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Antibacterial activity of some seaweeds from the Red Sea coast of Egypt.

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ABSTRACT

Some commonly occurring brown algae Turbinaria ornata, Cystosiera myrica and Padina pavonica have been collected from the Red Sea coast of Egypt. Three different solvents namely acetone, isopropyl alcohol and ethanol were used for preparing crude algal extracts. The antibacterial activities of crude extracts were evaluated against three pathogens: Grampositive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) by disc diffusion method, and then inhibition zones were compared. Strong antibacterial activity was observed for *T. ornata*, giving high potent with isopropyl alcohol against *S*. aureus and E. coli, with acetone against P. aeruginosa and with ethyl alcohol against S. aureus (inhibition zone >14 mm). Extracts of P. pavonica and C. myrica showed lower antibacterial activity zones, with no inhibition for most tested trials. Among solvents, isopropyl alcohol showed the highest inhibitory action, followed by acetone with moderate antimicrobial activity while ethanol revealed low antibacterial activity, giving inhibitory action only against Gram positive bacteria (S. aureus). Gram positive bacteria were more susceptible to the algal extracts than Gram negative bacteria. The present study revealed that T. ornata is a promising alga to act as an antimicrobial agent in food products, pharmacology and medical applications.

INTRODUCTION

Pathogenic bacteria is one of the most nuisance organisms because it causes many serious diseases for human and animals. Of the pathogenic bacteria for instance, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications (Jawetz *et al.*, 1995). *P. aeruginosa* is a prevalent pathogen among burned patients capable of causing life-threatening illness (Kandhasamy and Arunachalam, 2008). Recently, the use of antimicrobial drugs has a great challenge due to their side effects and changing strategy of resistance in pathogens. Therefore, there is a need for continuous searching for new antimicrobial compounds to develop more effective and safe drugs. Accordingly, pharmaceutical industries are giving importance to the compounds derived from traditional sources (Solomon and Santhi, 2008).







Traditional and modern medicines have relatively exhausted most of their resources in land plants. Nowadays, the marine environment is considered a rich source of new agents used in medicine (Bazes *et al.*, 2006; Chew *et al.*, 2007; Mayer *et al.*, 2007). Recently, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities (Duarte *et al.*, 2002; Faulkner, 2002; Ely *et al.*, 2004; Dubber and Harder, 2008).

Actually, seaweeds are the most interesting marine organisms that traditionally used in human and animal nutrition (Dawczynski *et al.*, 2007). Also, they are known to produce structurally diverse and biologically active secondary metabolites which act as defense mechanism against fouling organisms and herbivores (Bhadury and Wright, 2004). Most bioactive compounds obtained from seaweeds are used in pharmaceutical industries, such as antimicrobial, antiviral, antifungal, anticancer and antioxidant activities (Shanab 2007; Kolanjinathan *et al.* 2014; Lavanya *et al.* 2017).

The algal richness of Egyptian Red Sea coasts is undeniable in terms of diversity and quantity (Chiffings, 2003). The antimicrobial potential of seaweeds from Red Sea coasts of Egypt (Shanab 2007; Salem *et al.* 2011; El-Sheekh *et al.* 2014) and of Saudi Arabia (Omar *et al.* 2012; Al-Saif *et al.* 2014; Moubayed *et al.* 2017) remains partially unexplored.

The present study was undertaken to examine the antibacterial effect of crude extracts of three species of brown seaweeds, *Turbinaria ornata, Cystosiera myrica* and *Padina pavonica*, collected from the Egyptian Red Sea coasts, against pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Also, the efficacy of the three solvents, acetone, isopropyl alcohol and ethanol to obtain the most effective crude extract from each seaweed was evaluated.

MATERIALS AND METHODS

Algal materials

Samples of three species of algae belong to the Phaeophyta; *Turbinaria ornata* (Turner) J.Agardh, *Cystosiera myrica* (S.G.Gmelin) C.Agardh, and *Padina pavonica* (Linnaeus) Thivy (Fig. 1), were collected from Egyptian Red Sea at Wadi El Gemal National Park. The algae were identified according to Aleem (1978, 1981) and Abbott and Hollenberg (1976).



