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NUTRITIVE AND ANTIMICROBIAL PROFILES OF SOME SEAGRASSES FROM BARDAWIL LAKE, EGYPT

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ABSTRACT

Seagrasses species in Bardawil Lake have been defined through broad quantitative sampling. From the biochemical constituent's point of view, two species namely; *Cymodocea nodosa* and *Ruppia cirrhosa* were evaluated as feed additives and antimicrobial active compounds. The obtained results showed an obvious increase in chlorophyll "*a*", carbohydrate and lipid contents in the *C. nodosa* than *R. cirrhosa* while protein contents was higher in *R. cirrhosa* than in C. *nodosa*. Whole plant tissues powder substance (10.0%w/v) and ethanolic extracts (200µg/ml) of *C. nodosa* and *R. cirrhosa* were evaluated against some microorganisms using pour plate technique. Powdered substance of *C. nodosa* was exhibited the highest antifungal activity than that of *R. cirrhosa,* also its ethanolic extract inhibited all of the tested bacterial isolates except *E. coli*. *R.cirrhosa* whole plant tissues powder showed antibacterial effect on all the used bacterial isolates in addition to its reduction the radial growth of *Alternaria alternatium.* The inhibitory effects of the whole powder and ethanolic extracts varied considerably between the assays microorganisms.

1. INTRODUCTION

Seagrasses particulary *Posidonia oceanica* are very sensitive with regard to variation in environmental quality. They were use as "classical" bio-indicator species providing many valuable ecological and economical services within coastal and estuarine areas (Ferrat *et al.*, 2003 and Maria *et al.,* 2006). Moreover, *P.oceanica* collected in a contaminated site of the Western Mediterranean, showed increased GST (glutathione sulfotransferase) enzyme activity as an important antioxidant in plants (Rijstenbil *et al.*, 1994 and Hamoutene *et al.*, 1995). The distribution, biomass and biochemical contents of the seagrasses species collected from Bardawil Lake *R. cirrhosa* and *C.nodosa* were studied by Geneid and Abd El-Hady (2006). Also Haroon (2006) showed the effect of methanol extracts of some common and widely distributed macrophytes (Leaves and stems) collected from Manzalah Lake on the growth of toxigenic strain of *Aspergillus parasiticus* in a chemically defined media.

Pathogenic bacteria in water bodies are responsible for several diseases as well as heavy mortality in wild and cultured fish. The use of antimicrobial agents has increased in aquaculture practices. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture (Choudhury *et al.,* 2005). Decreased efficacy and resistance of pathogens to antibiotics has necessitated the development of new alternatives (Smith *et al.*, 1994). The antifungal chemical defenses and physiological responses of seagrasses collected from the Indian River Lagoon, were investigated against a panel of co-occurring

marine fungi (Ross *et al*., 2007), also the extracts from marine plants –collected from Indopacific marine – were tested for antimicrobial effects against marine microorganisms. About 95% of the tested extracts were active against one or more ,and 77% were active against two or more assay microorganisms (Puglisi *et al*., 2007).There for the main aims of this study were to investigate the biochemical constituents of *C. nodosa* and *R.cirrhosa* and their effect as antimicrobial agent against some tested microorganisms.

2. MATERIAL AND METHODS

2.1. Area of study

Bardawil Lake (Fig. 1) is situated in the north of Sinai Peninsula between W 32°40` and E 33°30` and between N 31°19` and S 31°03`, about 90 Km long with a maximum width of 22 Km, the wet area about 650 Km^2. The lake is a natural depression separated from Mediterranean Sea by long arrow – shaped sand bar, 300-800 m wide. The main connection between the Mediterranean Sea and the lake through two man-made inlets: the western inlet (Boughaz I) and the Eestern inlet (Boughaz II). There is also a third small Boughaz at the lake easternmost corner.

