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REGULAR ARTICLE

ANTIBACTERIAL ACTIVITY OF *SARGASSUM CRISTAEFOLIUM* AND *DICTYOTA CERVICORNIS* AGAINST TO BACTERIA

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ABSTRACT

Nowadays the treat infection of bacterial diseases faced limitations because of the harm of industrial artificial antibiotics for the digestive system and immune system. Consequently, the scientists seek to replace conventional anti-biotics whit natural ones. The central thesis of this study is to identify potential antimicrobial properties of the *Sargassum cristaefolium* and *Dictyota cervicornis* extracts. Water-methanol, n-hexane, Ethyl acetat, methanol and water extracts of two marine macroalgae from chahbahar bay (Iran) were weighed up in terms of antimicrobial activity by Agar disk diffusion, MIC and MBC methods against (*L.monocytogens* as gram-positive bacteri, and (*E.coli* and *P. aeruginosa* as gram-negative bacteria). According to the obtained results, the hot water extract in *S. cristaefolium* and *D.cervicornis* none of the strains examined is of antibiotic effects. Ethyl acetat extract in comparison with other extracts displayed better antibacterial activity ($P<0.05$). Highest zone of inhibition (14 mm) was recorded for Ethyl acetat extract of *S. cristaefolium* contrary to *L. monocytogenes.* Maceration method extracts did not display any effect towards the studied bacteria. For extracting antimicrobial compounds, the ultrasound method was a successful method.

Keywords: *Senna alata; Senna tora;* skin infections; antioxidant; phytochemical; antimicrobial

INTRODUCTION

Human beings' morality is dependent on many causes that infection diseases among them are one of the main causes in the world particularly in developing countries **(Waldvogel, 2004)**. In recent years, life-threatening feature of illness has been increased considerably because of severe infections and resistance of the pathogenic bacteria to medicines as a result of indiscriminate use of antibiotics. Antibiotic resistor in bacteria is one of the main and important health factor in the world **(Patra *et al.*, 2008; Appelbaum, 2006)** . Algaes have been long used as food material in Asian diets because of containing carotenoids and other bioactive compounds. For inductrial manufacturing phycocolloids such as agar-agar, alginate and carrageenan, seaweeds are the main used material **(Rajasulochana *et al.*, 2009)**. For making medicine, seaweed is the most important material because of the abundance and variation of secondary metabolites. Moreover, most of the secondary metabolites existed in seaweed are in form of halogenated compounds having antimicrobial, antifungal and antiviral attributes.

MATERIAL AND METHOD

Sample collection

Sargassum cristaefolium and *Dictyota cervicornis* macroalgae (Phaeophyta) were collected in the chabahar coast of the Persian Gulf, Iran. In laboratory, algae were washed out with water and sand particles were removed. Algal samples were cleaned from epiphytes. Debries, extraneous matters, and necrotics parts were removed. The surface of algal samples was rinsed carefully with sea water and after that it was washed out with fresh water in order to remove salts from algae and to get better results. Distilled water was used was replaced several times. Algae were spread over in the shade seven days in order to be dried completely and then were grinded by an electric powder mill. After sampling, extraction procedure was performed in two following ways, namely ultrasound and maceration methods.

Maceration method

The powder was broken down in methanol, ethyl acetat, n- Hexane , water-methanol and boiling water (1:10 w/v) and kept at room temperature overnight **(Patra *et al.*, 2008).**

Ultrasound method

Ultrasound facilitated extraction experiments. Ultrasonic irradiation was run using an ultrasonic device (50 kHz, temperature of 25 °C,Type Pajohesh nasir Iran) equipped with a digital timer and a temperature controller. The ultrasonic pulse sequence was 180s on and 60 s off. In this study, solvents containing methanol, ethyl acetat, n- Hexane , water- methanol and distilled water were utilized **(Wang *et al.*, 2015).**

