Available online on 15.03.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Chemical composition and antibacterial activity of *Algerian propolis* against fish pathogenic bacteria

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ABSTRACT

Five different varieties of propolis from four sites from Sétif region (East of Algeria) (Babor, Setif; Ain-Abbassa and El-Hamma), and one site from the center of Algeria (Tizi-Ouzou) were chemically analysed by gas chromatography-mass spectrometry. One hundred and two compounds were identified including aromatic acids, linear hydrocarbons and their acids, terpenes and alcaloïdes. Furthermore, the in vitro bacteriostatic and bactericidal activities of the aqueous extracts were evaluated against one Gram positive (*Bacillus subtilis,* used as probiotics in aquaculture) and two Gram negative (*Vibrio anguillarum* and *Vibrio harveyi*, pathogenic for fish) bacteria. The obtained results showed that all aqueous extracts of propolis inhibit the growth of *B. Subtilis* while the growth inhibition of fish pathogens was achieved when using higher propolis concentrations. These antibacterial properties would warrant further studies on the clinical applications of propolis in aquaculture field.

Keywords: Bactericidal activity; Chemical characterization; Propolis; *Vibrio*.

Article Info: Received 08 Jan 2020; Review Completed 12 Feb 2020; Accepted 19 Feb 2020; Available online 15 March 2020

Cite this article as:

Soltani E, Mokhnache K, Mezaache-Aichour S, Charef N, Haro JPD, Zerroug MM, Esteban MA, Chemical composition and antibacterial activity of *Algerian propolis* against fish pathogenic bacteria, Journal of Drug Delivery and Therapeutics. 2020; 10(2):12-19 http://dx.doi.org/10.22270/jddt.v10i2.3904

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INTRODUCTION

Propolis is a plant resinous substance collected by honeybees (*Apis mellifera*) from cracks in bark and leaf buds of regional macroflora [1, 2]. This substance is masticated with bee salivary enzymes and mixed with bees wax, and used by bees for coating the hive, blocking holes and cracks in the hive and to protecting hive from -invading microbes and insects [2].

Propolis has been much popular as an agent for treatment of many diseases in folk medicine and food supplementary material for human health in the world [3]. Many studies in humans have shown that propolis has antimicrobial, anti-inflammatory, hepato-protective and anti-oxidative effects and stimulates immune system along with many biological activities [4].

Due to the variability of plant sources, the chemical composition of propolis is highly variable within the distinct geographic regions with changeable antimicrobial compounds. For example, flavonoids and cinnamic acid derivatives are found in European propolis samples, while diterpenic acids and prenylatedcoumaric acids in Brazilian ones [5]. More than 300 compounds have been identified in the propolis, including several polyphenols, flavonoids, phenolic acid and their esters, phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids, and others [6]. However, a good number of the studies were limited to some components of interest, particularly flavonoids [7]. However, a detailed investigation of propolis chemical composition can aid in a better understanding of its biological potentials.

To our knowledge, there is very few studies focus on the analysis of the composition and/or possible effects of propolis from Algeria [8,9].Taking into account these previous considerations, the aims of the present investigation were: 1) to report the chemical composition and antibacterial activity of five propolis samples collected from two different geographical regions of Algeria not

previously studied; 2) to analyze the *in vitro* bacteriostatic and bactericidal activities of the aqueous extracts of propolis against one Gram positive bacteria used as probiotics in aquaculture (*Bacillus subtilis*) and two Gram negative bacterial fish pathogens (*Vibrio anguillarum* and *Vibrio harveyi*).The possibility of using Algerian propolis extracts as source of antibacterial natural agents in fish farmed industry is discussed.

EXPERIMENTAL

Propolis samples

Five Algerian propolis raw samples were collected from different geographical region during the years 2011-2013, by scraping the sample off from the frames of beehives. The locations of hives were four sites from Sétif region (East of Algeria) Babor, 2011 (B), Setif, 2012 (S), Ain-Abbassa, 2013 (Ab) and El-Hamma, 2013 (Hm), and one site from Tizi-Ouzou, 2012 (T) in the center of Algeria. The propolis samples were kept in the dark and stored at 4°C until use.

