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Evaluation of Antifungal Potential of *Dunaliella salina* and *Phormidium autumnale* against Plant Pathogenic Fungi

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The antifungal potential of *Dunaliella salina* and *Phormidium autumnale* were evaluated against plant pathogenic fungi; *Alternaria alternata, Cladosporium* sp., *Fusarium solani, Fusarium sp.,* and *Fusarium oxysporum*. Water, acetone, methanol and Methanol: Acetone: Diethyl ether extracts of tested algae showed weak to strong activity against one or more than one tested plant pathogenic fungi. However, none was able to inhibit the growth of *A. alternata*. The extracts prepared with the mixture of methanol, acetone and diethyl ether were found highly effective in controlling the growth of most of the plant pathogenic fungi, followed by methanol, acetone and water. Extract with combination of the three solvents methanol, acetone and diethyl ether of *D. salina* caused maximum reduction in the growth of *F. oxysporum* (64.4%). Whereas, Methanol:Acetone:Diethyl ether extract of *P. autumnale* exhibits maximum growth reduction against *F. solani* (62.2%). Water extract of *D. salina* registered almost negligible reduction in the mycelial growth of *Cladosporium* sp (0.1%). The crude extracts of algal species were analyzed by gas chromatography-mass spectrometry (GC-MS) and the main component in the crude extract of *P. autumnale* was 1-Hexyl-2-Nitrocyclohexane (91.7%) whereas in the extract of *D. salina,* 3-Methyl-2-(2-Oxopropyl) Furan (90%) was detected. Some important compounds; Butanal and Octanal were also detected in these crude extracts. This study will be helpful in exploring the suitable environmental friendly fungicides to be used against plant pathogenic fungi.

Key words: Antifungal activity, Crude extracts, Algae, Plant pathogenic fungi, GC-MS.

Plant pathogenic fungi are responsible for pre and post-harvest diseases and considered as the major cause of yield losses in numerous economically important crops[1]. Presently the most convincing way of managing the fungal diseases are fungicides. To minimize the role of chemical pesticides in agriculture it is necessary to explore alternatives. There is a growing demand for sound, biologically-based pest management practices. The environmental awareness has encouraged the plant pathologist to evaluate natural compounds for use as a biological control of plant pathogenic fungi. Many algal species such as *Anabaena* spp.[2,3]. *Scytonema* spp. [4] and *Nostoc* spp. [5,6] have been reported to be efficient in the control of several soil borne fungi. Cyanobacteria and algae treated seeds were protected from *Fusarium* sp., *Pythium* sp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum*[7,6]. The algal extract of *Hydrodictyon reticulatum*, *Schizomeris leibleinii*, *Spirogyra plena* and *Plectonema gracillimum* showed absolute inhibition of mycelial growth of plant pathogenic fung[8].

The algae are a rich and almost untouched source of innumerable biologically active products[7]. Maximum species of algae are reported from freshwater or marine habitats, but around 147 genera have also been mentioned from soil[9]. The studies on biologically active products from algae

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largely deal with human pathogenic bacteria/fungi and only some with plant pathogenic fungi. Moreover research on isolation and identification of biologically active compounds from algae are confined to the marine algae[7,10]. Therefore, in the present study we evaluated the antifungal potential of crude extracts of *Dunaliella salina* and *Phormidium autumnale*, which were isolated from the desert of Saudi Arabia against plant pathogenic fungi. Further, gas chromatography-mass spectrometry (GC-MS) was performed to find out the chemical composition of biologically active compounds.

MATERIALS AND METHODS

Cultivation of Algal Species

Two species of algae (*Phormidium autumnale* and *Dunaliella salina*) were procured from the Department of Botany and Microbiology, King Saud University. The test species of algae used in the present study were previously isolated and identified from different desert soils of Saudi Arabia. All species used were sub cultured in BG 11 nutrition media[11] and allowed to flourish at 20-30°C under constant light for 2-4 weeks. Cells of the active growing test algae were harvested by filtering through Whatman no. 1 filter paper and the extracted biomass applied for extraction.