2.2. Seagrass plants

Samples of plants were collected from stations representing almost all the lake. The seagrass plants were collected from Bardawil Lake by diving using a 25 x 25 cm metal quadrate for collection of plants. All samples were brought to laboratory in plastic bags containing water to prevent evaporation and then washed thoroughly with distilled water to separate potential contaminants and identified. Samples were dried in oven at 37°C, till constant weight and ground in an electric mixer (Lima-Filho *et al.*, 2002). The collected seagrass plants were identified as *C. nodosa* and *R.cirrhosa*. Their areas of collection are represented on (Fig. 1).

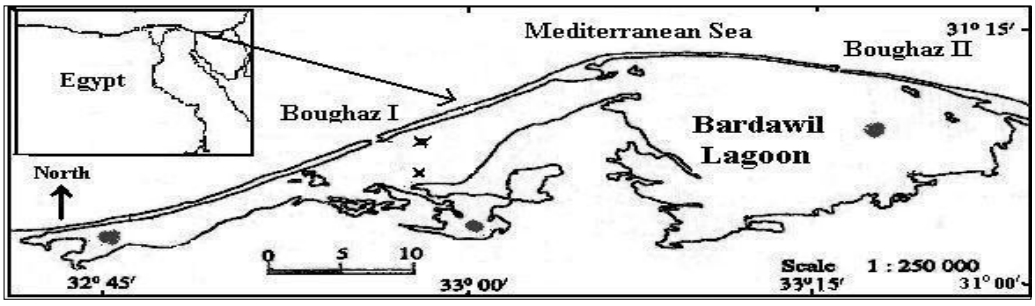

Fig (1): Map showing Bardawil Lake,

North Sinai, Egypt

***R.cirrhosa* (●) *C. nodosa* (x)**

2.2.1. Biochemical determination in seagrasses powder

Total lipid contents in seagrasses powder were determined by the sulphophosphovanillin procedure (SPV**)** (Chabrol and Castellano, 1961).Total cellular carbohydrates were measured as described by Dubois *et al.* (1956) .The total protein of seagrasses powder determined by Biuret method (David and Hazel, 1993) and chlorophyll "*a*" content was measured according to standard method (APHA, 1995).

2.2.2. Antimicrobial activity:

Bacillus sublilis NIOF, *Staphylococcus aureus* NIOF (Grame-postive), *E. coli* NIOF, *Pseudomonas eruginosa* ATCC 10145, (Grame-negative) and filamentous fungi as *Aspergillus flavus, A. fumigatus, a*nd *Alternaria alternatium*, were obtained from the culture collection of microbiology Lab. at NIOF, Cairo. All bacterial isolates were grown (10^8 cfu/ml) in nutrient broth and incubated at 37°C for 24h. and then plated into nutrient agar (NA 1-140, Scharulu Chemie,S.A.) medium (1 ml/20 ml NA) then Sterile discs of 5 mm. diameter were embedded with 100 µl of (10.0% w/v; whole seagrasses tissues powder) water or ethanol 95%. The plates were holed in the refrigerator for 2 h. to allow diffusion. The results were recoded as inhibition zoon of bacterial growth. The fungal isolates were grown for one week on potato dextrose agar (PDA, 1-451, Scharulu Chemie,S.A,),the spores were harvested in sterile water using sterile swab, 1ml of spores suspension (10^6 spores/ml) was plated and covered with 20 ml of potato PDA amended with 10.0% w/v seagrasses powder. The results were recoded as reduction of the fungal redial growth compare with the control one, (Daboor, 2001).

2.2.3. Extraction of antimicrobial material

C. nodosa and *R.cirrhosa* samples were dried in oven at 37-40°C, and 10 g of hammer milled samples were soaked in 100 ml each of water and ethanol 95% for 24h.then filtrated through Whatman No.1 filter paper (Adomi, 2006). The filtrate (aqueous and ethanol) was evaporated and dried at 55-60°C (Lima-Filho *et al.*, 2002) the final dried material was kept in the refrigerator at -20°C till use (Ergene *et al.*, 2006). The dried extracts were redissolved in each solvent to a final concentration 200 mg/ml. The bacterial (0.2 ml) and fungal spores suspensions (1.0 ml) in saline (0.85% NaCl), was mixed with 20 ml of NA, and PDA, respectively. Wells (5 mm in diameter) were punched in the agar medium using sterile stainless cork borer (Adomi 2006). Each well was filled with 50 µl of tested extracts or solvent (control). The plates were hold in the refrigerator for 2 h. to allow the extracts diffuse and then incubated for 24 h. at 37°C and 7 days at 30°C for bacterial and fungal isolates, respectively (Daboor, 2001). Antimicrobial activities were evaluated by measuring the diameter of inhibition zone (millimeters). Each test was carried out in duplicate.