Water extraction was carried out as described previously [10]. Twenty five g of air-dried propolis was ground into a fine powder in a blender and mixed with 400 mL boiling water by magnetic stirrer for 1h. Then the aqueous extract was filtered over cheese-cloth and Whatman No.1 paper, respectively. Filtrates were evaporated to dryness at 50°C. The extracts were stored at 4°C in the dark until use.

Gas chromatography-mass spectrometry

The Gas chromatography-mass spectrometry(GC/MS) analysis was carried out according toPopova [11]. Samples of 5 mg of the residue were mixed with 50 µl of dry pyridine and 75 µl N,O-bis(trimethylsilyl) trifluoracetamide, heated at 80°C for 20 min and analyzed by GC-MS. The GC-MS analysis was performed with a Hewlett Packard Gas Chromatograph 6890 Series II Plus linked to Hewlett Packard 6972 mass spectrometer system equipped with a 30 m long, 0.25 mm id, and 0.5 µm film thickness HP5-MS capillary column. The temperature was programmed from 100 to 325°C at a rate of 5°C/min. Helium was used as a carrier gas with a flow rate of 20 ml/min. Split ratio 50:1, injector temperature 280°C.

The identification of the compounds present in propolis samples was accomplished using computer searches on commercial libraries. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. If available, reference compounds were co-chromatographed to confirm GC retention times.

Bacterial strains and growth conditions

Vibrio harveyi isolated from diseased farmed Senegalese sole (*Soleasenegalensis*),[12]*Vibrio anguillarum* (ATCC 19264) and *Bacillus subtilis* (CECT 35, Valencia, Spain) were used to investigate antibacterial activity of aqueous extracts of the different propolis samples. Bacteria were cultured for 18 h at 26.5 ºC in tryptic soy broth (TSB) supplemented with 1.5% NaCl (*V. harveyi* and *V. anguillarum*) or nutrient broth (NB) (*B. subtilis*) and used as inoculums.

Antimicrobial activity assays

Micro-broth dilution method [13] was used with slightly modifications to evaluate the growth-inhibiting activity of aqueous extracts of propolis against the tested bacterial strains. Each of the propolis samples (0.8% w/v) was filtered (Millex-GV unit 0.22 mm Millipore pore size) and serial two-fold dilutions were prepared in a flat-bottomed 96-well plate (from 1: 2 to 1: 8192). Seventy-five microlitres of sterile deionized H_2O was added to the positive control wells. Bacterial inoculum was prepared with fresh cultures and compared with McFarland standards. A final inoculum of 75 µl containing approximately 2×10^5 CFU/ml was added to all wells and the plate was then incubated overnight at 26.5ºC.

The antimicrobial activity was determined by visual inspection (clear well contents). Minimum inhibitory concentration (MIC) was defined as the lowest concentration (µg/mL) of propolis at which there was no visible growth of bacteria. The minimal bactericidal concentrations (MBCs) were determined by plating the content of wells that showed no visible growth of bacteria and incubating at 26.5ºC for 18 h. The MBC was considered the lowest concentration (µg/mL) of propolis that prevented any colony formation. All microbial tests were performed in triplicate.

RESULTS

Chemical composition of Algerian propolis

Chemical composition of aqueous extracts of propolis collected from five locations in Algeria [Babor (B), Setif (S), Ain-Abbassa (Ab), El-Hamma (Hm) and Tizi-Ouzou, (T)] has been determined by GC-MS analysis. One hundred and two compounds have been determined such as aromatic acids (benzoic acid, cinnamic acid and its esters, ferulic acid and phloroglucinic acid), linear hydrocarbons and their acids (2-propenoic acid, pentanoic acid, palmitic acid, succinic acid, and 2,3,4-Trihydroxybutyric acid), terpenes (caryophyllene and germacrene A), and alcaloïdes (phenethylamine, thebaine, papaverine, 1,2,3,4-tetrahydroand (+)-salsolidine). In addition to these compounds, there are sugars and their acids, alcohols and other compounds. All the substances identified are listed in Table 1.

