Biomacromol. J., Vol. 4, No. 2, 78-92, December 2018.

Biomacromolecular Journal
www.bmmj.org

Searching for New Bioactive Metabolites from Marine Bacteria in the Persian Gulf: Antibacterial, Cytotoxic and Anti-inflammatory Agents

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(Received 20 January 2019, Accepted 7 March 2019)

ABSTRACT

Natural products have historically been considered as a rich source of therapeutic agents and play a pivotal role in the development of medicinal compounds. Among microorganisms, marine bacteria have been recognized as a rich, promising and untapped resource. In the present work, we focused on Gram-positive bacterial population of Iranian coastal area of the Persian Gulf and study on bioactive potentials of the organic extracts from the isolates. The disc diffusion, DNA-interaction analysis along with MTT assay, and red blood cell hemolysis analysis were performed to evaluate antibacterial, cytotoxicity and anti-inflammatory activities of the extracts. We succeed to identify 6, 39, 34 and 21 novel bacterial producers of the bioactive metabolite having antibacterial, DNA interaction ability, cytotoxic activity, and anti-inflammatory effects, respectively. Approximately 15.4% of the isolates produced antibacterial agents against *S. aureus* and *P. aeruginosa*, meanwhile the *Bacillus* genus revealed the strongest potential. Moreover, a significant correlation was observed between DNA binding ability of the extracts and their cytotoxicity effect on cancerous cell lines; although the chemical nature of the solvent used effects on it. The data of anti-inflammatory study showed that some bacterial extractions are more potent than dexamethasone by more than 90% RBC hemolysis protection. Our results demonstrate that the Gram-positive bacterial population of the Persian Gulf can be considered as a novel rich source of bioactive compounds with valuable therapeutic impact.

Keywords: Bacterial secondary metabolite, Bioactive compounds, Persian Gulf, DNA binding agents

INTRODUCTION

Nowadays, more than 30000 human diseases have been clinically described, while less than one third of them are curable. So, searching for new drugs is vital and much attention has been paid by medical scientist to this critical issue. Secondary metabolites are a rich source of biological-based drugs and many efforts have been recently focused to identify new natural compounds having bioactive properties. They are organic molecules that are frequently produced at highest level during a transition from exponential phase to stationary phase of microorganism life cycle [1]. Mostly, producer organism profits survival advantages from these compounds in various ways, for instance: supplying nutrient (*e.g.*, siderophores) [2], protection against environmental stress (*e.g.*, pigments and osmoprotectants) [3], improving competitive abilities (*e.g.*, antibiotics) [4], or acting as a metabolic defense mechanism (*e.g.*, many plant flavonoid and alkaloid toxins) [5,6]. Moreover, secondary metabolites have great importance in human life and widely used as active drug ingredients in medicine [7-9], as herbicides or phytotoxins in agriculture, or as food additives [10], and even as precursors for the synthesis of plastics [11]. Analysis of new medicines approved by the US Food and Drug Administration (FDA) [12] revealed that 34% of those medicines that were based on small molecules were natural products or direct derivatives of them [13-17]. Nevertheless, these natural products have several advantages over their synthetic ones. They are not only biologically active but also likely to be easily transport and deliver to their intracellular site of action [18]. Cancer as the second cause of death in all around the world is one the most important target of drug presentation by researchers. Despite the recent excitement about personalized anticancer therapies, such drugs currently represent a small fraction of anticancer agents [19]. Moreover, the clinical significance of DNA-binding compounds can hardly be overlooked, as many anticancer

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prescription include a compound that binds to and/or modifies DNA. Due to the large use of anticancer drugs, the further discovery and introduction of natural producers of them are of remarkable interest.

The ocean which covering more than 70% of the surface of earth, is believed to contain a total of almost 3.67×10^{30} microorganism [20] and shows an exceptional biological diversity, which accounts more than 95% of the whole biosphere. Microbial diversity constitutes an infinite pool of novel chemistry, making up a valuable source for discovery and identification of bioactive compounds [21,22]. A number of valuable antibiotics and metabolites have been derived from terrestrial microorganisms, and efforts in this area have diminished since the late1980s, because of the feeling that this resource has been exhaustively studied [23]. Rediscovery of the same compounds leads researchers switched over to new environments for novel pharmaceutical compounds specially for combating human pathogens. The ocean floor has been recently demonstrated as an ecosystem with many unique forms of bacteria [24]. Recently, natural products from marine organisms have shown many interesting activities, such as anti-microbial, cytotoxic, anticancer, anti-diabetic, anti-fungal, anti-coagulant, anti-inflammatory, and other pharmacological impacts which have continued to enter clinical trials or to provide leads for compounds that have entered clinical trials [25-28].

