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Green Biocides against Sulphate Reducing Bacteria and Macrofouling Organisms

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When waterborne bacteria congregate in sufficient numbers they form a film on the surface of pipelines, tanks, and industrial equipments. Sulfate-reducing bacteria (SRB) are the major contributors to microbial influenced corrosion (MIC), particularly in environments with high sulfate concentrations such as seawater. More problems occur when the biofilm builds up biofouling which is composed mainly of macrofouling organisms such as mollusks, barnacles and mussels. This work presents the results of biocidal effects of some readily available natural non-edible domestic wastes; the waste bitter water extract of Egyptian lupine seeds (L) and the hot water extracts of orange (O) and mandarin (M) peels against planktonic SRB and (*Brachidontes variabilis*). The three natural extracts express good biocidal activity against SRB and *B. variabilis* and show low toxic effect on non-target sea organisms (e.g. isopoda, amphipoda and decapoda) compared to chemical biocides reported in literature.

Key words: Biocides, sulphate reducing bacteria, macro-biofoulants, biomass extracts, toxicity, non-target sea organisms.

Metal surfaces immersed in seawater undergo a sequence of biological and inorganic changes that lead to biofouling and corrosion[1]. The biofouling process involves many interesting steps, from initial conditioning of the surface by organic and inorganic molecules to the colonization of microorganisms (microbial biofouling) and finally establishment of macrorganisms (macrobial biofouling)[2]. Microbiologically influenced corrosion (MIC) or biocorrosion results from the attachment and activities of aerobic bacteria and anaerobic bacteria mainly sulfate-reducing bacteria (SRB) on metal surfaces[3,4].

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The accumulation of macrofouling deposits (barnacles, hydroids, mussels, clams, etc.) impacts the performance of all materials exposed to marine environments. Most marine fouling invertebrates have a larval or pseudo larval form that is released into the water and although they possess some mobility in the water, they are carried by the currents which facilitate their distribution away from their place of origin, reaching their adult stage in the pipes and consequently result in clogging problems[5]. Predominant macro-biofoulants belong to the following main genera: *Brachidontes, Mytilus, Perna, Dreissena, Modiolus* and *Corbicula.*

Biofouling and MIC control methods can be broadly classified into five categories: mechanical, physical, thermal, paints and coatings, and finally chemical methods. Compared to other methods, the use of chemicals is the most widespread approach for controlling biofouling.

Chemical control compounds may be broadly classified as metals (Cu, Zn), oxidizing materials (chlorine, chloramines, bromines, chlorine dioxide, hydrogen peroxide, ozone and potassium permanganate) and non-oxidizing materials (quaternary amines, tertiary amines, glutarldhyde, etc.). Despite their remarkable inhibitive effect, the application of chemical biocides has been decreased due to their toxic nature[6]. Therefore, the use of biocides has gained global interest. Hence, it is a goal to make use of environmental friendly and less toxic/non toxic, extracts of naturally occurring materials as biocides[7-10].

The aim of this study is to develop one or more environmentally benign "Green" products that can be applied to eradicate planktonic SRB causing MIC and *Brachidontes variabilis* as one of the predominant macro-biofoulants. Their safety has been also studied on representatives of non-target sea organisms (isopodes, amphipodes and decapodes) which were selected because of their well-known high sensitivity.

MATERIALS AND METHODS

Preparation of biocides

The biocides used were hot water extracts of some readily available natural domestic wastes; the waste bitter water of Egyptian lupine (L), orange (O) and mandarin peels (M). Stock solutions of the extracts were prepared as follows: 100 g of orange and mandarin peels and Egyptian lupine were refluxed individually in 250 mL double distilled water for three hours at 100 °C. The refluxed solutions were filtered to remove any substrate debris and then dried under vacuum to a constant weight. The yield of the obtained extracts was calculated according to the following equation by[11]:

$$Yield\ \%\ (w/w) = \frac{\text{Weight of recovered extract}}{\text{Weight of dry biomass}} \times 100$$

Extracts were stored in dark and well sealed glass vials at 4°C until its chemical characterization and use to prepare the required concentrations (mg/L) to study their biocidal properties.

Identification of the chemical composition of each extract

An Agilent-Technologies 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector; GC/MS was used to identify the chemical composition of each extract (In: Holding Company for Drinking Water and Sanitation, Cairo, Egypt). Compounds were separated onV-5 MS capillary column (30 mm x 0.25 mm, film thickness 0.25 µm). A sample of 1.0 µL was injected in the split mode with split ratio 100:1. An electron ionization system, with ionization energy of 70 eV, was used for GC/MS detection. The column oven temperature was programmed from 40°C to 280°C at the rate of 5°C min^{-1}, initial and final temperatures were held for 3 and 5 minutes, respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass scanning range was varied over 50 –550 m/z while injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Compounds were identified using their MS data relevant to those in the (National Institute of Standards and Technology) NIST mass spectral library and/or published mass spectra in literature.

SRB bioassay

Microorganisms

In this study, two standard strains: non-halotolerant bacterium, *Desulfovibrio sapovorans* (ATCC 33892) and halophilic bacterium *Desulfovibrio halophilus* (ATCC 51179) and three mixed cultures of SRB (SRB1, SRB2 and SRB3) collected from different Egyptian oil fields were used. The salinity of the collected mixed cultures was measured as NaCl using ion chromatography (Dionex 1100, USA) and recorded 6500, 10000 and 30000 mg/L, respectively.

