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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS FROM SELECTED ALGAL SPECIES

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Abstract

Antioxidant and antimicrobial potential of oil extracts of four different algal species were evaluated. Microwave assisted extraction method was used for oil extraction and DPPH scavenging assay was used to determine the antioxidant activity. Higher antioxidant activities were observed in methanolic extract in the order *Oedogonium* sp. > *Stigeoclonium* sp. > *Ulothrix* sp. > *Nitzschia* sp. The IC_{50} values of *Oedogonium* sp., *Stigeoclonium* sp. and *Ulothrix* sp. were 19.47, 20.88 and 22.92 µg/ml, respectively. However for the *Nitzschia* sp. it was observed that ethanolic extracts had higher antioxidant potential as compared to methanolic extract. Antibacterial activity of all the ethanolic extracts was more against Gram-negative bacteria as compared to methanolic extracts on the concentrations used in the present study. Of the all species tested for antimicrobial activities, the maximum zone of inhibition was shown by ethanolic extract of *Ulothrix* sp. against *Staphylococcus aureus* at the concentration of 50 µg/ml.

Introduction

Algae are autotrophic and heterogeneous group of organisms. They may be unicellular as well as multicellular organisms in nature. Recently, algae have been used in several ways by humans, namely as soil conditioners, as fertilizers and as livestock feed. For biofuel production algae have evolved as potentially active next-generation feedstocks (Kilian *et al.* 2011). Agar a gelatinous material, is obtained from red algae and has numerous commercial applications. It also serves as a good medium for bacterial growth (Lewis *et al.* 1988). Aquatic organisms are considered as rich sources of structurally and biologically active metabolic compounds (Ely *et al.* 2004). During last decades, several unique compounds had been isolated from aquatic organisms and many of these compounds had been demonstrated to retain interesting biological activities (Dubber and Harder 2008).

The use of various extracts from animals and plants for medicinal purposes is an old practice in the history of mankind. Modern and traditional medicines have comparatively exhausted many resources in the land plants. Algae can be an alternative source of new types of agents against several cancerous and other infectious diseases because of their chemical and biological diversity (Chew *et al.* 2008). Algae are potentially active source of antimicrobial and antioxidant compounds. The antimicrobial compounds which are present in green algae have been accepted as animal medicine in Japan (Eguchi *et al.* 2004). Report on the material separation of algal material and antioxidant activity is still not enough. Attention has been focused on marine algae with very little on fresh water algae (Jaki *et al.* 2000). Free radical is accountable for causing several human diseases and aging. The research work demonstrates that antioxidant substance which scavenges free radical plays a vital role in the treatment of diseases caused by free radicals (Ismail and Hong 2002).

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Materials and Methods

Algal samples were collected from various localities of Lahore including different ponds and many damp soil places, and were kept in Biotechnology research laboratory of Lahore College for Women University under optimized conditions. Algal species were cleaned from epiphytes carefully and washed many times with tap and distilled water and then air dried and powdered. Algal contents were extracted by using microwave assisted extraction method as described by (Xia *et al.* 2011).

ATCC certified bacterial cultures of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were obtained from WTO-QOL-UVAS and confirmed by biochemical characterization as described by Bergey's Manual of Systematic Bacteriology. Antimicrobial and antioxidant activities were checked by this experimental design. Different concentrations of methanolic and ethanolic algal extracts (6.5, 12.5, 25 and 50 µg/ml) were used during this experiment.

Screening for the antimicrobial activity of all algal extracts under investigation was performed by using agar well diffusion method against bacterial species (Boyanova *et al.* 2005). To check antimicrobial activity, solvents such as methanol and ethanol were used as negative control. Antibiotic discs such as ampicillin, ciprofloxin and chloramphenicol were used against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*, respectively as positive control. Diameter of inhibitory zone was calculated in millimeters with the help of scale. Each test was conducted in triplicate.

Free radical scavenging activity of different algal extract concentrations was evaluated spectrophotometrically (at 517 nm) against the absorbance of the indicator DPPH (2, 2 diphenyl-1- picrylhydrazyl) solution by modified method (Braca *et al.* 2002). Ascorbic acid was used as reference free radical scavenger and percentage of DPPH - decolorization was calculated. The IC_{50} value (inhibition concentration with 50% radical scavenging activity) was calculated by linear regression analysis and expressed in µg/ml. All test samples were conducted in triplicate ($n = 3$).