Microbial Test

Bacterial strains used in this study contained *Listeria monocytogenes* (PTCC 1163), *Escherichia coli* (PTCC, 1399), *Pseudomonas aeroginosa* (PTCC 1430) respectively. Antibacterial effects were used of the modified disk diffusion method on agar antibiogram test (Agar Disk Diffusion). For this purpose, suspensions of bacteria to antimicrobial susceptibility testing standards with a concentration equivalent to 0.5 McFarland was prepared from overnight cultures. The medium was used for antibiogram Hinton agar (Muller Hinton Agar), with pH 7.2 and 5 mm in diameter, respectively. After providing a uniform, culture suspensions were prepared aseptically using sterile swabs. Blank CDs antibodies Medicine Company were placed on media and were inoculated with 100µl each of the algal extracts was prepared to help Sampler. Pre-release pellets for 30 min at 4° C were then incubated at 37° C were transferred were to diameter of inhibition zone (mm) after 24 h using a digital caliper to measure and record. For Each instance replicated three times. Gentamicin and Neomycin antibiotics were used as positive controls **(Tajbakhsh *et al.*, 2011; Soltani *et al.*, 2011).**

Determination of the MICs (the minimum inhibitory concentrations) and the MBCs (the minimum bactericidal concentrations)

The MICs against all the three bacteria were determined using the Microdillution method. The determinations of the MICs were done in triplicate and the mean values were used. The 96-well plates were scanned with ELISA reader at 630 nm **(Xiaoxi , 2011).**

The MICs were taken as the lowest concentration that caused optical density reduction by more than 90% compared with control growth results. All the MIC wells, which did not show any turbidity, were pureplated on Nutrient agar plates. The lowest concentrations that did not permit any visible growth on the plates after 24 h of incubation at 37°C were recorded as the MBCs **(Mohammadpour Vashvaei *et al.*, 2015).**

Statistical Analyses

The collected data were analyzed through SPSS version 21 software. The antibacterial activities of the data are expressed by means ±SD. Statistical analyses were run by ANOVA with Tukey test. A value of P≤ 0.05 was used to show statistical significance. Finally the ANOVA statistic Test was utilized to see whether there is any relationship between extracts effect and zone of inhibition against the bacteria.

RESULT

The results of this study revealed that extracts tested by maceration method did not show a zone of inhibition towards the bacteria. The inhibitory effects of the concentration of *S. cristaefolium* and *D.cervicornis* extracts displayed the growth of two gram negative bacteria and one gram positive bacteria using disc diffusion method is shown in Table 1. The extract showed both activity against gram positive and gram negative bacteria. And inhibitory impacts enhanced with the increase of extract concentrations. The *S. cristaefolium* extracts showed different degrees of antimicrobial activities on different bacteria. The *L. monocytogenes* was more sensitive one than others among the bacteria. *E.coli* as the gram negative bacteria was found to be more resistant than *P. aeruginosa*. Overall, the gram negative bacteria were more unaffected than the gram positive bacteria. In addition, it is noteworthy to say that the *E.coli* was found to be the most resistant one among all the bacteria. Other similar studies displayed the same type of results.

Antibacterial activity

Varying amounts of antibacterial inhibition against pathogenic bacteria were obtained in the extracts of *S. cristaefolium* and *D.cervicornis*. The measured growth inhibition zone ranged from 8.66 to 14.0 mm for all the sensitive bacteria. The ethyl acetat extract indicated the highest antibacterial activity with inhibition zone of 14.0 mm against *L. monocytogenes*. As it was shown in Table 1, the methanol, ethyl acetat and n-hexan extracts demonstrated activity against all the tested pathogens. On the other hand, the degree of water extracts activity was practically zero.
Table 2 exhibited the MICs and MBCs of seaweed extracts. According to the obtained results, the MIC for *Listeria monocytogene* (gram-positive) was lower than the MIC for gram-negative bacteria.

Table 1 Antibacterial Activity Extracts of Algae Against Pathogens Bacteria (inhibition of growth expressed as mm diameter of inhibition zone).