Preparation of the Algal Crude Extracts

For the extraction of metabolites 5 g each of the test algae biomass was mixed with 100 ml of methanol: acetone: diethyl ether (5: 2: 1) (Sigma Aldrich, USA) in a glass flask and shaken for 3 days at 20 °C. The mixture was separated from biomass through filtration with Whatman no. 1 filter paper. The extract was evaporated to dryness by keeping it in the water bath at 40 °C under the fume hood. The obtained residue was dissolved in 2 ml distilled water to get the final concentration of 50 mg/ml of the crude extract. The same procedure applied to the solvent acetone, methanol and water[12].

Antifungal Assay

Pure cultures of fungal strains, *F. oxysporum, Fusarium* sp., *F. solani, A. alternata,* and *Cladosporium* sp. were used in the present study. Details of their source and host are provided in the Table 1. Stock cultures of test fungi were maintained on potato dextrose agar (PDA) (Scharlau Chemie, Spain) slants and were stored at 4 °C.

Antifungal activity of algae extracts were tested by agar well diffusion method as well as by food poisoning method.

Agar well diffusion method

The antifungal activity was evaluated by measuring diameter of inhibition zone, formed around the well. From the plates of 5 days old actively growing test fungi the spores were collected and suspended in sterile distilled water @ 1x 10^6 spores/ml. Spores of test fungi were spread on the PDA with the help of sterilized cotton swab. The crude extracts (50 µl) were placed in wells of 5mm made on the pathogen inoculated agar plates. Wells containing only solvent served as the control. Plates were incubated for 3 days at 28±2°C, and inhibition zone of mycelial growth around the wells were measured.

Table 1. Host and source of plant pathogenic fungi

Name of Plant Pathogenic Fungi	Host	Source
Fusarium oxysporum	*Lavandula pubescens*	Department of Botany and Microbiology, King Saud University (ITCC. I.D. No.7532.09)
Fusarium sp.	*Solanum lycopersicum*	Department of Botany and Microbiology, King Saud University, (ITCC. I.D. No. 8189.11)
Fusarium solani	*Solanum lycopersicum*	Department of Botany and Microbiology, King Saud University
Alternaria alternata	*Verbesina encelioides*	Department of Botany and Microbiology, King Saud University, (ITCC. I.D. No.7912.10)
Cladosporium sp.	*Capsicum* spp.	Department of Botany and Microbiology, King Saud University

Food poisoning method

To determine the percent growth reduction of plant pathogenic fungi by crude extracts of tested algae, food poisoning method was employed. It was performed by using Petri plates containing 15 ml PDA medium supplemented with crude extract. The pathogenic fungi were point inoculated in above medium individually and incubated at 28° C for 72 hr. The diameter of mycelial colony developed on the crude extract containing PDA plates was compared with the diameter of colony obtained on control plates (devoid of the crude extract). The inhibition of fungal growth was calculated by the following formula:

$$I = (C-T/C) \times 100$$

where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate.

GC-MS analysis of Algal Crude Extracts

The crude extracts of three algae which showed strong positive antifungal activities were analyzed for its chemical composition. The analysis was done by using Perkin Elmer (Clarus 500, USA) gas chromatography coupled with (Clarus 500, USA) mass spectrometer (MS) equipped with RTx-5 column (30x0.32nm). The oven temperature was initially held at 75°C for 2 min, then increased to 75 to 175°C at a rate of 50°C per min and finally held at 175°C for 7 min. Helium (3 ml/min) was used as a carrier gas. Neither internal, nor external chemical standards were used in this chromatographic analysis. Interpretation of the resultant mass spectra were made using a computerized library-searching program (NIST database) and by studying the fragmentation pattern of such compound resulted from mass spectrometry analysis. Concentration of such compound was calculated by the following formula:

$$\text{Compound conc. percentage} = [P1/P2] \times 100$$

where, P1 is the peak area of the compound and P2 is whole peak areas in the fractionated extracts.

RESULTS

The antifungal activity of water, methanol, acetone and mixture of methanol, acetone and diethyl ether extracts of *Phormidium autumnale* and *Dunaliella salina* were assayed against *Alternaria alternata, Cladosporium* sp., *Fusarium solani, Fusarium sp.* and *Fusarium oxysporum*.