Fig. 1: The three tested algal species at a) collected location under water, and b) the laboratory after washing.

Algal materials were cleaned with seawater to remove all the extraneous matter such as epiphytes, and non-living matrixes and brought to the laboratory in plastic bags. The samples were thoroughly washed with freshwater, and rinsed many times in distilled water, then spread out at room temperature for drying. Shade dried seaweed materials were ground into powder in an electrical mill. The powdered samples were stored in the dark bottles at room temperature for further use.

Algal extract

The algal extract was prepared by soaking the algal powder in different solvents (acetone, isopropyl and methyl alcohols) in a conical flask (50 g dry algae/1000 ml solvent) for two days. The final extract was filtered by Whatman filter paper and the filtrate was concentrated in a rotary evaporator at room temperature (30°C) and the crude extract kept deep frozen for further assays.

Test microorganisms

The antimicrobial activity of acetone, isopropyl and methyl alcohols of marine algal extracts were examined *in vitro* against selected human pathogens such as Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The microorganisms used in this study were obtained from microbiology laboratory at Suez Canal University Hospital. The pathogenic bacteria were grown on nutrient agar medium, which was sterilized, and 25 ml was poured into sterile cabbed test tubes. Test tubes were cooled to room temperature and 0.5 ml of the uniform mixture of inoculum was introduced to each tube then well mixed. The content of each tube was poured into a sterile Petri-dish for solidification (Mtolera and Semesi, 1996).

Antibacterial assay

The antimicrobial activity was carried out using disc diffusion method (Tendencia, 2004). Paper disc of 6 mm in diameter prepared from Whatman filter paper was soaked with the crude extract and placed onto the bacterial lawn after it had solidified and incubated at 37° C for 24 h. After incubation, a clear zone was observed around paper disc and the diameter of the inhibition zone measured in millimeters. All assays were done in four replicates and data were expressed as mean values \pm SD.

RESULTS

Different extracts of *T. ornata, C. myrica* and *P. pavonica* were tested for their antimicrobial activity against three strains of microorganisms by disc diffusion method. However, the results of antimicrobial activity against tested pathogens are presented in Table (1) and Figures (2-4).

Table 1: Inhibition zone (mm) of different crude extracts of three species of seaweed extracts against selected pathogenic bacteria.

	Turbinaria ornata			Cystoseira myrica			Padina pavonica		
Tested bacteria	Acetone	Isopropyl	Ethyl	Acetone	Isopropyl	Ethyl	Aceton	Isopropyl	Ethyl
		alcohol	alcohol		alcohol	alcohol	e	alcohol	alcohol
P. aeruginosa	14.5±1.7	3.5±1.92	Nil	2.75 ± 0.5	15.5 ± 0.57	Nil	Nil	Nil	Nil
S. aureus	5.25 ± 0.95	15±1.4	14±1.15	Nil	4 ± 0.81	$4.25\pm0.$	15 ± 0.7	4.5 ± 1.2	3.29 ± 5
						5			
E. coli	1.75 ± 0.5	14.75±1.3	0.25 ± 0.5	Nil	Nil	Nil	2 ± 0.2	4.5 ± 1.1	Nil

The inhibition zones observed by different extracts of T. ornata against the pathogenic bacteria are represented in Figure (2). Turbinaria ornata showed high antibacterial activity with isopropyl alcohol extract against S. aureus and E. coli, with acetone extract against P. aeruginosa and with ethyl alcohol extract against S. aureus (inhibition zone >14 mm). On the other hand, ethyl alcohol extract of T. ornata had no effect on P. aeruginosa. Otherwise, low to moderate activity was observed for different solvents against the pathogenic bacteria.

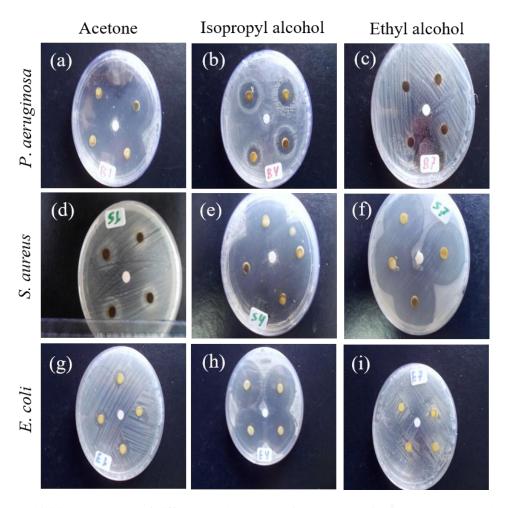


Fig. 2: Inhibition zone (mm) of different crude extracts of *T. ornata* against *P. aeruginosa* (a, b, c), *S. aureus* (d, e, f) and *E. coli* (g, h, i).

