Tetraselmis sp. *extracted with various solvents such as Methanol, Hexane, Diethyl ether. Antimicrobial potential of Tetraselmis* sp. *extracts was determined by Agar disc diffusion method against human pathogens of both Gram positive (E.coli, Proteus vulgaris) and Gram negative bacteria (Pseudomonas aeruginosa, Bacillus subtilis, Staphlococcus aureus) and three fungal pathogens such as Aspergillus fumigatus, Aspergillus niger, Candida albicans, were taken and studied against the standard streptomycin and chloramphenicol. All the extractions were subjected to evaluated for the presence of the active chemical compounds and alkaloids, tannins, saponins, flavonoids, and steroids are the major phytochemicals present in the extractions. Maximum inhibitory effect was noticed in Methanolic extracts against E.coli (16mm)and S. aureus (15mm) whereas fungal pathogens Aspergillus niger (7mm). Negative effect was observed against Proteus vulgaris, Candida albicans and Aspergillus fumigatus in all the extracts of Tetraselmis* sp. *The antibacterial effectiveness might be due to presence of the active compounds present in the Methanolic extract and having promising pharmaceutical applications.*

KEY WORDS

Tetraselmis sp*, Phytochemical, Antibacterial, Antifungal*

1. INTRODUCTION

Microalgae are natural resource with various biologically and pharmacologically compounds with structurally composite molecules. Marine microalgae constitute attractive sources of novel and active metabolites, comprising proteins, enzymes, pigments and polyunsaturated fatty acids (PUFA) that could be exploited in pharmaceutical, food, feed and cosmetic industries (Mendes *et al*., 2003; Cardozo *et al*., 2007; Surendhiran *et al*., 2014). Compounds with pharmaceutical importance, as anti-oxidative, anti-inflammatory, anti-microbial and antitumor properties have been identified (*Guedes et al*., 2011; *Kwak et al*., 2014). Based on its various bioactive compounds, *Tetraselmis* sp. have great potential as a source of functional foods. They are potential photoautotroph microorganisms that can be able to produce the secondary metabolites.

Secondary metabolites produced by microalgae is allelopathic compounds, that is able to inhibit the growth of both competitor and predator microorganisms. One species of microalgae that have secondary metabolites with such capabilities is *Tetraselmis* sp. (Makridis, *et al*. 2006). There is evidence from laboratory studies that phytochemicals in marine algae may reduce the risk of cancer, possibly due to dietary fibers, polyphone antioxidants and anti-inflammatory effects. The important molecules which have good antimicrobial activities are fatty acids such as saturated, unsaturated and monounsaturated acrylic

acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols (Kannan *et.al.* 2010).

Hence, our present study was focused on the growth rate of *Tetraselmis* sp. also biomass calculation, evaluating phytochemicals from various solvent extracts and determining their antibacterial potential of the extracts.

2.MATERIALS AND METHODS

Isolation and Culture

Tetraselmis sp. was collected from Vellar Estuary, Parangipettai, southeast coast of India. Microscopically identified Morphological characters (Tomas, 1997) and cultured the algal strain in F/2 medium under light intensity of 12h photoperiod and at 22°C. The wet biomass was obtained by centrifugation was done at 5000r/min for 6min. The obtained biomass were air dried and then pulverized with the help of blender.

Preparation of algal crude extract

The obtained biomass of *Tetraselmis* sp. powdered 5g were extracted with various solvent systems varying in their polarity such as Methanol, Diethyl ether and Hexane were taken for extraction. The sample were soaked in the solvent system for 24 hrs and every 2hrs it was stirred, after 24 hrs it was filtered and solvent system allowed to evaporate. The dried sample were refrigerated for further study.

Preliminary phytochemical analysis such as phenol, tannins, saponin, flavonoids, alkaloids, sterols, amino acids, protein and carbohydrate (Trease and Evans,1989 and Sofowora,1982.)

Test microorganisms

The cultures such as *E.coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphlococcus aureus, and Bacillus subtilis* and Three fungal pathogens such as *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*, were collected from Microbial Culture Maintenances Laboratory, Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamil Nadu, India.

Disc diffusion method

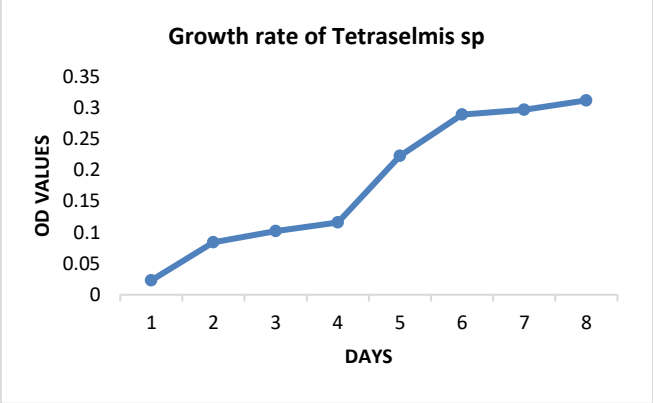
The antimicrobial activity was determined by disc diffusion method. The test pathogens of 1ml concentration were uniformly spread on the Muller-Hinton agar and Potato dextrose agar plates. The organic extracts were diluted with their respective concentration of 10mg/ml consider as stock. Sterile disc with 5mm diameter was loaded with (25 µg/disc) of the organic extracts. Streptomycin (25µg/disc) and Chloramphenicol (25 µg/disc) was used as the standard. The plates were incubated for 24 hrs at 37 °C. The inhibition zone was measured and compared against the standard.