Enrichment and enumeration of SRB

Postgate medium B (PMB)[12] was used for enrichment of SRB, optimum salinity of each SRB culture was adjusted by adding NaCl before sterilization. Salinity was adjusted to 10,000 and 60,000 mgL^{-1} for ATCC 33892 and ATCC 51179, respectively while that for SRB1, SRB2 and SRB3 was adjusted to 6500, 10000 and 30000 mg/L, respectively.

The enumeration of planktonic SRB in all experiments was done using the Most Probable Number (MPN) technique according to ASTM D-4412 (1990)[13]. MPN vials were incubated at 30°C for 21 d. In each inoculated vial, growth of sulfate reducers was indicated by the formation of a black

ferrous sulphide (FeS) precipitate.

The MPN method was used to study the biocidal effect of the three tested extracts, each extract was added individually to sterile PMB vials in a final concentration range of (100-10000 mg/L). Then inoculated with SRB under study. Non inoculated vials with biocides were used as -ve control, while inoculated vials without biocides were used as + ve control.

Macrobial biofouling assay

Adult marine mussels of *Brachidontes variabilis* were sampled from Suez Gulf water at Attaqah Mountain Beach (AMB), Suez, Egypt. Before the test study, the collected mussels were reared for two wks for adaptation, in glass aquaria of the dimensions 70 x 40 x 40 cm^3 filled with seawater collected from AMB (salinity 43,000 mg/L). Continuous aeration was provided using air compressors, the water was changed twice a week and dead bivalves were removed periodically. Adult mussels of the size 0.5-1.0 cm were used in all experiments. For all tests, ten adult mussels were placed in 1L beakers containing 500 mL seawater (collected from AMB) with the extracts under study in final concentrations 100-8000 mg/L. Control samples were applied using the same conditions but without tested extracts. The test duration was 15 d. Mortalities were observed periodically each 24 hrs. Mortality percentages were then calculated using Abbott's formula[14] as follows:

$$C = \frac{100\ (O-X)}{(100-X)}$$

where, C is the corrected mortality percentage, O and X are the percentage of observed mortality in biocides- injected beakers and control beakers, respectively.

Graphical analysis plotting percentage mortality as the ordinate against log concentration as abscissa was done using Probit method[15]. LC_{50} (lethal concentration producing 50% mortality) for 48 hrs was calculated for each extract. All studies were carried out in triplicates.

Toxicity against non-target sea organisms

Adult samples of each of the families: isopoda, amphipoda and decapoda as representatives of non-target sea organisms were aqua cultured using natural seawater from the AMB. Continuous aeration was maintained via air compressors. Regular water change was applied twice a week.

Twenty adult organisms of the selected families were placed in 1L beakers containing 500 mL natural seawater (collected from AMB) with the extracts under study with different concentrations (100-8000 mg/L). Control samples were applied using the same conditions but without test extracts and test duration was 8 d in all experiments. Mortalities were observed daily. Mortality percentages were then corrected using Abott's formula as previously mentioned. All studies were carried out in triplicates.

RESULTS AND DISCUSSION

Percentage yield of each extract

Hot water extract of orange peels and waste bitter water of Egyptian lupines yielded approximately the same amount of dry weight, recording 12 % (w/w), while hot water extract of mandarin peels yielded about 18 % (w/w).

Chemical composition of the extracts

GC/MS was used to depict the chemical composition of each extract (biocide). As revealed from Fig. (1a) and Table (1) lupanine was the major compound detected in the waste bitter water extract of Egyptian lupine.

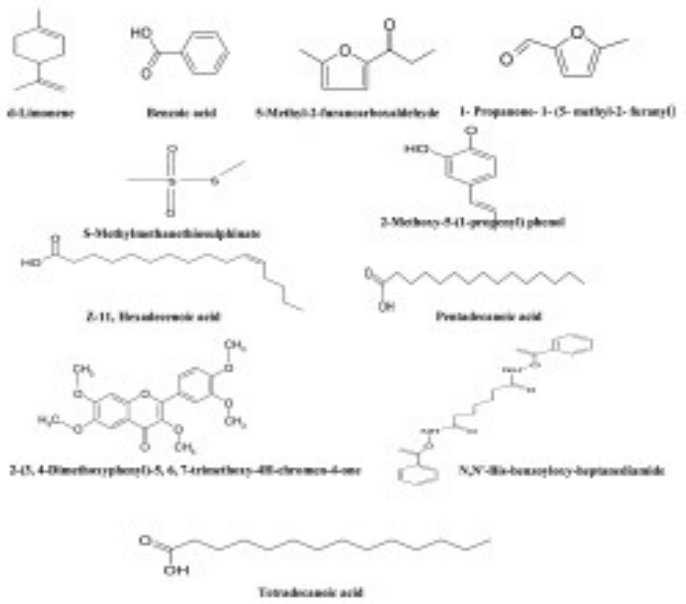
Seventeen compounds (Scheme 1 and 2) have been detected in hot water extract of orange peels. The following five compounds represented the major peaks as shown in Fig. (1b) and Table (2) and they are: Itaconic acid anhydride; 2, 4-dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one; 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP); 2, 3-dihydro-benzofuran and 2-methoxy-4-vinylphenol (4-vinyl guaiacol). Other twelve minor compounds have been also detected: glutaric