Cystosiera myrica showed either low antibacterial activity (inhibition zone <4 mm) or no inhibition zone for all solvents against all tested bacteria except for isopropyl alcohol extract against P. aeruginosa (inhibition zone: 15.5 ± 0.57 mm). The extracts of C. myrica with all solvents used in the present study did not give any inhibition zone against E. coli (Figure 3). Isopropyl alcohol extract of C. myrica showed a broad inhibitory activity, giving the highest activity against P. aeruginosa (inhibition zone: 15.5 ± 0.57 mm), moderate activity against S. aureus (inhibition zone: 4 ± 0.81 mm) and without activity against E. coli.

No extracts of *P. pavonica* affected the Gram negative bacteria *P. aeruginosa* (Figure 4). *Staphylococcus aureus* was the most sensitive bacteria for *P. pavonica* extracts, especially acetone extract (inhibition zone: 15 ± 0.7 mm), while isopropyl and ethyl alcohols extracts showed low activity (inhibition zone: 4.5 ± 1.2 and 3.29 ± 0.5 mm, respectively). Acetone and isopropyl alcohol extracts of *P. pavonica*

showed low activity against $E.\ coli$ (inhibition zone: 2 ± 0.2 and 4.5 ± 1.1 mm, respectively), while ethyl alcohol extract did not affect it.

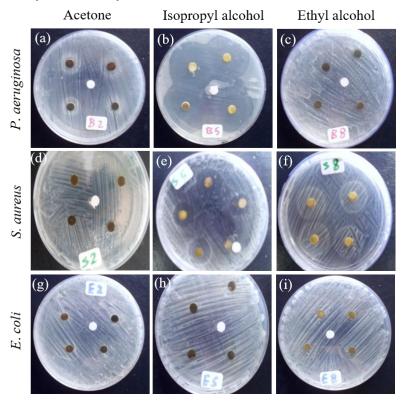


Fig. 3: Inhibition zone (mm) of different crude extracts of *C. myrica* against *P. aeruginosa* (a, b, c), *S. aureus* (d, e, f) and *E. coli* (g, h, i).

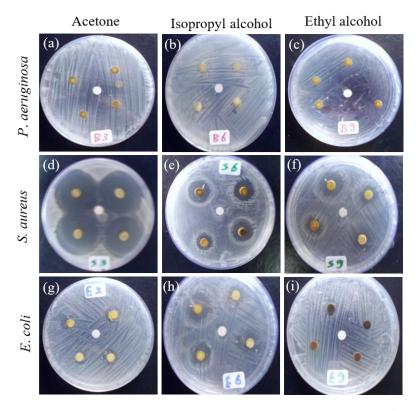


Fig. 4: Inhibition zone (mm) of different crude extracts of *P. pavonica* against *P. aeruginosa* (a, b, c), *S. aureus* (d, e, f) and *E. coli* (g, h, i).

DISCUSSION

Seaweeds act as potential bioactive compounds for pharmaceutical applications. This capability of seaweeds can be attributed to synthesis of various bioactive secondary metabolites which are biologically active against different types of pathogenic bacteria (Kolanjinathan *et al.* 2014; Abdel-Latif *et al.* 2018). The majority of the compounds isolated from marine algae that responsible for the antimicrobial activity are lipids (Rosell and Srivastava, 1987).