2.3. Statistical analysis

Mean and LSD were calculated using SPSS 10.0 for windows (1999).

3. RESULTS

Table (1) showed the biochemical constituents of the two seagrasses *C. nodosa* and *R.cirrhosa*. The data showed that, an obvious increase in chlorophyll "*a*",carbohydrate and lipid contents in *C. nodosa* (1.19, 130 and 36.5 mg/l, respectively) than in *R. cirrhosa*, while the protein content value in *R. cirrhosa* (28%) was higher than that recorded in *C. nodosa* (18 %).

The two seagrasses; *R. cirrhosa* and *C. nodosa* were found to show antimicrobial activities in vitro against tested bacterial and fungal species in their ethanolic extract (water extract data not shown). Table (2), represents the effect of whole seagrasses tissues powder of *C. nodosa* and *R. cirrhosa* on the assay microorganisms, where tissues powder of *C. nodosa* reduced the tested fungal growth with value ranged from 25.0 - 83.0% and inhibited the tested bacterial isolates with inhibition zones between 0.0 (ND) to 15.0 mm. On the other hand, the effect of whole tissues of *R. cirrhosa* have been reduced only *A. alternatum* by 25%, and inhibition zones ranged from 12.5 to 17.0 mm for the tested bacterial isolates. Significant differences were recorded for antibacterial activities of the two seagrasses. The effect of ethanloic extract of the two seagrasses as antimicrobial agent was represented in Table (3) where *C. nodosa* ethanolic extract showed antimicrobial activities against all the tested fungi and bacteria except *E.coli*, with value 10.0-12.8 mm and 0.0 -11.5 ,respectively. The ethanolic extract of *R. cirrhosa* showed antibacterial activities with inhibition zone ranged from 10.0-19.5 mm and 10.0 mm for *A. alternatum*. The tested microorganisms differed significantly ($P < 0.05$) in relation to their susceptibility to the different extracts.

Table (1): Biochemical constituents of some seagrasses from Bardawil Lake.

Biochemical parameters	Seagrasses species	
	R. cirrhosa	*C. nodosa*
Chlorophyll "*a*" mg/g	0.828	1.187
Proteins g /100 g	28.7	18.6
Carbohydrates mg/g	62	130
Lipids mg/g	15	36.5

Table (2): Antimicrobial activity of *C. nodosa* and *R. cirrhosa* extracts.

Tested micro-organism / seagrasses	Inhibition effect of whole seagrasses tissues						
	Fungi			Bacteria			
	Radial growth reduction (%)			Inhibition Zone (mm)			
	A. f	*A. fu*	*A. a*	*B. s*	*S. a*	*E. c*	*P. a.*
C. nodosa	83.3	41.67	25.0	15.0	10.0	N.D	11.0
R. cirrhosa	0.0	0.0	25.0	17.0	12.5	12.5	14.0
(LSD 5%)	14.0	12.74	2.09	2.7	1.74	2.3	2.0

N.D: not detected, A. f : *Asprogillus flavus*, A. fu: *Asprogillus fumigatus*, A. a: *Alternaria alternatium* B.S: *Bacillus subtilis* NIOF, ,S.a : *Staphylocococcus aurous* NTOF, E.C: *Echericha coli* NIOF and P. a: *Pseudomonas aeruginosa* ATCC 10145.

Table (3): Inhibitory activities (inhibition zone, mm) of ethanolic extracts of *C. nodosa* and *R. cirrhosa*.