Table 1: Chemical composition of aqueous extracts of *Algerian propolis* taken from Tizi-Ouzou, (T), Babor (B), Setif (S), Ain-Abbassa (Ab) and El-Hamma (Hm).

	Compounds	Area%				
		T	B	S	Ab	Hm
Linear hydrocarbons and their acids						
1	Butane	-	-	-	0.21	-
2	Butyrate	1.37	-	-	-	-
3	2-Methyl-propane	0.16	-	-	-	-
4	2-Propenoic acid	0.07	-	1.43	-	-
5	Propanoic acid	0.25	0.05	-	-	-
6	Butanoicacid	0.13	0.75	-	-	-
7	Pentanoic acid	-	-	-	-	2.12
8	Propanedioic acid	1.37	-	0.90	-	-
9	Hexadecanoic acid (palmitic acid)	0.23	-	0.38	-	-
10	Butanedioic acid (Succinic acid)	1.37	3.41	1.03	-	-
11	2,3,4-Trihydroxybutyric acid	-	0.03	-	-	-
Aromatic acids						
12	Ferulic acid	-	-	-	1.63	-
13	Hydrocinnamic acid	0.14	0.10	0.44	-	0.92
14	Benzoic acid	0.75	0.31	0.57	2.60	-
15	Cinnamic acid	1.01	0.81	5.37	1.63	-
16	Methyl cinnamate	33.23	30.08	13.23	34.94	-
17	Benzeneacetic acid	0.15	-	-	-	-
18	4-Hydroxymandelic acid, ethyl ester	-	0.17	-	-	-
19	2,4,6-Trihydroxybenzoic acid(Phloroglucinic acid)	-	0.19	-	-	-
20	5-Hydroxyindole-3-propionic acid	0.16	-	-	-	-
21	(4-Methoxyphenyl)octanoic acid	-	0.08	-	-	-
22	3-(4-N,N-Dimethylaminophenyl)propenoic acid, 2(diethoxyphosphinyl), ethyl ester	-	-	-	0.30	-
Sugars, sugar alcohols and sugar acids						
23	D-Glucose	2.16	-	7.73	0.35	-
24	Mannose	2.16	2.74	7.73	0.70	-
25	D-Ribose	0.27	0.06	-	-	-
26	Arabinose	0.27	-	-	-	-
27	d-Xylose	0.10	0.04	-	0.23	-
28	D-Altrose	0.10	-	0.83	-	-
29	D-Xylopyranose	0.75	0.06	-	-	-
30	D-Fructose	7.46	12.31	14.90	12.05	20.31
31	Sorbose	9.40	-	4.72	-	6.71
32	Arabinofuranose	1.03	-	14.90	-	20.31
33	L(-)-Fucose	-	4.61	-	0.76	-
34	Sorbopyranose	-	4.34	4.72	7.43	6.71