Results presented in Table 2 show that all extracts were able to inhibit the growth of one or more than one plant pathogenic fungi, however none of the extract was found effective against *A.*

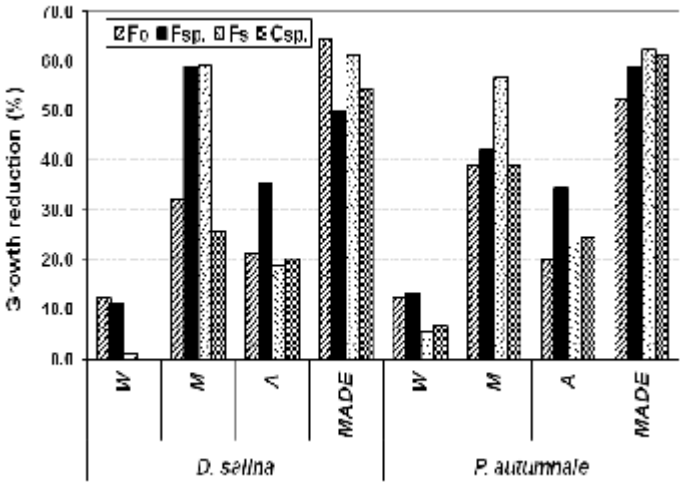
Table 2. Antifungal activity of crude extracts of *P. autumnale* and *D. salina* against plant pathogenic fungi

Algae	Extracts	Plant pathogenic fungi				
		F. oxysporum	*Fusarium* sp.	*F. solani*	*A. alternata*	*Cladosporium sp.*
		Diameter of zone of inhibition (mm)				
D. salina	Water	8.7±0.58	8.3± 0.58	6.3± 0.58	-	6.0± 0.0
	Methanol	14.7±0.58	22.7± 0.58	22.7± 0.58	-	12.7± 0.58
	Acetone	11.3±0.58	15.7± 0.58	10.7± 0.58	-	11.0± 0.0
	M ethanol, Acetone & Diethyl ether	24.3±0.58	20.0± 0.0	23.3± 0.58	-	21.3± 0.58
P. autumnale	Water	8.7±0.58	9.0± 1.0	6.7± 0.58	-	7.0± 0.0
	Methanol	16.7±0.58	17.7± 0.58	22.0± 0.0	-	16.7± 0.58
	Acetone	11.0± 0.0	15.3± 0.58	12.0± 0.0	-	12.3± 0.58
	M ethanol, Acetone & Diethyl ether	20.7±0.58	22.7± 0.58	23.7± 0.58	-	23.3± 0.58

Zone: mean ±SD for N = 3

alternata. Extract with combination of the three solvents methanol, acetone and diethyl ether, gave the highest bioactivity, followed by methanol, acetone and water. In the present study, methanol: acetone: diethyl ether extracts of *P. autumnale* and *D. salina* recorded the strong antifungal activity against *F. oxysporum, Fusarium* sp.*, F. solani,* and *Cladosporium* sp. Methanol extract of *D. salina* exhibited strong inhibition against *Fusarium sp.* and *F. solani;* weak inhibition against *F. oxysporum* and *Cladosporium* sp. Meanwhile, methanol extract of *C. vulgaris* was moderately effective in inhibiting the growth of *F. oxysporum* and *Fusarium* sp. whereas, growth of *Cladosporium* sp. and *F. solani* was weakly inhibited by the same extract. Methanol extract of *P. autumnale* strongly inhibited the *F. solani;* moderately inhibited *Fusarium* sp., *F. oxysporum* and *Cladosporium* sp. The acetone extract of both tested algal species has showed weak to moderate activity against tested plant pathogenic fungi except *A. alternata*. Whereas, water extracts of both algae showed weak zone of inhibition against all plant pathogenic fungi.

Fig. 1 show the percent reduction in the mycelial growth of plant pathogenic fungi. It may be noted that the percent growth reduction of *A. alternata* was not calculated, as none of the algal extract was able to inhibit the growth of *A.*



Fo: *Fusarium oxysporum;* Fsp: *Fusarium sp.;* Fs: *Fusarium solani;* Csp.: *Cladosporium* sp.
W: Water; A: Acetone; M: methanol; MADE: Mixture of acetone, methanol and diethyl ether