Tested micro-organism / Extract	Inhibition effect of ethanolic extracts						
	Fungi			Bacteria			
	A. f	*A. fu*	*A. a*	*B. s*	*S. a*	*E. c*	*P. a*
C .nodosa	12.8	10.45	10.0	10.0	11.5	N. D	10.0
R.cirrhosa	N. D	N. D	10.0	10.5	10.0	19.5	11.0
(LSD 5%)	1.7	4.0	2.5	0.8	2.1	4.7	2.0

4. DISCUSSION

Bardawil Lake is an important fishing area for many species of commercial fishes that use seagrass beds as nursery ground. Biochemical constituents and the antimicrobial activities of seagrasses; *R. cirrhosa* and *C.nodosa* collected from Bardawil Lake were studied. The biochemical contents measurements of *R. cirrhosa* and *C. nodosa* indicated that an obvious increase in carbohydrates, chlorophyll "*a*" and lipids of *C. nodosa* more than that found in *R. cirrhosa* but the protein contents of *R. cirrhosa* is mush higher than those recorded in *C. nodosa.* Concerning the results of this work, Geneid and Abd El-Hady (2006) stated that the biochemical analysis of *R. cirrhosa* and *C. nodosa* had the same pattern of distribution for lipids, carbohydrates and protein. In this connection, Olive *et al.* (2006) stated that *C. nodosa* collected from different stations in Cadiz Bay Natural Park (Southern Spain) showed maximum photo-synthetic rates. The plants also showed variation in photosynthetic parameters along the leaf. In general, middle portions of the leaf displayed maximum photosynthetic rate values than apical and basal portions. The present finding agreed with those of Geneid and Abd El-Hady (2006), who reported that during summer, both seagrasses plant, (*R. cirrhosa* and *C. nodosa*) were observed to be covered with a thick layer of epiphytic algae. This might explain the increased chlorophyll "*a*" reading during summer. Moreover, Mazzuca *et al*. (2006) reported that *P. oceanica* collected from Tyrrhenian Calabria coast (Italy) have low protein concentration of high-quality protein fractions. These results are in the line with Gobert *et al.,* (1995) who demonstrated that both lipid and total carbohydrate contents of *P. oceanica* leaves increase in summer.

One of the main objectives of this work is to evaluate and compare the ability of different seagrass extracts to produce bioactive compounds of potential therapeutic interest. The production of antimicrobial activities was considered to be an indicator of these seagrasses to synthesize bioactive secondary metabolites. In the line with the present results Haroon (2006) reported that the methanol extracts of some common and widely distributed macrophytes from Manzalah Lake, showed inhibitory effects on *A.parasiticus* growth. These inhibitory effects of the active and important substances (Tannins, Flavonoids, Saponins, Terpenes, Alkaloids and Glycosids) of the extracts were increased with increasing their concentrations as compared to the control. The effect of ethanol extract was active against both tested Gram positive and Gram negative bacteria. This may be attributed to the fact that cell wall in Gram positive bacteria consist of a single layer, whereas Gram negative bacterial cell wall is a multi layered structure bounded by an outer cell membrane (Yoa and Moellering, 1995). These results can be related to volatile antibacterial compounds in the extracts (Tüney *et al.,* 2006). Some of bacterial strains did not respond to extracts, where as the whole powder showed activity

against multiple strains. This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extracts (Choudhury *et al.,* 2005). The variation of antibacterial activity of the extracts might be due to distribution of antimicrobial substances, which varied from species to species. (Lustigman and Brown 1991). Adomi (2006) reported that, the water and ethanol extracts of the stem bark of some medicinal plants were tested on Gram-positive and Gram-negative bacteria, where ethanol extract was inactive against any of the bacterial tested while the aqueous extract was active.

On the other hand Ergene *et al*., (2006) investigated both ethanol and aqueous extract of *Heracleum sphondylium* sub sp. as antimicrobial activities against Gram-positive and Gram-negative bacteria and reported that, both extracts showed antimicrobial activity against Gram-positive bacterium *Staphylococcus aureus.* In addition, the two seagrasses are very sensitive with regard to variations in environmental quality thus used as classical bio-indicator, these results provide good evidence that the dried seagrasses could used as a source of antimicrobial and protein substances ,especially *R. cirrhosa* (contain ≈28%) for formulation of artificial feed instated of fish meal.

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