In the present study, the crude extracts of the three selected species of brown algae showed antibacterial activities against tested bacteria with various inhibitory actions depending on the seaweed species and the solvent used. Strong antibacterial activity was observed for T. ornata in all extracts with high potent against all tested bacteria. T. ornata demonstrated a broad spectrum of microbial growth inhibition for both Gram positive and Gram negative bacteria. Rajkumar (2017) stated that methanolic extract of T. ornata was worked well against P. aeruginosa and produced 24.02 ± 0.47 mm zone of inhibition. Vijayabaskar and Shiyamala (2011) tested the methanol extracts of brown algae Sargassum wightii and Turbinaria ornata against various Gram positive and Gram negative human pathogenic microbes. Their finding envisages that methanol extracts of T. ornata could be utilized as a good source of antimicrobial agent in pharmaceutical industry.

Cystosiera myrica and P. pavonica showed lower antibacterial activities, especially against Gram negative bacteria (P. aeruginosa and E. coli). Salem et al. (2011) found that Enterococcus feacalis was resistant to methanolic and ethyl acetate extracts of C. myrica. Our results also accords with the findings of El-Shoubaky and Salem (2014) who evaluated the potential antimicrobial activity of Padina pavonica collected from Red Sea of Saudi Arabia and their fatty acids content. They found that fatty acids extract of P. pavonica showed no inhibition effect against E. coli and S. aureus. The differential antibacterial activities of these marine algae may be attributed to the presence of different antibacterial active compounds which are easily extracted with organic solvents.

The highest inhibitory action among all extracts was obtained with isopropyl alcohol extract. Although isopropyl alcohol is known as a solvent for a wide range of non-polar compounds compared to other organic solvents (e.g. methanol), it was not seemed as a preferable solvent in the studies dealing with antimicrobial activities of algal extracts. The finding of the present study suggests that the isopropyl alcohol crude extracts of brown algae could be contained a new antibacterial compound(s) showed high activity against human pathogenic bacteria and further studies are required to purify the active principles.

Moderate antimicrobial activity was obtained with acetone extracts. Earlier studies on antibacterial screening using various solvent extracts revealed that the crude acetone extracts of different seaweeds reacted positively against selected human bacterial pathogens (Oumaskour *et al.*, 2012, Venugopal *et al.*, 2014; Moorthi and Balasubramanian, 2015; Rosaline *et al.*, 2017; Qari and Khan, 2019).

On the other hand, ethanol extracts revealed meager antibacterial activity, giving inhibitory action only against Gram positive bacteria (*S. aureus*). Ambreen *et al.* (2012) screened ethanol extract of 5 seaweeds for antibacterial activity, and 9 tested against root rotting fungi and found that the ethanol extract of most of the species was less active against tested fungi.

The study confirmed that susceptibility of Gram positive bacteria (S. aureus) to the algal extracts was more than those of Gram negative bacteria (P. aeruginosa and

E. coli). A similar observation was recorded by Salem et al. (2011) who found that Gram negative bacteria (E. coli, P. aeruginosa, Enterococcus feacalis and Salmonella sp.) were the most resistant bacteria to most tested seaweed extracts while Gram positive bacteria (S. aureus and Bacillus cereus) were the most sensitive to all seaweed extracts. In the same context, Oumaskour et al. (2012) and Al-Haj et al. (2009) reported that the Gram-positive bacteria presented a sensibility superior to the Gram-negative. Moreover, S. aureus was observed to be the most sensitive bacteria to most algal extracts (Oumaskour et al., 2012; Belattmania et al., 2016). This may be due to the more complex structure and composition of Gram negative bacterial cell wall (Rosell and Srivastava, 1987). Mendes et al. (2013) showed that the outer membrane and the thick murein layer of Gram negative bacteria act as a barrier, preventing the entrance of environmental substances such as antibiotics and inhibitors.

CONCLUSION

Conclusively, the current study showed appreciable antibiotic activity by all the three algae *T. ornata*, *C. myrica* and *P. pavonica* against certain human pathogens. These three species of algae that grow excessively at Wadi El Gemal National Park coast in Red Sea, Egypt can be used for development of anti-pathogenic drugs in the pharmaceutical industries. The present study revealed that *T. ornata* is a promising alga, as there may be a potential to utilize its extract in food products to act as antimicrobial agent, which could potentially increase the shelf life and safety of a wide range of food products, or in pharmacology as new agent for therapeutic medical and veterinary applications. However a detailed study in this field is required for isolation and characterization of the antibacterial compounds from these marine macro-algae.

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