Table 1. Chemical composition of the Egyptian lupine bitter water extract as identified by GC/ MS

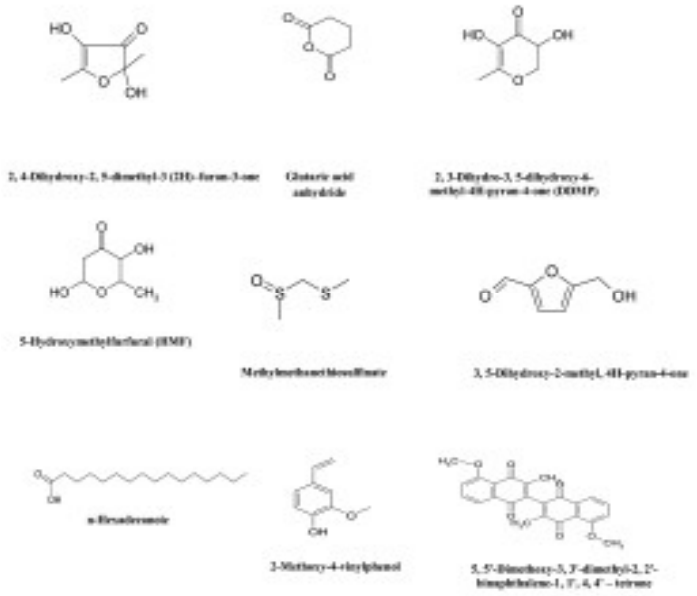
Peak number	Constituents	RT(min)	MS fragmentation pattern(m/z)
1	Lupanine	43.206	248, 233, 219, 205, 191, 145, 136

acid anhydride; 1,2- cyclopentanedione; 3,5-dihydroxy-2-methyl, 4H-pyran-4-one; methyl methane thiosulphinate (MMTSO); 5-hydroxymethylfurfural (HMF); eugenol; 2-(3,4-dimethoxyphenyl)-6-methyl-3,4-chromanediol; n-hexadecanoic acid; 13-heptadecyn-1-ol; phenol, 2,2'-methylenebis (6-tert-butyl-4-ethyl); androst-4-ene-3,17-dione, 12-[(trimethylsilyl)oxy]-, bis(O-methyloxime) and 5,5'-dimethoxy-3,3'-dimethyl-2,2'-binaphthalene-1,1',4,4'- tetrone.

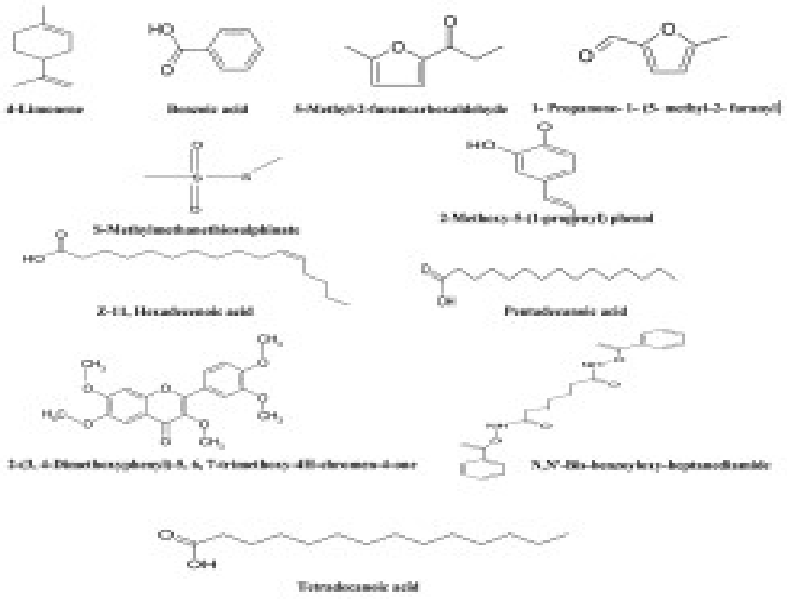
Twenty compounds (Scheme 1 and 3) have been detected in the hot water extract of mandarin peels as shown in Fig. (1c) and Table (3). The following eight compounds represent the major components: d-Limonene; 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP); 3,5-dihydroxy-2-methyl-4H-pyran-4-one; 5-



Scheme 1. Chemical structures of main common components of orange and mandarin peels hot water extracts



Scheme 2. Chemical structures of the main components of hot water extract of orange peels



Scheme 3. Chemical structures of the main components of hot water extract of mandarin peels

Table 2. Chemical composition of orange peels hot water extract as identified by GC/MS.

