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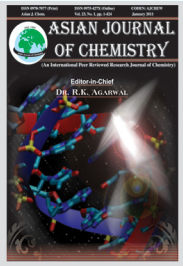

Antimicrobial Activity of Some Algal Species Belonging to Cyanobacteria and Chlorophyta

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In this study, antimicrobial activity of five algal species (*Oscillatoria limosa*, *O. limnetica*, *Phormidium tenue*, *Chlorella vulgaris*, *Spirulina major*) which were grown in proper culture condition was searched with their extracts that obtain by using different solvent (methanol, ethanol, *n*-butanol, acetone, hexane, 0.5 M *tris*-HCl pH 8.00). The antimicrobial activity of algal extracts were tested on *Staphylococcus aureus* ATCC 19213, *Bacillus subtilis* ATCC 6633, *Salmonella enteretidis* ATCC 13076 and *Escherichia coli* O157:H7 by using disc diffusion method. Among the investigated algae, *Spirulina major* has had the highest antimicrobial activity. Buffer extracts of this algal species (0.5 M *tris*- HCl, pH: 8,0) was found to be effective on *S. aureus* ATCC 19213, *B. subtilis* ATCC 6633, *S. enteretidis* ATCC 13076 and *E. coli* O157:H7. *S. aureus* ATCC 19213 and *E.coli* O157:H7 were the most sensitive species; *B. subtilis* ATCC 6633 was the most resistant bacterial strains against to antimicrobial activity of the algae extracts.

Key Words: Chlorophyta, Cyanobacteria, Antimicrobial activity, Disc diffusion method.

INTRODUCTION

In natural defense systems of all living forms, antimicrobial compounds has played an important role and there are quite a variety of these compounds[1]. Some of them are taken by people from natural products. Algae, nutrients and other purposes has been used by humans for thousands of years[2]. These organisms as food for humans and animals constitute important sources of bioactive molecules. In the last two decades, micro- and macro-algae derived from these molecules and their antimicrobial, antiviral, anticancer, antifungal, antiinflammatory and antibiotic effects are being investigated[3,4].

Today, the presence of bacteria resistant to conventional chemotherapeutic compounds and growth of pathogens, the use of these compounds makes them useless. Therefore algae are very beneficial raw materials for medicine and they have some foundations of compound less that are effective and less toxic. Along with these, they form a model for original medicine like physiological activities; therefore they are essential[5].

For years, algae have been used for therapeutic purposes. The microalgae started about 1980's and in the last 10 years microalgae became a focal point of sure investigations. The reason for that is because the samples of enzyme activity and cell cultures are tested to enhance and to be able to test for more extracts and compounds using less quantities of the material[6].

Algae are useful in even more fields. They contain active compounds as cyanobacteria, antibiotics, algaecide, toxins, pharmaceutical and compounds that have biological activity as part of growth regulator[7].

In this study possibility of having extracts of gram (+) and gram (–) in materials used against antimicrobial activity of the species *Chlorella* sp., *Oscillatoria* sp., *Phormidium* sp. and *Spirulina* sp., was investigated.

EXPERIMENTAL

Preparation of algae extracts: For the experiments of the antimicrobial activities, algae are disintegrated with liquid azotes and after each algae piece of 0.5 g measured, solute was added and extracted for 1 h in room temperature where it became centrifuged for 3 min in 13.000 rpm. The resulting supernatants protected at 4 ºC were used for 48 h at most[8].

Preparation of bacteria cultures: The bacterial strains were incubated in nutrient agar medium at 37 ºC over night. Bacterial strains taking a single colony immunized nutrient broth and the cultures were incubated at 37º in 18 h which was used in order to determine the antimicrobial effects[9].

Testing of antimicrobial affects: To find out the effects of algae extracts on bacteria, disk diffusion method is used. 40 µL algae extracts were saturated by 6 mm radius discs. These extracts were dried in 37 ºC for over night. For the

negative control, only methanol, ethanol, *n*-butanol, acetone, hexane and buffer (0.5 M *tris*-HCl pH: 8) were used in saturated disks. 37 ºC of incubation time was given for 24 and 48 h. At the end of these times, the discs were observed to check whether they had inhibited surrounded zones or not. The inhibition zones around the disks had seen measured with a ruler with mms and their photographs has been taken[10].