Since World War II, the Persian Gulf and the surrounding countries have come to account for a significant proportion of the world's oil production. In addition, the area has approximately two-thirds and one-third of the world's estimated proven oil and natural gas reserves, respectively. Although Persian Gulf geographically is rather a restricted area, the waters support many microorganisms, plants and animals, which makes it a fascinating pool for natural drug discovery. But unfortunately, there was not much attention to the medicinal potentials of the area. In the present work, we tried to do a primary study in bioactive compounds- producing ability of bacteria isolated from the Iranian border of Persian Gulf. Among diverse microbial species, isolates of marine *Bacillus* and *Actinomycetes* belong to phylogenetically and phenogenetically heterogeneous groups of bacteria and were our aim population. *Bacilli* are ubiquitous in the marine environment and can tolerate adverse conditions such as high temperature, pressure, salinity, and pH [29]. Generally, *Bacillus* strains need more nutrition and space for faster growing rate which they compete with other organisms. Needless to say, over 10,000 of 23,000 known bioactive compounds are produced by *Actinomycetes*, representing 45% of all bioactive microbial metabolites discovered in practical use [21]. So, these genera have considered as target sources and a secondary metabolite screening program was conducted. Overall, in present study we aimed to screen potent bacterial producers of bioactive compounds from Iranian coastal area of Persian Gulf as a way to discover targeted natural compounds from this almost unstudied ecosystem and introduce them at future.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Sediment samples were collected from 50 cm depth in 24 distinct Iranian coastal areas of the Persian Gulf (listed in Table 1). These samples were processed as soon as possible after collection using the selective method of dilution and heat shock and were inoculated into isolation media (M1 and M5). The dilution and heat-shock method were carried out as described by Mincer *et al.* [30]. Briefly 1 ml of wet sediment was added to 4 ml of sterile seawater, heated for 40 min at 55 °C while vigorously shaken, and further diluted (1:4) in sterile seawater. Then, 150 µl of each diluted sample was inoculated by spreading with a sterile glass rod onto agar-based isolation media.

The isolation media consist of the following: M1, 10 g of starch, 4 g of yeast extract, 2 g of peptone, 18 g of agar to one liter of natural seawater; and M5, 18 g of agar to 1 l of natural seawater. The isolation media were amended with filtered Cycloheximide (100 µg ml^{-1}) and Kanamycin (20 µg ml^{-1}), after autoclaving to avoid from fungi and gram-negative bacteria growth, respectively.

Secondary Metabolite Production Screening

All gram-positive isolates were cultivated in Tryptic Soy Broth media (Merck, Germany) for 5 days; then organic extraction was performed as follows: Supernatants were treated and shaken with equal volume of ethyl acetate for 90 min. The organic phase separated and concentrated using

Table 1. DNA Binding Potential of the Organic Extracts from Effective Isolates and Type of their Structural Changes on DNA