3. RESULT

Growth rate

The growth rate of optical density (OD) measured throughout the 8 days of culture at 560 nm. The growth rate of *Tetraselmis* sp. in the F/2 medium at the lab condition is shown in **(Fig.1).** The growth rate in *Tetraselmis* sp. was found to be at range between 0.312 during the 8th day.

Fig.1. Growth rate of *Tetraselmis* sp.



Biomass yield of *Tetraselmis* sp.

The biomass of *Tetraselmis* sp. is obtained from 5Litre F/2 (growth) medium. The well grown culture was filtered by using Whatman filter 0.2 *µm.* The harvesting, thickening and dewatering of marine culture are used for further study. The value of biomass is given in the Table 1.

Table 1. Biomass yield

S.NO	Marine algae	Wet biomass (5 L/g)	Dry biomass (5 L/g)
1.	*Tetraselmis* sp.	10. 57	4.934

Preliminary phytochemical Analysis

Crude organic extracts of Methanol, Diethyl ether, Hexane of *Tetraselmis* sp. Alkaloids, flavonoids and steroids are present in good concentrations. Sugar, amino acid and tannins are also identified. Methanol and hexane extracts showed the presence of larger phytoconstituents followed by Diethyl ether. Presence of Lipids was confirmed in all the extracts.

Antimicrobial activity of solvent extracts of *Tetraselmis* sp.

The antimicrobial potential of the organic extracts of *Tetraselmis* sp. was determined against the human pathogens. The solvent extracts showed remarkable inhibitory effect against both gram positive and gram-negative bacteria. Poor activity was recorded in fungal strains. The maximum zone of inhibition was found against *Aspergillus niger* (7mm). *Candida albicans* and *Aspergillus fumigatus* are resistant to the extracts. Hexane extract showed maximum activity against Gram - positive strains *S. aureus* (15mm), *pseudomonas* (10 mm), *Bacillus subtilis* (11 mm). Gram negative bacteria such as *E. coli* (16mm) showed higher inhibitory zone in Methanolic extract and significant inhibition are also observed in all other extracts. The zone diameter was similar with that of control antibiotic streptomycin. No activity was observed in *Proteus vulgaris* in all the extracts.

Table –2 Phytochemical screening of marine microalgae (*Tetraselmis* sp).

No. of Test	*Tetraselmis* sp.		
	Methanol	Diethyl ether	Hexane
Phenol	+	-	+
Tannins	+	-	+
Alkaloids	+	+	+
Sugar	+	-	-
Aminoacids	-	-	+
Steroids	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+

(+) indicates present (-) indicates absent

Antimicrobial activities of various extracts of *Tetraselmis* sp. and zone of inhibition against different human bacterial and fungal pathogens are depicted in Table 3.

Extraction	Zone of inhibition (mm in diameter)							
	E.coli	***Bacillus subtilis***	***Pseudaomonas aeruginosa***	***Staphylococcus aureus***	***Proteus vulgaris***	***Aspergillus fumigatus***	***Aspergillus niger***	***Candida albicans***
Methanol	16 mm	8 mm	9 mm	15 mm	6 mm	6 mm	7 mm	6 mm
Ethyl acetate	9 mm	6 mm	10 mm	7 mm	8 mm	4 mm	6 mm	8 mm
Chloroform	12 mm	9 mm	11 mm	13 mm	10 mm	9 mm	11 mm	7 mm
Hexane	10 mm	11mm	10 mm	15 mm	9 mm	--	--	5 mm
Acetone	7 mm	6 mm	7 mm	11 mm	5 mm	--	--	7 mm

Table 4. Anti-fungal activity of marine microalgae (*Tetraselmis* sp.)