Independent t-test was applied on the results of diameter of inhibitory zones of algae extracts against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* and antioxidant activity of methanolic and ethanolic algal extracts. Results were expressed as means ± Sd. Significance was measured at the level of $p < 0.05$.

Results and Discussion

Algal samples were identified based on morphological characters as given by (Sharma 1986) and a voucher specimen *Oedogonium* sp., *Stigeoclonium* sp. *Ulothrix* sp., *Nitzschia* sp. were preserved as BT-Od-05, BT-St-2, BT-Ul-2 and BT-N-1 in Algal Biotechnology Lab, Department of Biotechnology, Lahore college for women University, Lahore.

Comparison of all the tested species indicated that ethanolic extract of *Nitzschia* sp. and *Stigeoclonium* sp. showed more significant antibacterial activity than their methanolic extract against *Escherichia coli* while only the methanolic extract of *Ulothrix* sp. was significantly more active against the same bacterial strain whereas ethanolic extract of *Ulothrix* sp. and *Oedogonium* sp. had more significant antibacterial activity against *Salmonella typhi* than their methanolic extracts. Of the all species tested, the maximum zone of inhibition was shown by ethanolic extract of *Ulothrix* sp. against *Staphylococcus aureus* on the concentration of 50 µg/ml and minimum zone of inhibition was shown by *Oedogonium* sp. against *Salmonella typhi* on the same concentration. Results are presented in Tables 1, 2 and 3.

It was demonstrated that methanolic extract of algal species i.e. *Oedogonium* sp., *Stigeoclonium* sp. and *Ulothrix* sp. had significantly great antioxidant potential with very low oxidation index IC_{50} (19.47, 20.88 and 22.92 µg/ml, respectively) while one of the algal species

i.e. *Nitzschia* sp. had significantly high antioxidant potential in the ethanolic extract with its very low IC_{50} (22.49). The maximum value of % inhibition of ascorbic acid was 20.24 ± 1.00 at 50 µg/ml concentration. IC_{50} Value for ascorbic acid was 16.37 µg/ml. Results of antioxidant activity of different algal species are presented in Figs 1 and 2.

Table 1. Effect of antibacterial activity of methanolic and ethanolic extract of algal species against *Escherichia coli.*

Test compound	Solvent used	Concentration used in µl	Mean inhibition (cm)	Sd	% antibacterial activity
	Ethanol	Negative control	Nil	-	-
	Methanol		Nil	-	-
Ampicillin (disc)		Positive control	4.5	0.1	100
Nitzschia sp.	Methanol	6.5	1.03*	0.05	22.88
		12.5	1.16*	0.05	25.77
		25	1.26*	0.05	28
		50	1.36*	0.05	30.22
Nitzschia sp.	Ethanol	6.5	1.36*	0.05	30.22
		12.5	1.53*	0.05	34
		25	1.66*	0.05	36.88
		50	1.83*	0.05	40.66
Ulothrix sp.	Methanol	6.5	1.23*	0.05	27.33
		12.5	1.43*	0.05	31.77
		25	1.46*	0.05	32.44
		50	1.56*	0.05	34.66
Ulothrix sp.	Ethanol	6.5	1.06*	0.05	23.55
		12.5	1.23*	0.05	27.33
		25	1.33*	0.05	28.88
		50	1.43*	0.05	31.77
Stigeoclonium sp.	Methanol	6.5	1.03*	0.05	22.88
		12.5	1.16*	0.05	25.77
		25	1.33*	0.05	29.55
		50	1.46*	0.05	32.44
Stigeoclonium sp.	Ethanol	6.5	1.26*	0.05	28
		12.5	1.43*	0.05	31.77
		25	1.63*	0.05	36.22
		50	1.73*	0.05	38.44
Oedogonium sp.	Methanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Oedogonium sp.	Ethanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-

Data were analyesd by applying t-test with significance $p < 0.05$. *Showing significance of mean inhibition values.