Peak number	Constituents	RT(min)	MS fragmentation pattern(m/z)
1	2,5-Furandione, dihydro-3-methylene (Itaconic acid anhydride)	14.900	68, 53, 44, 41, 40
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	15.420	144, 101, 73, 55
3	Glutaric acid anhydride	15.893	112,84, 66, 55
4	2,3-Dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one(DDMP)	19.116	144, 130, 115, 101, 98, 73
5	1, 2- Cyclopentanedione	19.820	98, 82, 69, 55
6	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	20.168	144, 101, 73, 58, 55
7	Methyl methane thiosulphinate (MMTSO)	20.752	63, 61
8	2,3-Dihydro- benzofuran	21.120	120, 111, 105, 92, 91
9	5-Hydroxymethylfurfural(HMF)	21.820	126, 109, 97, 81, 69, 55, 53
10	2-Methoxy-4-vinylphenol(4-Vinyl guaiacol)	23.195	150, 135, 107, 77
11	Eugenol	25.24	164, 149, 147, 137, 133, 131,103
12	2-(3,4-Dimethoxyphenyl)-6-methyl-3, 4-chromanediol	29.383	270, 180, 165, 151, 137, 119
13	n-Hexadecanoic acid	37.695	256, 227, 213, 185, 157, 143, 129, 115, 97
14	13-Heptadecyn-1-ol	38.474	241, 234, 219, 207, 191, 179, 163, 149, 135, 123, 109, 96
15	Phenol, 2,2'-methylenebis (6-tert-butyl-4-ethyl)	47.254	368, 353, 312, 297, 256, 191, 178, 175, 169, 163, 155, 135, 105, 57
16	Androst-4-ene-3,17-dione, 12-[(trimethylsilyl) oxy]-, bis(O-methyloxime)	73.98	432, 401, 387, 342, 327, 311, 296, 280, 268
17	5,5'-Dimethoxy-3,3'-dimethyl-2,2'-binaphthalene-1,1',4,4'- tetrone	74.46	402, 387, 371, 357, 343, 315, 287, 255, 201

Table 3. Chemical composition of mandarin peel hot water extract as identified by GC/MS

Peak number	Constituents	RT(min)	MS fragmentation pattern(m/z)
1	d-Limonene	14.195	136, 133, 121, 115, 107, 93
2	2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one	15.309	144, 101, 73, 55
3	Glutaric acid anhydride	15.615	112,84, 66, 55
4	S-Methyl methane thiosulphinate(MMTS)	17.021	126, 111, 95, 81, 63
5	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4 one(DDMP)	17.960	144, 130, 115, 101, 98, 73
6	Benzoic acid	19.415	122, 105, 94, 77, 57, 51
7	N,N'-Bis-benzoyloxy- heptanediamide	19.749	143, 122, 105, 94, 77, 51
8	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	20.070	144, 101, 73, 58, 55
9	Methyl methane thiosulphinate (MMTSO)	20.154	63, 61
10	5-Hydroxymethylfurfural(HMF)	21.381	126, 109, 97, 81, 69, 55, 53
11	2-Methoxy-4-vinylphenol(4-Vinyl guaiacol)	23.160	150, 135, 107, 77
12	1- Propanone, 1- (5-methyl-2- furanyl)	27.751	138, 109, 95, 81, 65, 53
13	2-Methoxy-5-(1-propenyl) phenol	29.023	164, 149, 131, 103, 91, 77, 55
14	Tetradecanoic acid(Myristic acid)	34.527	228, 199, 185, 171, 143, 129
15	Pentadecanoic acid	36.476	242, 225, 213, 199, 185, 157, 143, 129
16	Z-11, Hexadecenoic acid	37.572	254, 136, 192, 152, 123, 111, 97
17	n-Hexadecanoic acid	37.799	256, 227, 213, 185, 157, 143, 129, 115, 97
18	5-Methyl-2-furancarboxaldehyde	38.799	110, 109, 81, 55
19	2-(3,4-Dimethoxyphenyl)-5,6,7-trimethoxy-4 H-chromen-4-one	67.266 181	372, 357, 341, 329, 311, 273, 191,
20	5,5'-Dimethoxy-3,3'-dimethyl-2,2'-binaphthalene-1,1',4,4' – tetrone	74.165	402, 387, 371, 357, 343, 315, 287, 255, 201

Table 4. Biocidal activity of the extracts against planktonic non-halotolerant *Desulfovibrio sapovorans* ATCC 33892 and the low salinity mixed cultures SRB1 and SRB2

Extract		Total viable count		
Concentration(mg/L)		ATCC 33892(cells / mL)	SRB1(cells / mL)	SRB2(cells / mL)
	+ ve Control	>24 X 10^6	>24 X 10^6	>24 X 10^6
L	100-1000	>24 X 10^6	>24 X 10^6	>24 X 10^6
	2000	>24 X 10^6	>24 X 10^6	>24 X 10^6
	3000	>24 X 10^6	>24 X 10^6	>24 X 10^6
	4000	4.3 X 10^2	4.3 X 10^2	91
	5000	NIL	9.3	NIL
	6000	NIL	NIL	NIL
O	100-1000	>24 X 10^6	>24 X 10^6	>24 X 10^6
	2000	>24 X 10^6	91	36
	2500	NIL	2.3	NIL
	3000	NIL	NIL	NIL
M	100-1000	>24 X 10^6	>24 X 10^6	>24 X 10^6
	2000	>24 X 10^6	4.6 X 10^3	>24 X 10^6
	2500	NIL	2.3	NIL
	3000	NIL	NIL	NIL

Table 5. Biocidal activity of different extracts against planktonic halophilic *Desulfovibrio halophilus* ATCC 51179 and the high salinity mixed culture SRB3