Bacterial	Solvent	*Sargassum cristaefolium*	*Dictyota cervicornis*
E.coli	Methanol	8.66±0.5	9±0
	N-Hexan	9±1	8.66±0.5
	Ethyl Acetat	9.75±0.2	7.83±0.7
	Water-Methanol	-	-
	Water	-	-
P. aeruginosa	Methanol	11.83±0.7	9.33±0
	N-Hexan	11±0	8.83±0.7
	Ethyl Acetat	11±1	9.16±0.7
	Water-Methanol	-	-
	Water	-	-
L.monocytogenes	Methanol	10.66±1	12±1
	N-Hexan	12±1	11±1
	Ethyl Acetat	14±1	11.66±0.5
	Water-Methanol	10±1	-
	Water	-	-

Zone of inhibition (mm), including of the agar – disk (6 mm), Mean value of three replicate ± SD
(-), no activity

Table 2 MICs and MBCs extracts of marine Algae Against Bacteria Tested

Bacterial	Solvent	*Sargassum cristaefolium*		*Dictyota cervicornis*	
		MIC	MBC	MIC	MBC
E.coli	Methanol	0.1	0.3	0.2	0.4
	N-Hexan	0.05	0.2	0.2	0.4
	Ethyl Acetat	0.05	0.1	0.2	0.4
	Water-Methanol	-	-	-	-
	Water	-	-	-	-
P. aeruginosa	Methanol	0.05	0.2	0.2	0.4
	N-Hexan	0.05	0.2	0.2	0.4
	Ethyl Acetat	0.05	0.2	0.1	0.2
	Water-Methanol	-	-	-	-
	Water	-	-	-	-
L.monocytogenes	Methanol	0.05	0.1	0.05	0.1
	N-Hexan	0.05	0.1	0.05	0.1
	Ethyl Acetat	0.05	0.1	0.05	0.1
	Water-Methanol	0.05	0.2	-	-
	Water	-	-	-	-

MIC:mg/ml

DISCUSSION

The present study was an attempt to assess the antimicrobial activity of the different macro-algae in terms of their bioactive potentials **(de Quirós *et al.*, 2010; Priyadharshini *et al.*, 2012)**. have reported that seaweeds are an outstanding source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities **(Al-Saif *et al.*, 2014; Kausalya and Narasimha Rao, 2015).**
Antimicrobial activity of the seaweed extraction depends on the utilized solvents. Many researchers reported the influence of different extraction solvents on the content of compounds in extracts. Solvents solubility effectiveness is strongly hanged on material used for extraction **(Zhou and Yu, 2004; Michiels *et al.*, 2012; Mahianeh *et al.*, 2014).** Organic solvent is of a higher capability in extracting compounds for antibacterial activities in comparison with water based methods **(TÜney *et al.*, 2006; El-Sheekh *et al.*, 2014).**
Based on the results, the n-hexane and methanol extracts of the algae *Sargassum cristaefolium* did not show antibacterial activity. Similarly, **Febles *et al.* (1995)** reported that the n-hexane and methanol extracts prepared by maceration method of *Sargassum desfonrainesii* which was gathered during winter and autumn did not show antibacterial activity against *P. aeruginosa,* **(Febles *et al.*, 1995)**. A similar study conducted by **Demirel *et al,*(2009)** reported that the methanol and n-hexan extracts *of Dictyota dichotoma var. implex* and *D. dichotoma* prepared by maceration method showed no antibacterial activity against *E. coli* (ATCC 29908) and *E. coli hemorrhagic* (O157:H7) **(Demirel *et al.*, 2009)**. *Pseudomonas*

aeruginosa was resistant to boiling water extracts of *Dictyota sp*. and *S. ramifolium*. In another study, methanol extract of *Dictyota cervicornis* could not create zone of inhibition against the studied bacteria **(El-Sheekh *et al.*, 2014)**. In the same vein, **TÜney *et al* ,(2006)** reported that the methanol extracts prepared by maceration method of *Dictyota linearis* do not create halo-zones against bacteria. The water extracts of *sargassum cristaefolium* and *dictyota cervicornis* did not manifest any antibacterial activity against all the tested pathogenic bacteria **(TÜney *et al.*, 2006)**. **Kausalya and Narasimha Rao, (2015)** study revealed that solvents in comparison with water are always better for extraction **(Kausalya and Narasimha Rao, 2015).** The result of the present study indicated the less efficiency of the extracts prepared by maceration method of *sargassum cristaefolium* and *dictyota cervicornis.* Moreover, it did not display any antibacterial activity against *E. coil,p.aeruginosa* and *L.monocytogenes. Sargassum sp*. and *Dictyota sp.* Extracts. And also, no growth of the bacteria inhibition was detected by **(Ballantine *et al.*, 1987; Chowdhury *et al.*, 2015)**. Positive and favorable antimicrobial effects were shown by extracts prepared by ultrasonic method (see Table 1 and 2).