Antibacterial activity of all the ethanolic extracts was more against Gram negative bacteria as compared to methanolic extracts on the concentrations used in the present study. This might be due to the reason that polar components of all the species under investigation were more soluble in ethanol than in methanol (Yi *et al.* 2001). In present work, all of the four algal species inhibited Gram-positive and Gram-negative bacteria except *Staphylococcus aureus* which was resistant to

all algal species; only the ethanolic extracts of *Nitzschia* sp. and *Ulothrix* sp. showed antibacterial activity against this strain. Furthermore, all the species showed inhibitory activity against *Salmonella typhi* except for *Nitzschia* sp. and ethanolic extract of *Stigeoclonium* sp. All the species showed antibacterial activity against *E. coli* but this bacterial strain was resistant to both the methanolic and ethanolic extract of *Oedogonium* sp. Therefore, Gram-negative bacteria were more susceptible to the algal species than those of Gram-positive bacterial strain.

Table 2. Effect of antibacterial activity of methanolic and ethanolic extract of algal species against *Staphylococcus aureus.*

Test compound	Solvent used	Concentration used in µl	Mean inhibition (cm)	Sd	% antibacterial activity
	Ethanol	Negative control	Nil	-	-
	Methanol		Nil	-	-
Ciprofloxin (disc)		Positive control	3.2	0.1	100
Oedogonium sp.	Methanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Oedogonium sp.	Ethanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Nitzschia sp.	Methanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Nitzschia sp.	Ethanol	6.5	1.23	0.05	38.43
		12.5	1.26	0.05	39.37
		25	1.33	0.05	41.56
		50	1.46	0.05	45.62
Ulothrix sp.	Methanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Ulothrix sp.	Ethanol	6.5	1.23	0.05	38.43
		12.5	1.33	0.05	41.56
		25	1.63	0.05	50.93
		50	1.86	0.05	58.12
Stigeoclonium sp.	Methanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-
Stigeoclonium sp.	Ethanol	6.5	Nil	-	-
		12.5	Nil	-	-
		25	Nil	-	-
		50	Nil	-	-

More susceptibility of Gram-negative bacterial strain to the algal species might be due to the differences in the structure of their cell wall and its composition. This observation is in agreement with the observations made by Demirel *et al.* (2009) and Ibtissam *et al.* (2009). It was also noted

that ethanolic extracts of all species had more inhibitory activity against bacteria than their methanolic extracts and the most powerful inhibitory extracts were ethanolic extracts of *Nitzschia* sp. and *Ulothrix* sp. (1.76 cm against *Escherichia coli*, 1.86 cm against *Staphylococcus aureus*, respectively). The present results are in accordance with the results reported earlier by Yi *et al.* (2001).

Table 3. Effect of antibacterial activity of methanolic and ethanolic extract of algal species against *Staphylococcus aureus.*

Bacterial strain	Test compound	Solvent used	Concentration used in µl	Mean inhibition (cm)	Sd	% antibacterial activity
Salmonella typhi		Ethanol	Negative	Nil	-	-
		Methanol	Control	Nil	-	-
	Chloramphenicol (disc)		Positive Control	5.56	0.1	100
	Nitzschia sp.	Methanol	6.5	Nil	-	-
			12.5	Nil	-	-
			25	Nil	-	-
			50	Nil	-	-
	Nitzschia sp.	Ethanol	6.5	Nil	-	-
			12.5	Nil	-	-
			25	Nil	-	-
			50	Nil	-	-
Salmonella typhi	*Ulothrix* sp.	Methanol	6.5	1.13*	0.05	20.32
			12.5	1.26*	0.05	22.66
			25	1.23*	0.05	22.12
			50	1.33*	0.05	23.92
	Ulothrix sp.	Ethanol	6.5	1.16*	0.05	20.86
			12.5	1.33*	0.05	23.92
			25	1.43*	0.05	25.71
			50	1.53*	0.05	25.51
Salmonella typhi	*Stigeoclonium* sp.	Methanol	6.5	1.06	0.05	19.06
			12.5	1.16	0.05	20.86
			25	1.23	0.05	22.12
			50	1.26	0.05	22.66
	Stigeoclonium sp.	Ethanol	6.5	Nil	-	-
			12.5	Nil	-	-
			25	Nil	-	-
			50	Nil	-	-
Salmonella typhi	*Oedogonium* sp.	Methanol	6.5	1.03*	0.05	18.52
			12.5	1.16*	0.05	20.86
			25	1.18*	0.05	21.22
			50	1.23*	0.05	22.12
	Oedogonium sp.	Ethanol	6.5	1.06*	0.05	19.06
			12.5	1.26*	0.05	22.66
			25	1.43*	0.05	22.66
			50	1.46*	0.05	26.25

Data were analyesd by applying t-test with significance $p < 0.05$. *Showing significance of mean inhibition values.