RESULTS AND DISCUSSION

In present study, antimicrobial effect of *Oscillatoria limosa*, *O. limnetica*, *Phormidium tenue*, *Chlorella vulgaris*, *Spirulina major* was determined with different solvents against to bacteria strains of gram-negative and gram-positive.

***O. limosa*:** Extracts of *O. limosa* prepared in ethanol were found the most effective against to *S. enteridis* ATCC 13076. Except prepared in methanol extract, others showed antimicrobial effect against *S. enteridis*. All extracts showed antibacterial effect against *E. coli*. In response to this, extracts of *O. limosa* showed no effect to *S. aureus* ATCC 19213 and *B. subtilis* ATCC 6633 (Fig. 1a, Table-1).

TABLE-1
ANTIMICROBIAL ACTIVITY OF ALGAL EXCRACTS OBTAINED IN DIFFERENT SOLVENTS (mm)

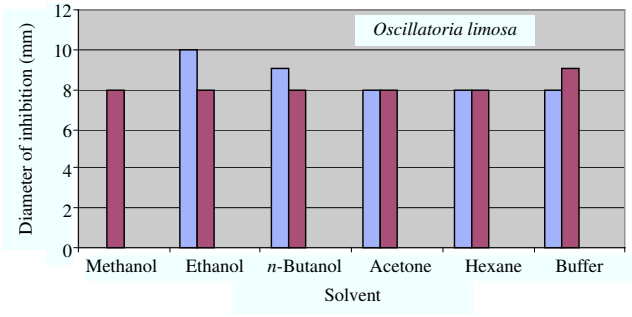
Oscillatoria Limosa	*Salmonelle enteritidis*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus Subtilis*
Methanol	–	8.0	–	–
Ethanol	10.0	8.0	–	–
n-Butanol	9.0	8.0	–	–
Acetone	8.0	8.0	–	–
Hexane	8.0	8.0	–	–
tris-HCl	8.0	9.0	–	–
Oscillatoria limnetica				
Methanol	–	–	8.0	-
tris-HCl	9.0	–	10.0	11.0
Phormidium tenue				
Methanol	–	8.0	–	–
Ethanol	–	8.0	–	–
n-Butanol	7.0	8.0	8.0	–
Acetone	–	9.0	–	–
tris-HCl	–	10.0	9.0	–
Chlorella vulgaris				
Methanol	–	–	–	–
Ethanol	–	9.0	7.0	–
n-Butanol	9.0	9.0	7.0	8.0
Acetone	–	9.0	8.0	–
Hexane	–	9.0	8.0	–
tris-HCl	–	11.0	9.0	–
Spirulina major				
Methanol	–	8.0	7.0	–
Ethanol	–	9.0	7.0	–
n-Butanol	8.0	9.0	12.0	–
Acetone	–	8.0	7.0	–
Hexane	–	8.0	7.0	–
tris-HCl	8.0	11.0	10.0	9.0

***O. limnetica*:** Extracts of *O. limnetica* prepared in buffer were found the most effective against *B. subtilis* ATCC 6633. Extracts of *O. limnetica* prepared in methanol and buffer showed antimicrobial effect against *S. aureus* ATCC 19213. Besides, *E. coli* 0157:H7 was not been affected (Fig. 1b, Table-1).

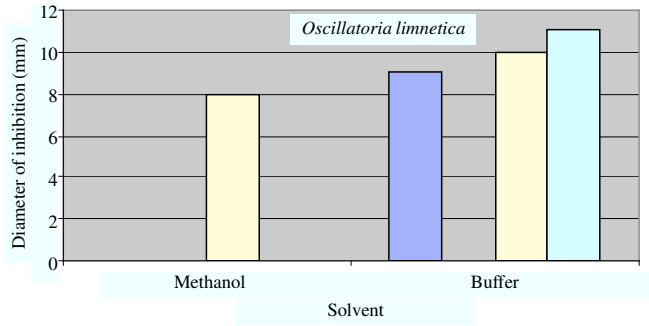
***P. tenue*:** Extracts of *P. tenue* prepared in buffer were found the most effective against *E. coli* 0157:H7. Extracts of *P. tenue* prepared in *n*-butanol buffer showed antimicrobial effect against *S. enteritidis* ATCC 13076 and *S. aureus* ATCC 19213, except *B. subtilis* ATCC 6633 (Fig. 1c, Table-1).