35	Galactopyranose	0.09	4.61	4.72	-	-
36	Lyxose	-	0.04	-	-	-
37	beta.-L-Arabinopyranose	0.10	0.06	-	-	-
38	D-Mannopyranose	1.59	4.61	4.24	0.70	-
39	alpha.-D-Glucopyranose	1.59	2.74	0.83	0.70	-
40	Levoglucosan	-	0.06	-	-	-
41	1,6-Anhydro-.beta.-d-glucose	-	0.06	-	-	-
42	Galactose	1.49	-	-	0.35	-
43	D-Altro-2-Heptulose	0.75	1.26	1.74	-	-
44	Inositol+ *Myoinositol*	0.33	0.23	-	-	-
45	Arabitol	0.19	-	0.13	-	1.42
46	Ribitol	0.31	0.05	-	-	1.42
47	Xylitol	0.31	0.05	0.13	0.37	1.42
48	D-Mannitol	0.11	-	0.20	-	-
49	beta.-DL-Arabinofuranoside	0.58	0.06	-	-	-
50	alpha.-D-Mannopyranoside	-	-	0.83	0.76	-
51	Xylopyranoside	-	-	-	0.23	-
52	alpha.-D-Galactopyranoside	-	1.65	-	-	-
53	D-Glucuronic acid	0.15	0.06	-	-	-
54	beta.-D-Glucopyranuronic acid	0.15	0.06	-	-	-
55	Galacturonic acid	-	-	-	0.89	-
56	DL-Malic acid	-	0.75	-	-	-
57	Malic acid	0.43	-	1.03	-	-
58	Talonic acid	0.16	-	-	-	-
59	Gluconic acid	1.75	-	-	-	-
60	Ribonicacid	0.17	-	-	-	-
Terpenes						
61	Caryophyllene	-	-	-	-	0.90
62	Neoisolongifolene	-	0.12	-	-	-
63	Germacrene A	-	-	-	-	0.90
Alkaloïds						
64	Phenethylamine	0.15	-	-	-	-
65	Thebaine	-	-	1.60	-	-
66	Papaverine, 1,2,3,4-tetrahydro	-	-	1.60	-	-
67	(+)-Salsolidine	-	-	1.60	-	-
68	Aspidodispermine	-	0.12	-	-	-
69	Isovanillin	-	0.04	-	-	-
70	Morpholine	-	-	-	0.76	-
71	Aspidofractinine, 3-methylene-,alpha.,5.alpha.)	-	0.03	-	-	-
72	Demecolcine	-	-	-	-	1.12
Others						
73	Ether of glycerol	6.67	8.72	4.89	8.80	16.21
74	Ether of glucitol	0.11	-	0.20	-	-
75	Morphinan-3-ol, 6,7,8,14-tetradehydro-4,5-epoxy-6-	0.26	-	-	-	-

	methoxy-17-methyl, (5.alpha.)					
76	Barbituricacid	-	-	-	-	0.44
77	Cannabinol	0.09	-	-	-	-
78	l-Alanine	0.06	-	-	-	-
79	L-Proline	0.30	0.20	0.23	-	-
80	Acetophenone and its derivatives	-	0.18	-	-	-
81	1-Propanone, 1,3-diphenyl	-	0.04	-	-	-
82	1,1'-Binaphthalene, 3,3'-dimethyl-	-	0.04	-	-	-
83	1H-Pyrazole, 3,5-bis(1,1-dimethylethyl)-4-ethyl	-	0.18	-	-	-
84	Benzenamine, N,N-diethyl-4-[2-(4-nitrophenyl)ethenyl]	-	0.86	-	-	-
85	1,3-Dioxolane, 2-(4-methoxyphenyl) -2-methyl	-	-	7.48	-	-
86	8-Furan-2-yl-3,3-dimethyl-6-morpholin-4-yl-3,4-dihydro-1H-thiopyrano	-	-	-	0.30	-
87	1-Dimethyl (phenyl) propane	-	-	-	1.30	-
88	1,3-Benzoxazine, perhydro-4-phenyl -2-thioxo-, cis	-	-	-	0.69	-
89	2,5-Cyclohexadien-1-one, 4-[[4-(dimethylamino)phenyl]imino]-2,5-dimethyl	-	-	-	0.36	-
90	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	-	-	-	0.27	-
91	Methylhydroquinone	-	-	-	0.71	-
92	dimethyldi(3-fluorophenoxy	-	-	-	0.70	-
93	Pentacene, 6,13-dihydro-	-	-	-	0.70	-
94	Dibenz(a,h)anthracene, 7,14-dihydro	-	-	-	0.70	-
95	Thiazolo[3,2-a]pyridinium, 2,3-dih ydro-8-hydroxy-5-methyl-2-phenyl-,hydroxide, inner salt	0.17	-	0.30	0.63	4.19
96	Dibenz[b,d]cycloheptane, 3,4,7-trimethoxy-11-oximido	-	-	-	0.31	-
97	Sarcosine	0.06	-	-	-	-
98	11H-Cyclopenta[a]phenanthren-17-ol	-	0.07	-	-	-
99	1H- -1,3(2H)-dione	-	0.08	-	-	-
100	Indene Tranylcypromine, pentafluorobenzoyl ester	-	-	-	-	1.57
101	Scopolin	-	-	0.32	-	-
102	6,7-Dihydroxy-1-oxotetrahydronaphthalene	-	0.08	-	-	-