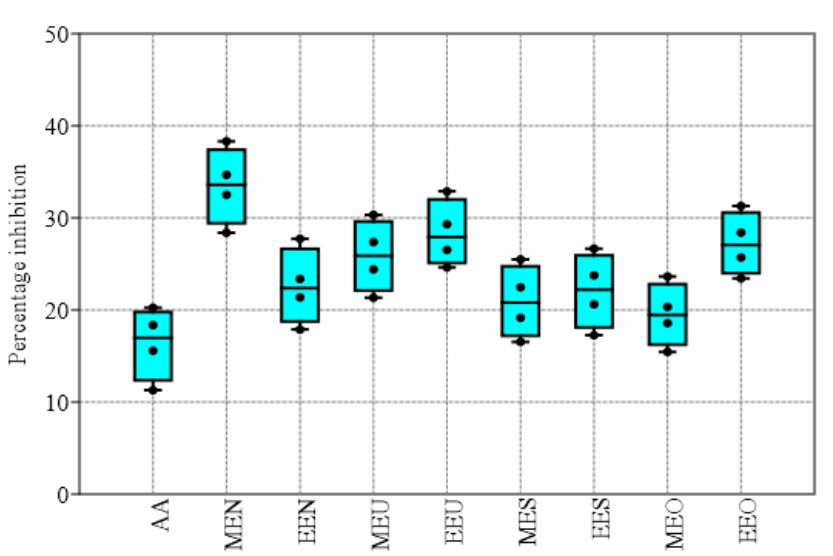

Fig. 1. Inhibitory activity of extracts (AA- Ascorbic acid, MEN- Methanolic extract of *Nitzschia* sp., EEN- Ethanolic extract of *Nitzschia* sp., MEU- Methanolic extract of *Ulothrix* sp., EEU- Ethanolic extract of *Ulothrix* sp., MES- Methanolic extract of *Stigeoclonium* sp., EES- Ethanolic extract of *Stigeoclonium* sp., MEO- Methanolic extract of *Oedogonium* sp., EEO- Ethanolic extract of *Oedogonium* sp.) at 6.5, 12.5, 25 and 50 µg/ml concentrations of extracts.

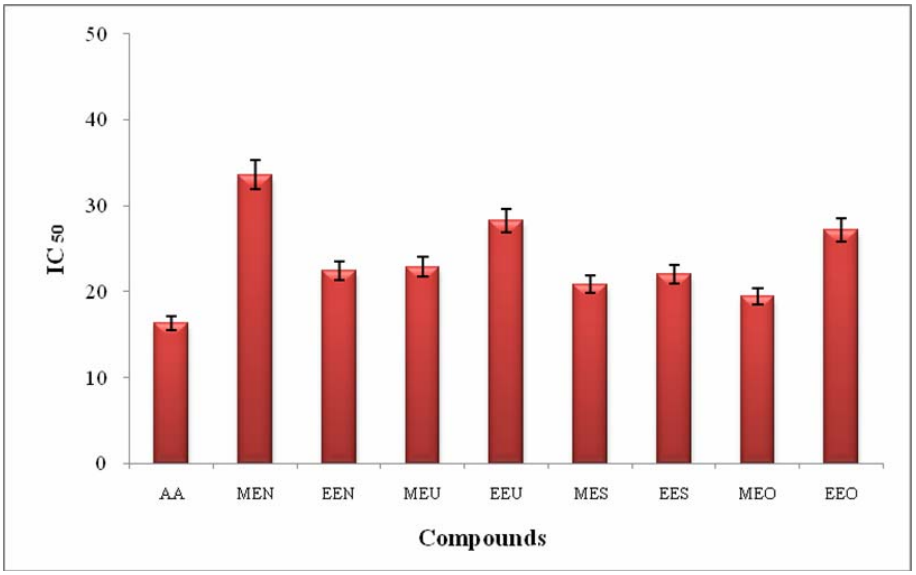

Fig. 2. IC 50 values of algal extracts ((AA- Ascorbic acid, MEN- Methanolic extract of *Nitzschia* sp., EEN- Ethanolic extract of *Nitzschia* sp., MEU- Methanolic extract of *Ulothrix* sp., EEU- Ethanolic extract of *Ulothrix* sp., MES- Methanolic extract of *Stigeoclonium* sp., EES- Ethanolic extract of *Stigeoclonium* sp., MEO- Methanolic extract of *Oedogonium* sp., EEO- Ethanolic extract of *Oedogonium* sp.) at 6.5, 12.5, 25 and 50 µg/ml concentrations of extracts).