Isolate code	Fraction type	Solvent used	Type of DNA binding in the presence of		
			Ni^{2+} (5 µM)	Cu^{2+} (15 µM)	H_2O_2 (50 µM)
13	Supernatant	EtOAc	Intercalation	Intercalation	DSBs
13	Biomass	MeOH	DSB_S+ Intercalation	Intercalation	Intercalation
31	Supernatant	EtOAc	Intercalation	Intercalation	No effect
31	Biomass	EtOAc	Intercalation	Intercalation	No effect
31	Biomass	MeOH	Intercalation	Intercalation	Intercalation
32	Supernatant	EtOAc	Intercalation	Intercalation	No effect
32	Biomass	MeOH	Intercalation	SSB+Intercalation	Intercalation
41	Supernatant	EtOAc	DSBs+Intercalation	DSBs	DSBs+Intercalation
41	Biomass	EtOAc	Intercalation	Intercalation	No effect
41	Biomass	MeOH	Intercalation	Intercalation	Intercalation
43	Supernatant	EtOAc	DSBs	DSBs	Intercalation
43	Biomass	EtOAc	No effect	DSBs+Intercalation	No effect
44	Supernatant	EtOAc	No effect	Intercalation	DSBs+Intercalation
44	Biomass	MeOH	No effect	DSBs	Intercalation
45	Supernatant	EtOAc	No effect	No effect	DSBs
45	Biomass	MeOH	Intercalation	Intercalation	Intercalation
71	Supernatant	EtOAc	Intercalation	No effect	DSBs
71	Biomass	EtOAc	No effect	No effect	SSB
71	Biomass	MeOH	Intercalation	Intercalation	No effect
91	Supernatant	EtOAc	No effect	No effect	Intercalation
101	Biomass	EtOAc	SSB	DSBs	Intercalation
551	Supernatant	EtOAc	DSBs	Intercalation	Intercalation
561	Supernatant	EtOAc	DSB	DSB	Intercalation
562	Supernatant	EtOAc	No effect	SSB	Intercalation
581	Biomass	EtOAc	Intercalation	Intercalation	Intercalation
591	Biomass	EtOAc	No effect	No effect	Intercalation
593	Biomass	EtOAc	No effect	No effect	Intercalation
121	Supernatant	EtOAc	No effect	No effect	DSBs+Intercalation
121	Biomass	MeOH	Intercalation	Intercalation	Intercalation
122	Biomass	EtOAc	No effect	No effect	Intercalation
122	Biomass	MeOH	Intercalation	Intercalation	No effect
131	Biomass	EtOAc	SSB	SSB	Intercalation
133	Biomass	EtOAc	No effect	SSB	DSBs+SSB
133	Biomass	MeOH	No effect	Intercalation	Intercalation
134	Supernatant	EtOAc	SSB	SSB	No effect

Table 1. Continued

134	Biomass	EtOAc	Intercalation	Intercalation	No effect
135	Supernatant	EtOAc	Intercalation	Intercalation	Intercalation
135	Biomass	MeOH	Intercalation	Intercalation	Intercalation
137	Supernatant	EtOAc	No effect	Intercalation	No effect
137	Biomass	EtOAc	SSB	SSB	No effect
137	Biomass	MeOH	Intercalation	Intercalation	Intercalation
012	Supernatant	EtOAc	No effect	No effect	SSB
012	Biomass	MeOH	Intercalation	Intercalation	No effect
013	Supernatant	EtOAc	SSB	No effect	No effect
013	Biomass	EtOAc	Intercalation	Intercalation	SSB
013	Biomass	MeOH	SSB	SSB	Intercalation
014	Biomass	MeOH	DSB	DSB	No effect
024	Supernatant	EtOAc	SSB	No effect	No effect
024	Biomass	MeOH	DSBs	Intercalation	Intercalation
026	Supernatant	EtOAc	Intercalation	Intercalation	Intercalation
026	Biomass	MeOH	DSBs	DSBs	Intercalation
031	Supernatant	EtOAc	SSB	SSB	No effect
031	Biomass	EtOAc	No effect	No effect	SSB
031	Biomass	MeOH	Intercalation	Intercalation	Intercalation
033	Supernatant	EtOAc	SSB	SSB	No effect
033	Biomass	EtOAc	No effect	No effect	SSB
033	Biomass	MeOH	No effect	DSB	DSB
041	Biomass	MeOH	Intercalation	Intercalation	Intercalation
042	Supernatant	EtOAc	Intercalation	No effect	Intercalation
042	Biomass	EtOAc	SSB	SSB	SSB
042	Biomass	MeOH	Intercalation	Intercalation	Intercalation
043	Biomass	EtOAc	SSB	Intercalation	Intercalation
043	Biomass	MeOH	Intercalation	Intercalation	Intercalation
055	Biomass	EtOAc	No effect	No effect	SSB
055	Biomass	MeOH	SSB	SSB	Intercalation
072	Supernatant	EtOAc	Intercalation	No effect	Intercalation
072	Biomass	MeOH	Intercalation	Intercalation	Intercalation
073	Supernatant	EtOAc	No effect	SSB	No effect
073	Biomass	EtOAc	SSB	No effect	No effect
073	Biomass	MeOH	Intercalation	Intercalation	No effect
2116	Biomass	EtOAc	Intercalation	No effect	Intercalation
2121	Biomass	EtOAc	Intercalation	SSB	No effect
0264	Biomass	EtOAc	SSB+ Intercalation	SSB	Intercalation
0264	Biomass	MeOH	Intercalation	Intercalation	Intercalation