Extraction preparation using solvents	Zone of inhibition (mm in diameter)				
	Aspergillus fumigatus	***Aspergillus niger***	***Candida albicans***	***Rhodotorula* sp**	***Cryptococcus neoformans***
Methanol	7 mm	5 mm	6 mm	8 mm	--
Ethyl acetate	4 mm	6 mm	8 mm	--	--
Chloroform	9 mm	11 mm	7 mm	8 mm	5mm
Hexane	--	--	5 mm	6 mm	--
Acetone	--	--	7 mm	7 mm	--

4. DISCUSSION

Microalgae are high sustenance including all primary and secondary metabolites, polyunsaturated fatty acids (PUFAs), vitamins, minerals and non-caloric dietary fibers. These beneficial were transferred to animals through the food cycle (Kumar and Singh, 1976). Microalgae are in practice as live feed for bivalve larvae and spat in aquaculture industries stated by Knauer and Southgate, 1999) and have wide applications in energy Production (Chisti, 2007; Schenk et al. 2008; Hu et al. 2008), reported as healthy food by Natrah et al. 2007; Plaza et al. 2008, as animal feed and also impending as fertilizers (Spolaore et al. 2006), blooming nutraceuticals and pharmaceuticals (Wanasundara and Shahidi, 1998; Horrocks &Yeo, 1999) and bioplastics industries (Murphy, 2004).

Growth rate

Algae as a producer of numerous primary and secondary metabolites with potential activities which act as chemical defense and gain more attention towards pharmaceutical industries. According to our present findings, growth rate and biomass calculation of *Tetraselmis* sp. the growth rate was found to be higher in the 8thday (0.312 Day^{-1}). The biomass was obtained in *Tetraselmis* sp. (4.934 g/5L) after the final day harvesting of biomass.

Preliminary phytochemical

The present study is to determine the existence of phytoconstituents present in *Tetraselmis* sp. and also, to assess the antimicrobial potential of various organic extracts of *Tetraselmis* sp. against human pathogenic strains. In our study, Preliminary phytochemical screening of the extracts *Tetraselmis* sp. showed the presence of alkaloids, saponins, flavanoids, phenols, proteins, carotenoids, sugars, fatty acids, carbohydrates, and glycosides in most of the organic solvent extracts, hexane and Methanol extracts showed the presence of a larger group of molecules followed by diethyl ether extracts. The presence of carbohydrates, amino acids and lipids was confirmed in almost all the extracts, In agreement with our findings, Rajendran *et al*. (2014) reported the maximum content of flavonoids in the secondary metabolites from *Tetraselmis* sp. and *Oscillatoria* extracted on four types of solvents *viz*, acetone, ethanol, methanol and chloroform respectively. Similarly, Adhoni *et al*. (2016) screening the phytochemical profile of *C. vulgaris* with various polar and non-polar solvent extracts and reported 14 phytochemical constituents and the high concentration was obtained in polar solvents extracts. Diethyl ether extract showed less significance in the preliminary phytochemical analysis as compared to other solvent extracts. Similar qualitative phytochemical results were obtained (Pratt et al.,1944, Prashanth kumar et al., 2006, Singh et al., 2010, Uma et al., 2011; Bhagavathy et al., 2011).

Antimicrobial Assay

According to Martinez Nadal 1963, the organic solvents benzene and diethyl ether were the most suitable solvents for extracting antibiotic. Hornsey and Hide 1976 experienced in extracting antimicrobial compounds acetone solvent was suitable from British Marine algae. In the present study, organic extracts of *Tetraselmis* sp. showed significant effect against the bacterial strains as compared to that of the fungal pathogens *A.niger, A.fumigatus* and *C.albicans.* Gram negative bacteria *Proteus vulgaris* and fungal strains *A.fumigatus* and *C.albicans* are resistant to the organic extracts of *Tetraselmis* sp. was observed.

In the present investigation, antibacterial assay of a *Tetraselmis* sp. organic extracts showed moderate anti-bacterial activity against five pathogens *viz, E. coli, B. subtilis, Pseudomonas aeruginosa, S. aureus* and *P. vulgaris.* Maximum inhibition was observed in Methanolic extracts against the pathogen *E. coli* (16mm) followed by *Staphylococcus aureus* (15mm) in hexane

extract. *E.coi* an intestinal Pathogenic Gram negative bacteria causes diarrhea, fever, intestinal infection, Urinary tract infection, meningitis, pneumonia and S. *aureu, B. subtilis, Pseudomonas* are the Gram positive bacteria which are the causative agents for the ailments, such as paryngitis, sinusitis, bacteraemia, osteomyelitis, endocarditis *etc.,* The organic extracts of Hexane, Methanol, Diethyl ether showed a promising antibacterial effect against the pathogens, hence these can be used for the treatment of the above-mentioned infections caused by the pathogens of human and animal origin. The phytochemicals present in the extracts might be responsible for the antibacterial activity of the extracts of *Tetraselmis* sp.

From the overall results *Tetraselmis* sp. showed inhibitory activity against both the Gram positive and Gram-negative bacteria. The maximum inhibition was closely related to the standard streptomycin which served as control.

5. CONCLUSION

Our study reveals that, emerging trends to replace synthetic drugs or chemical by organic sources from marine microalgae. The drug from *Tetraselmis* sp. can serve as broad spectrum antibiotic to replace synthetic or chemical drugs. The importance phytochemicals and anti-microbials have emerged the researchers to identify the active compound responsible. The study concluded that the *Tetraselmis* sp. play an important role in solving the problem between the production of food, Pharmaceutical industry in the near future.

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