***C. vulgaris*:** Extracts of *C. vulgaris* prepared in buffer were found the most effective against *E. coli* 0157:H7. Except the methanol extract, other extracts showed antimicrobial effect to *E. coli* 0157:H7 and *S. aureus* ATCC 19213. Extracts of *C. vulgaris* in *n*-butanol were effective to all test bacteria (Fig. 1d, Table-1).

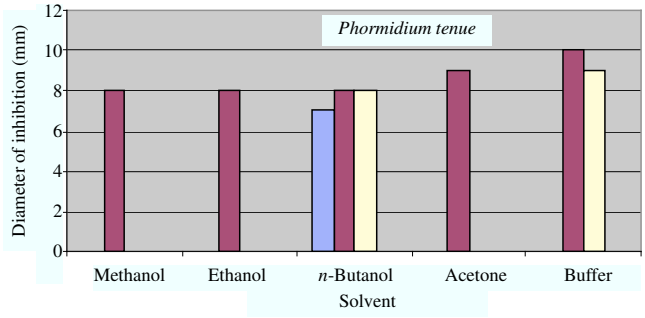
***S. major*:** Extracts of *S. major* prepared in buffer were found the most effective against to *E. coli* 0157:H7. Also extract of buffer were effective against to all test bacteria. All extracts of *S. major* showed antimicrobial effect to *E. coli* 0157:H7 and *S. aureus* ATCC 19213. However, except the buffer extract other extracts were not effective to *B. subtilis* ATCC 6633 (Fig. 1e and Table-1).



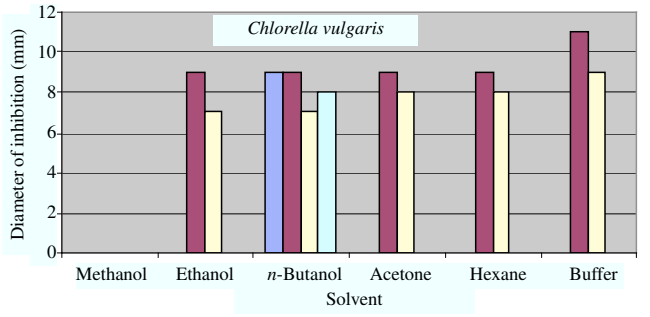
(a)



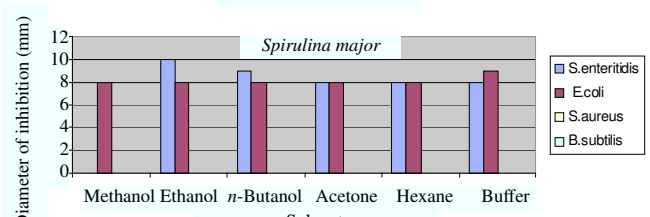
(b)



(c)



(d)



(e)

Fig. 1. Diameter of inhibition of algal excracts obtained different solvents

Among the investigated algae *Spirulina major*, has had the highest antimicrobial activity. Buffer extracts of this algal species (0.5 M tris-HCl, pH: 8.0) was found to be effective on *S. aureus* ATCC 19213, *B. subtilis* ATCC 6633, *S. enteretidis* ATCC 13076 and *E. coli* O157:H7 (Table-1). When the number of effective antimicrobial extracts examined, it is noticed that *Esherichia coli* 0157:H7 strain and *Staphylococcus aureus* ATCC 19213 strains in the examined strains have the highest susceptibility. In response to this, it is noted that *Bacillus subtilis* ATCC 6633 strain and *Salmonella enteritidis* ATCC 13076 strains have low susceptibility.

In the report of Scheuer[11], Chlorophyta and Rhodophyta algae groups, it was noted that the *n*-hexane of methanol extracts and ethyl acetate had more antimicrobial activity. In response to this, it is noted that methanol and similar extracts have a higher antimicrobial activity compared to chloroform extracts[12,13]. In another research, it is noted that the extracts obtained from water with organic solvents have better antimicrobial activity[14].

Some species of belonging to Chlorophyta and Cyanobacteria, in terms of antimicrobial were tested by other investigators and similar effects to our results were found[15-19].

Conclusion

First time to investigate the antimicrobial effects of these species will shed light to other studies in this issue and the discovery of active components and their optimization will be important for new drugs.

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