rotary evaporator. Biomass was treated and shaken with acidic methanol (1:24 V/V, HCl 1M: Absolute Methanol) for 45 min. After evaporation of methanol, collected cell organic extracts washed with ethyl acetate and then with methanol to achieve two separate solvent extractions for each isolate. Finally, the three distinct organic extracts of each isolate: the ethyl acetate extract of supernatant, the ethyl acetate extract of biomass, and the methanolic extract of biomass were tested for bioactivity.

BIOACTIVITY TESTS

Antibacterial Activity Test

Antibacterial activity of the extracted secondary metabolites were tested against gram-positive pathogen *Staphylococcus aureus* ATCC 25923 and gram-negative pathogens, *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 using agar disc diffusion assay [31]. Briefly, pathogens were swabbed uniformly across Muller-Hinton agar plate (Merck, Germany). Then a filter-paper disk, impregnated with the defined amount of organic extracts was placed on the surface of the agar and incubated at 37 °C overnight (16 h). The antibacterial activity of each organic extract was reported by measuring the diameter of inhibition zone.

DNA Interaction Analysis

DNA interaction or cleavage study was conducted by agarose gel electrophoresis technique. Three distinct experiments were carried out using different additives (H_2O_2, Cu^{2+}, and Ni^{2+}) as follow: 300 ng of the circular plasmid DNA, pBluescript II KS (+), was treated with organic extracts in a simulated physiological condition using phosphate buffer saline, pH 7.4, supplemented with H_2O_2 (50 µM) or $CuSO_4$ (15 µM) or $NiCl_2$ (5 µM), and incubated at 37 °C for 1 h. Finally, samples were electrophoresed in 1% agarose gel in Tris-acetic acid-EDTA (TAE) buffer and the gels stained by ethidium bromide.

Cytotoxicity Assay

Jurkat (immortalized line of T cells) and HepG2 (Hepatocellular carcinoma) cell lines were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C in DMEM high medium supplemented with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 10% fetal bovine serum. Cell viability assay was performed using MTT method [32]. Briefly 2.5×10^4 cells were seeded in each well of 96 well plates and incubated at 37 °C for 24 h in the presence of 5% CO_2. Then a defined number of cells exposed to the organic extracts of isolates (2 mg ml^{-1}) in a period of 48 h. Afterward, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to achieve a final concentration of 0.45 mg ml^{-1} per well and incubated at 37 °C for another 4 h. Finally, equal volume of solubilization solution (40% (vol/vol) dimethylformamide (DMF) in 2% (vol/vol) glacial acetic acid and 16% sodium dodecyl sulfate (SDS), pH~4.7) was added to each well to dissolve formazan crystals. The absorbance of each well was recorded at 570 nm and 630 nm to omit the turbidity using SPECTRO $star^{Nano}$ (BMG, Germany) microplate reader. Cell viability percentage was calculated as follow:

$$A_T = A_{570} - A_{630}$$

$$\text{Cell viability\%} = [A_T \text{ sample}/A_T \text{ control}] \times 100\%$$

Anti-inflammatory Test

Anti-inflammatory assay was performed using human red blood cell membrane stabilization method [33]. The blood sample was collected from Shiraz blood transfusion center from healthy volunteers. The blood was mixed with isosaline and centrifuged in 3000 g for 10 min. The pellet further washed with isosaline twice and a suspension of 2% V/V of RBC was prepared. The assay mixture comprised of 1 volume of each hyposaline and RBC suspension, 2 volume of phosphate saline buffer pH ~ 7.4 treated by the organic extracts of the isolates in a final concentration of 10 mg ml^{-1} and incubated at 37 °C for 30 min. Finally, the absorbance of samples read at 560 nm. Anti- inflammatory activity calculated as follow:

$$\text{Hemolysis percentage} = (A_{560} \text{ sample}/A_{560} \text{ control}) \times 100$$

$$\text{Anti-inflammatory activity percentage} = 100 - \text{hemolysis percentage}$$

Where the control is RBCs incubated in hypotonic condition

(maximum lysis) for 30 min at 37 °C. Both cytotoxicity and hemolysis assays were performed in triplicate.