Extract		Total viable count	
Concentration(mg/L)		ATCC 51179 (cells / mL)	SRB 3(cells / mL)
	+ ve Control	>24 X 10^6	>24 X 10^6
L	100-8000	>24 X 10^6	>24 X 10^6
	9000	36	>24 X 10^6
	10,000	NIL	NIL
O	100-8000	>24 X 10^6	>24 X 10^6
	9000	91	>24 X 10^6
	10,000	NIL	NIL
M	100-8000	>24 X 10^6	>24 X 10^6
	9000	91	>24 X 10^6
	10,000	NIL	NIL

hydroxymethylfurfural (HMF); 2-methoxy-4-vinylphenol (4-vinyl guaiacol); n-hexadecanoic acid and 5-methyl-2-furancarboxaldehyde. Also, twelve other minor compounds have been detected and they are: glutaric acid anhydride; S-methyl methanethiosulphinate (MMTS); benzoic acid; N,N'-bis-benzoyloxy- heptanediamide; methyl methane thiosulphinate (MMTSO); 1- propanone, 1- (5-methyl-2- furanyl); 2-methoxy-5-(1-propenyl) phenol (isoeugenol); tetradecanoic acid (myristic acid); pentadecanoic acid; Z-11, hexadecenoic acid; 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-4H-chromen-4-one and 5,5'-dimethoxy-3,3'-dimethyl-2,2'-binaphthalene-1,1',4,4' – tetrone.

Biocidal activity against planktonic SRB

In laboratory biocorrosion experiments, *Desulfovibrio* species usually serve as model microorganisms[16]. *Desulfovibrio* species have been revealed as the most abundant population in corrosive biofilms[17] and several sulfate reducers isolated from corrosive environments have been identified as *Desulfovibrio* species[18].

In this study, the biocidal effect of three water extracts was assessed against planktonic non-halotolerant *Desulfovibrio sapovorans* (ATCC 33892), halophilic *Desulfovibrio halophilus* (ATCC 51179) and the mixed cultures; SRB1, SRB2 and SRB3.

It was obvious from results listed in Tables 4 and 5 that the biocidal activity of the three extracts against the halophilic Desulfovibrio *halophilus* (ATCC 51179) and the high salinity mixed culture SRB3 was lower than that against the non-halotolerant *Desulfovibrio sapovorans* (ATCC 33892) and low salinity mixed cultures SRB1 and SRB2. There is no recorded biocidal effect at low concentrations (100-1000 mg/L) of the three extracts on non-halotolerant ATCC 33892, low salinity mixed cultures SRB1 and SRB2, while there is no recorded biocidal activity against the halophilic ATCC 51179 and the high salinity mixed culture SRB3 at concentration range (100-8000 mg/L).

Halophilic microorganisms have strategies that allow them not only to withstand osmotic stress, but also to function better in the presence of high salt concentrations, partly due to the synthesis of compatible solutes that allow them to balance their osmotic pressure as well as the enzymes which become active in solutions of very high ionic strength[19]. This might explain their higher resistance to biocides, as they are extremophile microorganisms, which can survive under stress conditions.

Generally the biocidal activity of the three extracts against the non-halotolerant *Desulfovibrio sapovorans* (ATCC 33892) and low salinity mixed cultures SRB1 and SRB2 can be ranked in the following order O>M>L. While, the three tested extracts expressed nearly the same biocidal activity against the halophilic *Desulfovibrio halophilus* ATCC 51179 and high salinity mixed culture SRB3.

There is a sharp decrease in microbial count at high concentration (4000 mg/L) of (L) and the maximum biocidal activity of (L) on non-halotolerant and low salinity cultures SRB1 and SRB2 was recorded at high concentration (≥ 5000 mg/L).

Water extract of orange peels at

concentration of 2000 mg/L showed no biocidal effect on non-halotolerant *Desulfovibrio sapovorans* ATCC 33892 while showed a sharp biocidal activity against low salinity mixed cultures SRB1 and SRB2.

Water extract of mandarin peels at concentration of 2000 mg/L showed no biocidal activity on non-halotolerant *Desulfovibrio sapovorans* ATCC 33892 and low salinity mixed culture SRB2 while showed a high biocidal activity against SRB1.

The maximum biocidal activity of orange and mandarin peels on non-halotoerant ATCC 33892 and low salinity mixed cultures SRB1 and