Fig. 1. Effect of crude extracts of *P. autumnale* and *D. salina* on the percent growth reduction of plant pathogenic fungi

alternata. It has been observed that the extract with combination of the three solvents methanol, acetone and diethyl ether of *D. salina* gave the maximum percent reduction of the mycelial growth of *F. oxysporum* (64.4%), next it was *F. solani*, followed by *Cladosporium* sp. and *Fusarium* sp. Whereas, Methanol:Acetone:Diethyl ether of *P. autumnale* showed maximum mycelial growth reduction of *F. solani* (62.2%), followed by *Cladosporium* sp. (61.2%), *Fusarium* sp. (58.9%) and *F. oxysporum* (52.2%). However, water extract of *D. salina* registered almost negligible reduction in the mycelial growth of *Cladosporium* sp (0.1%). The result of percent reduction in the mycelial growth of plant pathogenic fungi by algal extracts was found in synchronisation with the result of well diffusion method. Further, it can be noted that these extracts were much more effective in controlling the growth of genera *Fusarium* than others.

The GC-MS analysis was performed to determine the chemical composition of crude extract of tested algal species. Chemical composition and concentrations of the analyzed fractions are presented in Table 3. The main component in the crude extracts of *P. autumnale*

Table 3. The GC-MS analysis of crude extracts of *P. autumnale* and *D. salina*

Algal species	Compound	Rev	Bioactive compounds reported earlier
P. autumnale	Boronic acid, Ethyl-, Dimethyl ester	843	
	2-Butanol, 3-Methyl-, (S)-	882	
	2-Butanol, 3-Methyl-	862	
	Butanal	874	Gregory *et al.*,[31]
	3,4-Hexanediol, 2,5-Dimethyl-	772	
	2-Thiophenecarboxylic acid, 5-(1,1-Dimethylethoxy)-	854	
	Cyclopropanepentanoic acid, 2-Undecyl-, Methyl Ester, Trans-	804	
	1,6-Anhydro-3,4-Dideoxy-.Beta.-D-Gluco-Hexopyranose	773	
	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	903	
	1-Hexyl-2-Nitrocyclohexane	917	
	1-Hexyl-1-Nitrocyclohexane	870	
D. salina	2-Aminononadecane	837	
	1,6;3,4-Dianhydro-2-Deoxy-.Beta.-D-Lyxo-Hexopyranose	792	
	Octanal	785	Singer, [30]
	2-Hexanol, Acetate	811	
	3-Methyl-2-(2-Oxopropyl)Furan	909	
	2-Octadecyl-Propane-1,3-Diol	888	
	1-Hexyl-2-Nitrocyclohexane	906	
	Pentadecanal-	887	

was 1-Hexyl-2-Nitrocyclohexane (91.7%) and *D. salina* was 3-Methyl-2-(2-Oxopropyl) Furan (90%). The biomedical values compound Butanal and Octanal were also detected in the crude extract of *P. autumnale* and *D. salina* respectively.

DISCUSSION

The result clearly showed that all the extracts of algal species had the potential to inhibit the growth of one or more than one tested plant pathogenic fungi but none of them were found to be fungicidal. Methanol: acetone: diethyl ether extract of different algae species were observed highly effective in controlling the growth of most of the plant pathogenic fungi. However the affected fungal species were not equally susceptible to the bioactive compounds of algal species; the reason for this may be the phylogeny of the microorganism species[13]. The difference in antifungal activity was noticed between different extracts within the same algal species. This suggested that the effectiveness of algal bioactivity depends on the type of solvents used in extraction[14,15]. All these solvents showed the most pronounced inhibitory effect against plant pathogenic fungi[7,16,17]. However, the extract used in our study failed to control the growth of *A. alternata,* on the contrary these extract had stimulatory effect on the fungus. Recently, Kamble *et al.*[8] also reported that *Hydrodictyon reticulatum, Schizomeris leibleinii, Spirogyra plena* and *Plectonema gracillimum aqueous extract* had inhibitory effect on *A. alternata*, *A. flavus* and *F. roseum*, whereas extract of *Nitella batrachosperma*, *S. platensis* and *P. corium* stimulated the growth of same plant pathogenic fungus.