Propolis antimicrobial activity

Antimicrobial activities of the five Algerian aqueous propolis extracts were tested against one Gram positive bacteria used as probiotics in aquaculture (*B. subtilis*) and two Gram negative bacterial fish pathogens (*V. anguillarum* and *V. harveyi*). The bacteriostatic (MIC) and bactericidal (MBC) activities of the five propolis samples are shown in Table 2.

The obtained results showed that all aqueous extracts inhibit the growth of *B. subtilis* at 31.25 µg/ml. At this concentration, samples T, B, S and Hm prevent bacterial growth after plating treated bacteria on extract-free medium (Table 2).

Growth inhibition of the fish pathogen *V. Anguillarum* requires concentrations of 1000 µg/ml for extracts T, B and Ab, and 2000 µg/ml for extracts S and Hm. Propolis T, Ab and Hm effectively inhibit growth of *V. harveyi* at 500 µg/ml, while propolis S and B require 250 µg/ml and 125 µg/ml, respectively. Solutions of extract B containing 250 µg/ml have bactericidal activity against this fish pathogen (Table 2).

Table 2. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of *Algerian propolis* against *V. anguillarum, V. harveyi* and *B. subtilis*. Tizi-Ouzou, (T), Babor (B), Setif (S), Ain-Abbassa (Ab) and El-Hamma (Hm)

	V. anguillarum		*V. harveyi*		*B. subtilis*	
Propolis varieties	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
T	1000	2000	500	500	31.25	31.25
B	1000	1000	125	250	31.25	31.25
S	2000	2000	250	500	31.25	31.25
Ab	1000	2000	500	500	31.25	62. 5
Hm	2000	4000	500	1000	31.25	31.25

DISCUSSION

Differences were recorded between the five selected Algerian propolis samples regarding their chemical composition. The highest number of different compounds (52) was recorded with T sample (taken from the Tizi-Ouzouzone in the center of the country; 48 substances were identified in B sample, 33 compounds in S and Ab samples and only 16 compounds in Hm sample. Regarding the difference registered in the number of the compounds among the propolis extracts, some compounds were found to be common. For example, *D*-Fructose, xylitol and ether of glycerol were common between the five extracts, benzoic acid, cinnamic acid, methyl cinnamate, mannose, *D*-mannopyranose and *alpha.-D*-glucopyranose were common between T, B, S and Abpropolis extract. Thiazolo[*3,2-a*]pyridinium, *2,3*-dihydro-*8*-hydroxy-*5*-methyl-*2*-phenyl-,hydroxide and inner salt were found in T, S, Ab and Hm extract. Also, hydrocinnamic acidwas common between T, B, S and Hm extract. Furthermore, seven compounds (*2*-Propenoic acid, propanedioic acid, palmitic acid, *D*-altrose, *D*-mannitol, malic acid and ether of glucitol) were common only between T and S extracts.

On the other hand, T and B extracts has in common nine compounds (propanoic acid, butanoic acid, *D*-ribose, *D*-xylopyranose, *beta.-L*-arabinopyranose, (Inositol+ Myoinositol), *beta.-DL*-arabinofuranoside, *D*-glucuronic acid and *beta.-D*-glucopyranuronic acid) and only four compounds (succinic acid, galactopyranose, *D-Altro-2*-Heptulose and *L*-proline)were common among T, B and S extracts. Some other compounds were found only in one extract (*e.g.* cannabinol and sarcosine in T, *2,3,4*-trihydroxybutyric acid and phloroglucinic acid in B, *1,3*-dioxolane, *2*-(*4*-methoxyphenyl) *-2*-methyl and scopolin in S, ferulic acid and *1*-dimethyl(phenyl) propane in Ab and barbituric acid and tranylcypromine, pentafluorobenzoyl ester in Hm).