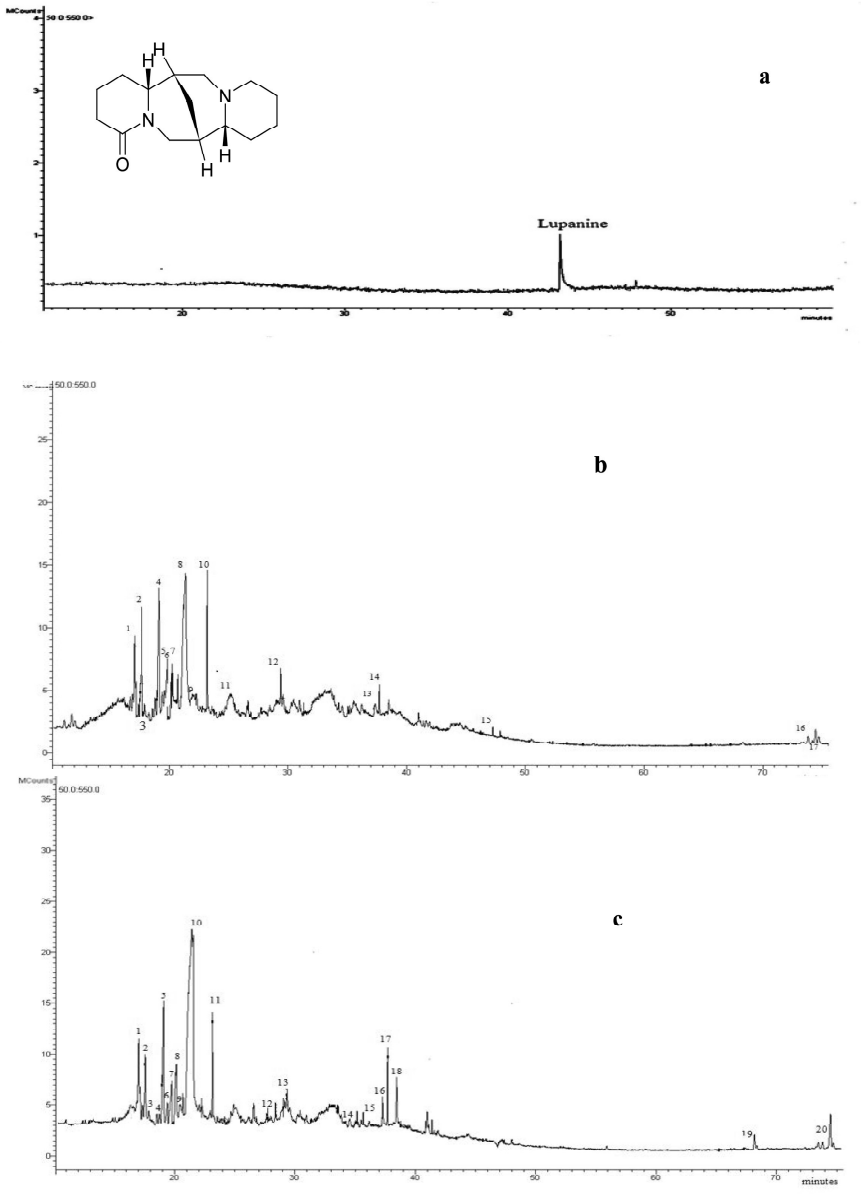


Figure 1. GC-MS chromatogram showing the chemical analysis of the extract of waste bitter water of Egyptian lupines (a), hot water extract of orange (b) and mandarin (c) peels

SRB2 extract was recorded at concentration (≥ 2500 mg/L).

There was a sharp recorded biocidal activity of the three tested extracts at concentration (≥ 9000 mg/L) on the halophilic ATCC 55179. While the concentration 10000 mg/L recorded maximum biocidal activity against halophilic ATCC 51179 and high salinity mixed culture SRB3.

Biocidal activity against *B.variabilis*

Fig. 2 illustrates the biocidal effect of the three extracts on *B.variabilis* as a model organism of macro-biofoulants.

For waste bitter water extract of Egyptian lupine, complete mortality of the mussels under investigation at the concentrations 3000-4000 mg/L was obtained after 2 d. LC_{50} was 2000 mg/L (equivalent to log = 3.3. Particularly, no mortalities were observed at the concentrations 100-500 mg/L which may be referred to low concentrations of biocide. Complete mortality was achieved at the concentrations (1000-2000 mg/L) after 3 d and at 5000-8000 mg/L after 1 d of exposure.

For orange peel extract, total mortality percentage of the tested mussels was achieved at the concentrations 1000-4000 mg/L after 2 d of exposure time and LC_{50} was 1000 mg/L (equivalent to log = 3.0). Furthermore, 20% total mortality was observed at 100 mg/L after 8 d of exposure, while 80% total mortality was obtained at 500 mg/L. Otherwise, no mortalities were observed at the concentrations 5000-8000 mg/L which may be referred to the quenching potentials of the tested extract.

Mandarin peel extract expressed 100% total mortality of *B. variabilis* at the concentrations 3000-4000 mg/L after 2 d of exposure and LC_{50} was at 1400 mg/L(equivalent to log = 3.15). At 100 mg/L, no observed mortality was detected for the test duration which may be referred to lower concentration used. At 500 mg/L, 40% total mortality was obtained after 7 d, while 80% total mortality was recorded at 1000 mg/L after 4 d and