Another important finding of the present study was that gram positive organism are more affected by the used algae extracts. Similarly,**(TÜney *et al.*, 2006; Taskin *et al.*, 2007).** suggested that gram positive bacteria were more practically dominated by the extracts of the algae used in their study than gram negative bacteria **(Salvador *et al.*, 2007; Salem *et al.*, 2011; Kavita *et al.*, 2014).**

Ultrasound-assisted extraction process employs sonic energy and solvents in order to extract the intended compounds from various plant matrices. It is generally accepted that solvents increase the extraction resulting in an acceptable level. Cavitation phenomenon occurs as a result of using diffusion of ultrasound pressure, accordingly cell membrane will be destroyed and the contents of the cell will be evacuated into the extraction medium **(Ebringerová and Hromádková, 2010).**

Interestingly, UAE was detected appropriate for the extraction of aroma compounds. UAE was also utilized for extraction of oil from soybean **(Li *et al.*, 2004)**, rapeseed **(Ibiari *et al.*, 2010)**, and Monopterus albus **(Abdullah *et al.*, 2010).** Studies on effect of different solvents and their compound, effect of solvent content, sonication power, and sonication time reported that UAE has the potential to ameliorate extraction output. The sonication-assisted extraction can be implemented at lower temperatures suitable for the thermally instable compounds **(Wu *et al.*, 2001; Gupta *et al.*, 2012).**

In a study, **Kadam *et al,* (2015)** kdam et al, reported that ultrasound technique was better in comparison with liquid-solid method in terms of observed laminarin content and molecular weight distribution in the extract **(Kadam *et al.*, 2015).**

Ultrasound may raise extraction efficiency and enhance the quality of extracts **(Altemimi *et al.*, 2016).** Ultrasound-assisted extraction was appeared to be an acceptable and appropriate method for extracting bioactive compounds from Solvia officinalis **(Salisova *et al.*, 1997).** Nowadays, UAE is widely utilized for the extraction of worthy molecules such as proteins **(Qu *et al.*, 2012)**, sugars **(Karki *et al.*, 2010)**, polysaccharides-protein complex **(Cheung *et al.*, 2012)**, oil **(Adam *et al.*, 2012)**, phenolic compounds **(Ince *et al.*, 2014; Kuo *et al.*, 2014).** oils and lipids **(Gil-Ch´avez *et al.*, 2013)**. Dent et al reported that among different extraction methods, the ultrasound-assisted extraction using an ultrasonic device with straight incitement gave rise to the increased improvement of total polyphenols combined with the used lower solvent than conventional extraction **(Dent *et al.*, 2015)**. In addition, ultrasound-assisted extraction is cheaper and easier than other new extraction techniques **(Wang and Weller, 2006)**. In their study, **Kavitha *et al*,(2015)** found that Ultrasonic-assisted extraction enlarges the efficiency of protein extraction from White button Mushroom, Agaricus bisporus **(Kavitha *et al.*, 2015)** .

CONCLUSION

Minimal inhibitory concentration (MIC) or zone diameter showed susceptible, intermediate, and resistant values as defined below**(Cockerill *et al.*, 2012).**

Table 3 Reference table for compare the results

	MIC (µg/mL)	Zone Diameter (mm)
Susceptible	≤ 4	≥ 20
Intermediate	8–16	15–19
Resistant	≥ 32	≤ 14

(Cockerill *et al.*, 2012).

Based on the above mentioned standard values, the bacteria involved in the present study were resistant against the extracts.

Recommendation: There are some cases which can be considered in future research as follows: First, Determining phytochemical compounds in algae by using GC-Mass. Second, Utilizing other extraction methods such as enzyme and soxhlet methods. Third, Decontaminating the bioactive compounds in the extract and examining the antibacterial effects of these compounds individually. Last but not the least, Assessing the antibacterial mechanism of phytochemical compounds against bacteria

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