According to the obtained results, it could be deduced that the active compounds of propolis of Tizi-ozou were the aromatic acids while terpenes (0.90% Germacrene and 0.90% Caryophyhllene) were the active compounds in propolis of El- Hamma. Alkaloids represent 4.8 % in S extract (1.6% Thebaine, 1.6% Papaverine and 1.6% *(+)* -Salsolidine). It is represent also by 0.15 % (Phenethylamine), 0.16 % (0.12% Aspidodispermine, 0.04% Isovanillin and 0.03 % Aspidofractinine), 0,76 % (Morpholine) and 1.12% (Demecolcine) in the composition of T , B , Ab and Hm extract respectively.

These differences in the chemical composition between the five tested samples can be attributed to geographical locations which influence the vegetation of the regions and even the vegetation between different places inside the same region. In fact, it is known that the chemical composition of propolis depends on the vegetation of the area where it was collected [3,8,14,15]. Furthermore, the different results obtained regarding the chemical composition of propolis may also be explained by the manipulations of the samples (*e.g.* extract procedures and chemical techniques) [15-16]. We have used water extracts of propolis, while usually ethanolic, [17-20] methanolic [16]or diethyl ether [17]extracts were studied. The aqueous extract was chosen firstly on the basis of the traditional use of propolis in Algeria (as decoction and in boilling water), secondly because there is a previous work on the propolis in Algeria, about alcoholic extracts and mainly about essential oils of propolis. In comparison with other extracts, the present propolis water extract contained some compounds which were also found in ethanolic, methanolic or diethyl ether extracts. For example, benzoic acid, ferulic acid, succinic acid, malic acid, fructose, sorbose, inositol, glucose, phloroglucinic acid, scopolin, xylose, mannose, palmitic acid, hydrocinnamic acid, *D*-xylopyranose, gluconic acid and galactose were common in ethanolic extracts.[16]Ferulic acid was common in methanolic extracts and diethyl ether extracts [17, 20]while cinnamic acid was common in the three extracts [16, 17, 20].

After a detailed review of the available literature, there is only one work comparing the chemical composition and antimicrobial activity of propolis from different Mediterranean countries including Algeria (in addition to Bulgaria, Turkey and Greece). All the propolis sampled contained mainly flavonoids and esters of caffeic and ferulic acids, found significant amounts of a hydroxyditerpenic acid. Furthermore, all propolis samples showed significant antibacterial and weak to moderate antifungal activity [9]. There is two more papers focus on some effects of Algerian propolis in rats. Nadia *et al.*[8]reported the ability of propolis extracts to restore the fall of mitochondrial membrane potential and to prevent apoptotic process induced by ferulenolin in rat liver mitochondria. More recently, Piccinelli *et al.*[9]make an analytical and pharmacological study which evaluated the effects of propolis extract on renal oxidative stress induced by doxorubicin.

To our days, there are very few papers related to the possible applications of propolis in aquaculture although protective effect of propolis on growth performance, haematological parameters, oxidative damage and immune system have been demonstrated in different teleost fish species [21, 22] In this sense, diseases in farmed fish are a common problem, which makes the fish often have to be treated with antibiotics. It is known, that the repetitive use of antibiotics in different fields (veterinary and human medicine) improves the emergence and occurrence of the

resistance phenomenon in pathogenic bacteria. Furthermore, the use of antibiotics in food-producing animals has generated considerable interest because the wide spread administration of the may lead to the development of resistant human pathogens [23]. Regarding the problem of microbial resistance, there is an urgent need to find useful natural alternatives (namely, new compounds) to the use of antibiotics, in order to control (both, to prevent or even treat) bacterial diseases in aquaculture field. A very abundant substance in nature, with a very complex chemical composition and properties and with proven health and wellness for humans is propolis. Owing the ability to contain many different substances, the propolis can be considered as one of the top richest sources of new drugs [24]