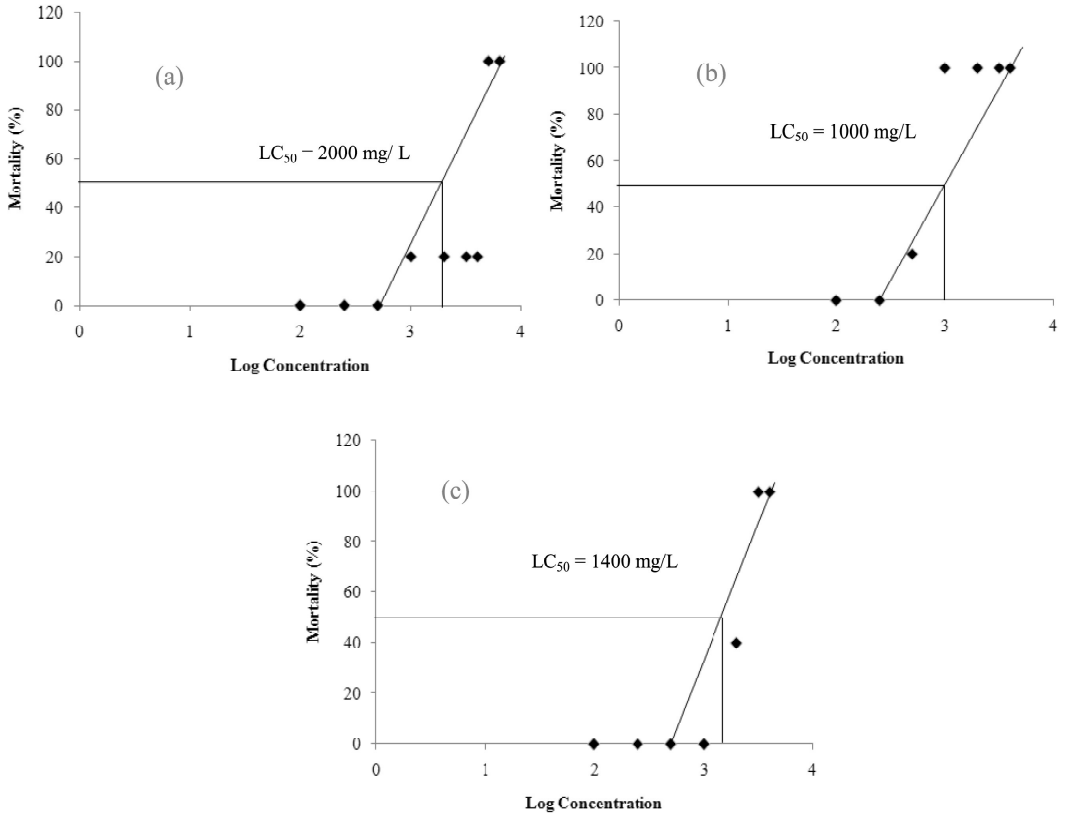


Fig. 2. Mortality % of *Brachidontes variabilis* at different concentrations of Egyptian lupine waste bitter water extract (a), hot water extract of orange (b) mandarin (c) peels after 2 d

100% total mortality was achieved at 2000 mg/L after 3 d. The concentrations (5000-8000 mg/L) caused 100% mortality after 1 d.

It is worth noting that; in case of mandarin peels and Egyptian lupine bitter water extracts, as the concentrations increased, the time of exposure was shortened which is obvious in higher concentration 5000-8000 mg/L.

Concerning mode of action of extracts under investigation on tested SRB and *Brachidontes variabilis*, lupines represent a monophyletic sub tribe of the family Fabaceae. A characteristic trait of all lupines is the production of quinolizidine alkaloids (QAs). Lupanine is one of the most abundant QAs. QAs are known to play a role in ecological interrelationships by expressing chemical defense against phytophagous animals (nematodes, mollusks, insects and vertebrates), microorganisms (bacteria and fungi) and other competing plant species[20]. The main molecular targets which are affected by (QAs) are nicotinic acid, muscarinic acid, acetylcholine receptors[21] as well as Na^+ and K^+ channels[22]. In addition, protein biosynthesis and membrane permeability is modulated at higher concentrations of lupanine[23]. As described by Wink[24], QAs constitute a potential defense system of legumes against mollusks and other herbivores and since they are also effective against bacteria, these compounds have evolved as molecules of general biotoxcity.

One of the possibilities for the action of *Citrus* active constituents is that they exert their bactericiodal effects at the membrane level[25] where they cause an increase in the permeability of the cell membrane by inducing material losses (cytoplasmic), leakage of ions, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore to its death[25-26]. Disruption of membranes results in bacterial cell death, due to proton-motive force depletion[26]. Thus these active constituents can be bactericidal in high concentrations, but can slow the growth of bacteria at lower concentrations (bacteriostatic).

In the present study, the presence of phenols (4-vinyl guaiacol), furanones (2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one), furfurals (5-hydroxymethylfurfural) and saturated fatty acids (hexadecanoic acid), 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and methyl methane thiosulphinate (MMTSO) in orange and mandarin peels hot water extracts might explain their biocidal activity against SRB and macro-biofoulants:

4-Vinyl guaiacol (4-VG) has a smoky, spicy and clove-like aroma. In addition to its commercial value as aroma and flavor substances, it has antioxidant properties[27] and serves as a precursor for the bioproduction of vanillin which is reported to have antimicrobial activity against bacteria[28].

Furanones are natural aroma compounds which occur naturally in fruits such as pineapples and strawberries. Martinelli *et al.,*[29] reported that naturally occurring furanone such as 2, 4-dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one plays a role in inhibiting bacterial infections and biofilm formation.

5-Hydroxymethylfurfural (5-HMF) is an important bio-sourced intermediate, formed from carbohydrates such as glucose or fructose[30]. Sindhu and Manorama[31] reported the presence of 5-HMF in *Polycarpaea corymbosa* (Caryophyllaceae). According to Seidel and Taylor[32] 5-HMF possesses antibacterial and antifungal activities.

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) was first identified in *Phaseolus vulgaris* by Yoshiki et al.[33]. DDMP belongs to the flavanoid fraction, a group of complex natural products of current medicinal interest in which its antimicrobial properties have been recognized by Taylor *et al.*,[34] and Chaturvedi *et al.,*[35].

Seidel and Taylor[32] reported that hexadecanoic acid, which is among the saturated fatty acids, has potential antibacterial activities. Saturated fatty acids are soluble in water at elevated temperature[36] and the hydrophobic groups of those saturated fatty acids play an important role in bioactivity[37].