The variation in the present findings might be due to certain factors such as experimental method, growth conditions, season for algal collection and habitat of algae under investigation. In addition, the difference in methanolic and ethanolic extract potential in the present results might be due to place and time of sample collection and difference in species used, as well as; differences in susceptibilities of targeted strains might be due to assay method. Furthermore, there might be differences in the ability of extraction protocols to get bioactive compounds.

It is important to discuss that in some algal species (for example *Nitzschia* sp. and *Ulothrix* sp.) the antimicrobial potential was detected only in the extract obtained from one solvent but not demostrated in the extract obtained from another solvent. The reason for such result could be associated with the presence of bioactive compounds which were found in these two algal species that are soluble in one kind of solvent but are not soluble in another one. Karthikaidevi *et al.* (2009) made similar conclusions and suggested that to obtain antimicrobial metabolites from algae, a specific solvent is needed. There are biologically active compounds in algae which are responsible for antimicrobial activity. According to researchers, most of the active metabolites of algae possess antibacterial capability (Lavanya and Veerappan 2011).

Hence all extracts of algal species showed promising activity against the tested pathogens and ethanolic extracts of *Oedogonium* sp., *Ulothrix* sp., *Nitzschia* sp. and *Stigeoclonium* sp. were best against the tested bacterial strains. Among all the algal extracts tested, some seemed to be specific in their capability against the tested bacteria. This idea may be the chief key for the use of algae in pharmaceutics in future.

During the present work, the algal species exhibited a strong antioxidant potential. These results suggest that antioxidants in algal extract act as electron or hydrogen donors for DPPH. The ethanolic extracts of *Stigeoclonium* sp., *Nitzschia* sp., *Oedogonium* sp. and *Ulothrix* sp. showed IC_{50} 22.05, 22.49, 27.19 and 28.30 µg/ml, respectively. Among the four algal species tested, highest IC_{50} value was recorded in methanolic extract of *Nitzchia* (33.61 µg/ml) showing that it has least antioxidant activity while the highest antioxidant potential was recorded in *Oedogonium* sp.

IC_{50} value of the reference standard was 16.37 µg/ml. IC_{50} value of ecperimental extracts was comparable with standard reference (Ascorbic acid). Thus, all the species had more or less antioxidant potential so they can be used as free radical scavengers. The significance is important as radical scavengers do not allow free radicles to scavange the tissues of cell. Hence these natural antioxidants may be helpful in pharmaceutical industry. As IC_{50} value for ascorbic acid was 16.37 so the ascorbic acid had more antioxidant activity. Methanolic extracts of all species had more free radical scavenging activity than the ethanolic extracts except for *Nitzschia* sp. which had contradictory results. This might be due to the reason that algal extracts varied strongly in their antioxidant potential between the species. They are also dependent on conditions during their growth and the solvent used for extraction.

Methanolic extracts of almost all the species under investigation had more antioxidant capacity than the ethanolic extracts. This means that the solubility of active compounds was more in methanol than the ethanol. Differences in the polarity of the solvents used may cause this. There are earlier reports indicating better antioxidant potential in methanolic extracts (Ganesan *et al.* 2008). Algal species which exhibited high antioxidant activity in their methanolic extracts belong to the class Chlorophyta. It is also in accordance with work of (Takamatsu *et al.* 2003) who demostrated a high antioxidant potential in the same class.

Antioxidant potential of algal species observed is due to biologically active compounds. Hemalatha *et al.* (2013) suggested that phenolic content is responsible for antioxidant potential. In addition, it was also reported by Pant *et al.* (2011) that algae contain variety of compounds such as

Catechin, phlorotannins flavonols, glycosides flavonol and phenolic compounds; the key compounds found in algae which had highest free radical scavenging activity were phenolic compounds among all of its compounds.

Therefore algae can be potentially used in pharmaceutics as a natural source to produce particular antibiotics. However, further research work is required for the identification of the compounds which have ability for antioxidant and antimicrobial activity against pathogens especially those which cause human diseases.

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