The data show that nearly all the extracts of algal species gave positive results but the effectiveness of these extracts varied considerably. Antimicrobial activity of methanol, acetone and diethyl ether extracts of blue green algae and various algae have been reported earlier[12, 18-20]. The antimicrobial activity of ethanol, acetone, diethyl ether and methanol extracts of *Anabaena oryzae*, *T. ceytonica, S. platensis, C. pyrenoidosa* and *Scenedesmus quadricauda* was reported against *A. niger*, *A. flavus*, *Penicillium herquei*, *F. moniliforme*, *Helminthosporium* sp., *A. brassicae*,

Saccharomyces cerevisiae, *Candida albicans*[16]. *In vitro* and *in vivo* fungal growth of *F. oxysporum* f. sp. *lycopersici* was significantly inhibited by methanol extract of *N.* commune[21]. Acetone extract of *P. corium,* methanol extract of *Lyngbya martensiana* and diethyl ether extract of *Microcystis aeruginosa* gave the maximum inhibition of the growth of tested fungi[22]. Our present results are in line with these investigations.

Recently, Al-wathnani *et al.*[12] studied the antibacterial activity of different extracts of *S. platensis, N. linckia, P. autumnale, T. distorta, M. aeruginosa, C. vulgaris* and *D. salina*. They observed that the methanol, acetone and diethyl ether mixture was the most effective solvent system among the others and showed strong activity against *S. sonnei*, *S. aureus,* and *B. subtilis*. Whereas ethanol extract of *P. autumnale* was found effective against only *C. albicans*.

The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes – including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons[23,24]. The antimicrobial activity observed in various pressurized extracts from *D. salina* was suggested to be due to several fatty acids or due to other compounds as á- and â-ionone, â-cyclocitral, neophytadiene and phytol[25]. Pressurized liquid ethanol extracts of *H. pluvialis* were assayed against *C. albicans* and *A. niger*; all extracts showed antifungal activity against the former, but not against later. Butanoic acid and methyl lactate were claimed to be the main compounds responsible for such antifungal activity[26]. Some compounds detected in the crude extracts such as Butanal and Octanal have been reported for biomedical values, these compounds may have played some role in the inhibition of fungal growth[27,28].

CONCLUSION

The findings of the present study clearly showed that *P. autumnale* and *D. salina* have the potential to be utilized as biocontrol agents. However, further studies can be done to identify the compounds directly responsible for antifungal properties and to determine the *in vivo* potential of these compounds, so that these can be formulated into environmental friendly fungicides.

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REFERENCES

1. Fletcher, J., Bender, C., Budowle, B., Cobb, W.T., Gold, S.E., Ishimaru, C.A., Luster, D., Melcher, U., Murch, R., Scherm, H., Seem, R.C., Sherwood, J.L., Sobral B.W., Tolin, S.A. Plant pathogen forensics: capabilities, needs and recommendations. *Mol. Biol. Rev.,* 2006; **70**: 450–71.
2. Moore, R.E., Patterson, G.M.L., Myndrese, J.S., Barchi, J. Jr., Norton, T.R. Toxins from cyanophyte belonging to the Scytonematoceae. *Pure Appl. Chem.,* 1986; **58**: 263-271.
3. Farnkmolle, W.P., Larsen, L.K., Caplan, F.R., Patterson, G.M.L., Knubel, G. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. I. isolation and biological properties. *J. Antibiot.,* 1992; **45**: 1451-7.
4. Chetsumon, A., Fujieda, K., Hirata, K., Yagi, K., Miura, Y. Optimization of antibiotic production by the cyanobacterium Scytonema sp. TISTR 8208 immobilized on polyurethane foam. *J. Appl. Phycol.,* 1993; **5**: 615-622.
5. Bloor, S., England, R. R. Antibiotic production by cyanobacterium *Nostoc muscorum*. *J. Appl. Phycol.,* 1989; **1**: 367-72.
6. Kim, J.D., Lee, C.G. Diversity of heterocystous filamentous cyanobacteria (blue-green algae) from rice paddy fields and their differential susceptibility to ten fungicides used in Korea. *J. Microbiol. Biotechnol.,* 2006; **16**: 240-5.
7. Kulik, M. M. The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant Pathol.,* 1995; **101**: 585-99.
8. Kamble, S.M., Rokde, A.U., Ashok, M.C. Antifungal activity of algal extracts against plant pathogenic fungi. *Int. Multidis. Res. J.,* 2012; **2**: 23-4.
9. Metting, B. The systematics and ecology of soil algae. *Bot. Rev*., 1981; **47**: 195-311.
10. Amaro, H.M., Guedes, A.C., Malcata, F.X. Antimicrobial activities of microalgae: an invited review. In: *Science against microbial pathogens: communicating current research and technological advances,* A. Méndez Vilas (Ed.), Formatex; Badajoz, Spain, 2011; pp. 1272-1280.