Marks *et al.,*[38] reported that methyl methane thiosulphinate (MMTSO) is produced as a result of an enzymatic reaction in *Brassica.* According to Small *et al.*,[39], MMTSO has the same functional group and antibacterial activity of allicin which is present in garlic and onion.

Orange peels hot water contained characteristic compounds which might aid in

exerting biocidal activity against SRB and macro-biofoulants: benzofurans are bicyclic ring system with multiple applications. Kamal *et al.*,[40] demonstrated that the compounds having benzofuran nucleus possess broad range of biological activities. Furthermore, Singh *et al.,*[41] reported that phenolic compounds such as eugenol, phenol, 2, 2'-methylenebis (6-tet-butyl-4-ethyl) and 2- (3, 4-dimethoxyphenyl) - 6-methyl-3, 4- chromanediol are among the most active aromatic components and potentially toxic. Phenolic compounds in their many forms are the main components responsible for the functional properties associated with many natural products having antibacterial capacity[42]. In addition, Montemayor *et al.,*[43] revealed that 1, 2-cyclopentanedione is among the phytochemical constituents of *Citrus aurantifolia* which may aid in exerting antimicrobial activity.

Mandarin peels hot water extract also contained some compounds which were reported to have biocidal activity against SRB and macrobiofoulants: limonene is a natural aroma compound. Di Pasqua *et al.*[25] and Gulaykirbalar *et al*.,[44] reported that limonene might exert a strong broad spectrum of antimicrobial activity against Gram positive and Gram negative bacteria. Benzoic acid is found naturally in many plants as an intermediate in the formation of other compounds. It is characterized with highly fragrant odor. According to Papatsiros *et al*.,[45] benzoic acid possesses antibacterial activity against Coliforms (*E. coli*). Similarly, the presence of long chain saturated fatty acids in mandarin peels extract might have also aided to its biocidal activity. Tetradecanoic (myristic) acid was reported by Liu *et al.,*[46] to have biocidal properties against *Alternaria solani* and *Fusarium oxysporum.* Wei *et al.,*[47] reported that pentadecanoic acid found in Malaysian *Andrographis Paniculata* leaf extract has antioxidant activity.

S-Methaneothiosulfinate (MMTS) has been demonstrated by Kyung and Fleming[48] to function as an important antibacterial compound in fresh cabbage. Jadhav *et al.*[49] reported that the tamarind (*Tamarindus indica*) pulp extracts contain 5-methyl-2-furancarboxaldehyde which may help in exerting antimicrobial activity against many pathogenic bacteria.

Phenolic compounds are secondary plant metabolites found in numerous plant species and they play key roles in the biochemistry and physiology of plants. According to Riyaz *et al*.,[50] recent studies indicate that *Citrus* peels yield 1000 folds more phenolic compounds than pulp. Friedman *et al*.,[51] reported that phenolic compounds such as 2-methoxy-5-(1-propenyl) phenol have antibacterial and antifungal activities.

Toxicity against non-target sea organisms

Though the biocides of biological origin are presumably eco-friendly, it is highly preferable to be familiar with their toxicity. Toxicity experiments can give a solid evidence for the biocompatibility of the biocides.

For orange peels hot water extract, safe concentrations against the selected non-target sea organisms were in the range 100-4000 mg/L for the 8 d of exposure. Regarding mandarin peels hot water extract, its safety range was; 100-500 mg/L and 1000-4000 mg/L up to 8 d and 4 d of exposure, respectively. Concerning the extract of waste bitter water of Egyptian lupines, the safe concentrations were 100-1000 mg/L up to 7 d of exposure.

From the foregoing data, the tested extracts showed less toxicity against non-target sea organisms compared to commercial chemical biocides most commonly used such as Bayluscide and copper sulphate. Waller *et al.,*[52] reported that Bayluscide and copper sulphate possessed great toxicity against adult fish as non-target sea organisms after 48 h of exposure. In addition, Hosea and Finlayson[53] reported that copper sulfate applied at a rate of 1 part per million (ppm) was toxic for some non-target fishes.

CONCLUSION

The tested hot water extracts of orange, mandarin peels and the extract of the waste bitter water of Egyptian lupine showed a promising biocidal activity against sulfate reducing bacteria (SRB): the non-halotolerant *Desulfovibrio sapovorans* ATCC 33892, halophilic *Desulfovibrio halophilus* ATCC 51179, low salinity mixed cultures SRB1 and SRB2 and the high salinity mixed culture SRB3 collected from different Egyptian oil fields. The three extracts also showed a good biocidal activity against *Brachidontes variabilis* as an example of macro-biofoulants. The three extracts expressed less toxic effects towards non-target sea

organisms (isopodes, amphipodes and decapodes) relevant to chemical biocides published in literature.

Therefore, this study is an approach to open up a scope for future utilization of the fruit peel wastes and bitter waste water of Egyptian lupine as green agents with biocidal properties against corrosion associated micro- and macro-organisms. The raw biomass of the tested biocides is readily available and can be supplied from unwanted domestic and food industrial wastes.

In Egypt, solid municipal wastes density average 300 Kg/m^3. The organic materials constitute up to 60 % of these wastes. The use of these wastes for production of biocides would offer an effective and promising solution for waste management, microbial induced corrosion (MIC) and biofouling problems in Egypt, therefore such application would have a double positive impact on economic and environmental problems.

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