The available reports about antimicrobial effect of propolis in aquaculture are particularly scarce [18, 19]Abd-El-Rhman[18]study propolis taken from High Egypt and calculated the MIC of propolis-ethanolic-extract against *Aeromonashydrophila*. Besides that, in an *in vivo* test he concluded that propolis-ethanolic-extract enhanced the growth, immunity and resistance of *O. niloticus* against *A. hydrophila* more than the crude propolis. In the former study, the *in vitro* antibacterial activity of ethanol extract of propolis from Iran was investigated against three prevalent species of fish bacterial pathogens: *A. hydrophila*, *Yersinia ruckeri* and *Streptococcus iniae.* Growth inhibition was observed for the three studied bacteria when incubated a 1: 128, 1: 256 and 1: 512 dilution of the 10% ethanol extract, respectively. In the present study, the aim was to investigate the *in vitro* antimicrobial (bacteriostatic, MIC and bactericidal, MBC) activity of the aqueous extracts of propolis from Algeria against one Gram positive bacteria (*B. subtilis*) and two Gram negative fish pathogenic bacteria that are often the cause of bacterial diseases in Mediterranean aquaculture, V. *anguillarum* and *V. harveyi*. *B. subtilis* was chosen because is one of the bacteria most widely used as probiotic in fish studies [25-29].The obtained results showed that all aqueous extracts inhibit the growth of *B. subtilis*. It can be concluded must exercise caution if you want to manage propolis extracts at thesame time that live probiotics because the extracts could affect the viability of the seprobiotics. Regarding effects of propolis on Gram negative bacteria, growth inhibition of the fish pathogen *V. Anguillarum* required high concentrations of propolis extracts (1000 µg ml^{-1}forextracts T, B and Ab, and 2000 µg ml^{-1}forextracts S and Hm). On the other hand, T, Ab and Hm propolis samples effectively inhibit growth of *V. harveyi* at 500 µg/ml, while S and B propolis required 250 µg/ml and 125 µg/ml, respectively. Solutions of extract B containing 250 µg/ml have also bactericidal activity against this fish pathogen. The present results are particularly significant due to the fact that the development of antibiotic resistance has already been reported in *V. anguillarum*and *V. salmonicida* as well as for other bacteria also pathogenic for fish, such as *A. hydrophila*, *A. salmonicida, Edwardsiellatarda, Edwardsiellaicttaluri, Pasteurellapiscida* and *Y. ruckeri* [30].It has been described that the antimicrobial activity of propolis is basically against Gram-positive bacteria [31].Burdock [2] attributed this capacity to the presence of aromatic acids and esters, while [32,33].Takaisi and Scjoncjer[32] and Cushnie and Lamb [33] suggested that it is due to the action of the flavononepinocembr in and the flavonolgalangin, and caffeic acid phenethyl ester, whose action mechanism is based on the inhibition of bacterial RNA polymerase. The action mechanism involves degrading the cytoplasm membrane of the bacteria, which leads to a loss of potassium ions and the damage caused provoking cell autolysis. More studies are needed to understand the exact mechanism of action of propolis on Gram negative bacteria as well as the compounds involved in this process.

CONCLUSION

To conclude, at the best of our knowledge, this study is the first report of chemical composition and antibacterial activity of five different Algerian propolis against fish pathogenic bacteria. The antibacterial properties against *V. anguillarum* and *V. harveyii* would warrant further studies on the clinical applications of propolis in aquaculture field.

ACKNOWLEDGMENTS

V. anguillarum and *V. harveyi* strains were kindly donned by Dr. MA Moriñigo from the University of Málaga (Spain). The financial support of the Spanish Ministry of Economy and Competitiveness under Grant no. AGL-2011-30381-C03-01 and of the *Fundación Séneca de la Región de Murcia* (Grant no. 04538/GERM/06, Grupo de Excelencia de la Región de Murcia, Spain) is gratefully acknowledged.

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