11. Rippka, R., Deruelles J., Waterburg, J.B., Herdman, M., Stanier, R.Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteira. *J. General Microbiol.,* 1979; **111**: 1-16.
12. Al-Wathnani, H., Ara, I., Tahmaz, R.R., Bakir, M.A. Antibacterial activities of the extracts of cyanobacteria and green algae isolated from desert soil in Riyadh, Kingdom of Saudi Arabia. *Afr. J. Biotechnol.,* 2012; **11**: 9223-9.
13. Philip, K., Sinniah, S.K., Muniandy, S. Antimicrobial peptides in aqueous and ethanol extracts from microbial, plant and fermented sources. *Biotech.,* 2009; **1**: 1-16.
14. Mian, P., Heilmann, J., Burgi H.-R., Sticher, O. Biological screening of terrestrial and freshwater cyanobacteria for antimicrobial activity, brime shrimp lethality and cyanotoxicity. *Pharm. Biol.,* 2003; **41**: 243-7.
15. Salvador, N., Gomez-Garreta, A., Lavelli, L., Ribera, L. Antimicrobial activity of Iberian macroalgae. *Sci. Mar.*, 2007; **71**: 101-13.
16. Rania, M.A.A.; Taha, H.M. Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by placket-burman design for antimicrobial activity of S*pirulina platensis*. *Global J. Biotechnol. Biochem*., 2008; **3**: 22-31.
17. Cosveanu, A., Axine, O., Iacomil, B. Antifungal activity of macroalgae extracts. Scientific papers, UASVM Bucharest, Series A, Vol. LIII, 2010; pp. 442- 447.
18. De Mule, M., Caire, Gde., Cano, Mde., Haperin, D. Bioactive compound from *Nostoc muscorum* (cyanobacteria). *Cytobios,* 1991; **66**: 169-72.
19. Tassara, C., Zaccaro, M.C., Storni, M.M., Palma, M., Zulpa, G. Biological control of lettuce white mold with cyanobacteria. *Int. J. Agric. Biol.,* 2008; **10**: 487-92.
20. Ostensvik, O., Skulberg, O.M., Underdal, B., Hormazabal, V. Antibacterial properties of extracts from selected planktonic fresh water cyanobacteria- a comparative study of bacterial bioassays. *J. Appl. Microbiol.,* 1998; **84**: 1117-24.
21. Kim, J., Kim, J.D. Inhibitory effect of algal extracts on mycelial growth of the tomato-wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici*. *Mycobiol.,* 2008; **36**: 242-8.
22. Madhumathi, V., Deepa, P., Jeyachandran, S., Manoharan, C., Vijayakumar, S. Antimicrobial activity of cyanobacteria isolated from freshwater lake. *Int. J. Microbiol. Res.,* 2011; **2**: 213-6.
23. Mayer, A.M.S., Hamann, M. Marine pharmacology in 2001–2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp. Biochem. Phys. C,* 2005; **140**: 265-86.
24. Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcão, V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P., Pinto, E. Metabolites from algae with economical impact. *Comp. Biochem. Phys. C,* 2007; **146**: 60-78.
25. Herrero, M., Ibañez, E., Cifuentes, A., Reglero, G., Santoyo, S. *Dunaliella salina* Microalga pressurized liquid extracts as potential antimicrobials. *J. Food Prot.,* 2006; **69**: 2471-7.
26. Santoyo, S., Rodríguez-Meizoso, I., Cifuentes, A., Jaime, L., García-Blairsy, R.G., Señorans, F.J., Ibáñez, E. Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. *LWT-Food Sci. Technol.,* 2009; **42**: 1213-8.
27. Singer, M.S. Analysis of the molecular basis for octanal interactions in the expressed rat I7 olfactory receptor. *Chem. Sense,* 2000; **25**: 155-65.
28. Gregory, N., Craggs, L., Hobson, N., Krogh, C. Softening of cattle hoof soles and swelling of heel horn by environmental agents. *Food Chem. Toxicol.,* 2006; **44***:* 1223-7.