Phylogenic Phenotyping by 16S rDNA Sequence Alignment

16S rDNA gene of the bioactive compounds producing isolates was amplified by PCR using forward primer HRK1 (5'-ACTCCTACGGGAGGCAGCAG-3') and revers primer HRK2 (5'-TGACGGGCGGTGTG TACAAG-3'), which were designed for a 1000 bp DNA fragment amplification. Amplified fragments were purified from 1% agarose gels and sequenced by Macrogen company. The obtained sequences were submitted to the GenBank database at national center for biotechnology information (NCBI). The 16S rDNA sequence data were analyzed by MEGA 6.0 software, and phylogenetic tree constructed using neighbor-joining algorithm.

RESULTS AND DISCUSSION

Samples were collected from Persian Gulf coastline through Bushehr and Hormozgan states in a total of 24 regions. Those were mostly sediments, but a few samples of crustaceans and marine plants were among them (Fig. 1). After isolation, a total of 100 Gram-positive, spore forming bacteria were selected and purified for secondary metabolite production experiments. The bacterial isolates and their respective origins are listed in supplementary Table S1.

DNA Binding Study

Cancer is a leading cause of death worldwide and anticancer agents that target DNA are one of the most effective agents in clinical use. They mostly caused significant increase in the survival of cancer patients when used along with other drugs that have different mechanisms of action [34]. But the most disadvantage of their medication is that they are extremely toxic for normal cells too [35]. Consequently, much effort has been put into finding compounds that are more selective, and there is great enthusiasm towards the identification of cancer-specific molecular targets which yield a new generation of less toxic therapeutics. Compounds which target DNA can trigger cell death in a variety of ways. DNA intercalators mostly interfere with the recognition site of proteins such as DNA/RNA polymerases, DNA topoisomerases, *etc.* [36], and inhibition of those protein leads to double strand break induction [37]. Some of compounds act like a chemical nuclease and cleave DNA, so produce either single or double strand breaks. In all cases, unrepairable damages induced cell death by apoptosis [38]. Cancer cells acquire some unique characteristics such as increase of Cu^{2+} and Ni^{2+} ions levels in their cytoplasm [39]. Furthermore amount of reactive oxygen species (ROS) especially H_2O_2 increases significantly due to high rate of growth and metabolism [40,41]. Consequently, compounds which interact with or affect DNA via mechanisms involved these mediators can act as specific drugs to treat cancer with [42]. So, DNA interaction potential of the organic extracts in the presence of H_2O_2 (50 µM), Cu^{2+} (15 µM) and Ni^{2+} (5 µM) ions, were investigated separately. Circular plasmid DNA is naturally found in different topological forms, which can be distinguished by gel electrophoresis assay due to their different mobility rates. Bacterial plasmid DNA molecules are mostly found in the positive supercoil (SC) form which has the highest mobility speed, linear DNA (L) with moderate mobility and the relax form or open circular plasmid (OC) which is less frequent and shows the lowest mobility rate in agarose gel electrophoresis. DNA binding ligands can induce DNA structural changes in various ways including: typical single (SSB) and double strand breaks (DSB) and various type of intercalations. The mode of each type of interactions can be characterized by evaluating the electrophoretic behavior of a circular DNA. In a SSB event, the positive supercoil converts to relax form, so the ratio of SC/OC decreased. In some cases of DSBs, the DNA turn to low molecular weight fragments which were observed as smear and in the specific DSB, only a single band with the exact molecular weight of the corresponding DNA observed. In the case of intercalations (Both classical and non-classical models), the mobility rate of DNA is decreased. As shown in Fig. 2, in lane 1, 2 and 5 the mobility of plasmid DNA decreased significantly when treated by the organic extracts which can identify them as intercalators. In lane 6 the ratio of SC/OC decreased meaningfully in compare to the control (lane 8) due to SSB and in lane 4, a low molecular weight smear was observed shown several DSBs occurred. Table 1 shows the data of DNA binding activity of different organic extracts from a

Fig. 1. Samples collected from the coastlines of Persian Gulf, sediments, marine plants and crustaceans.

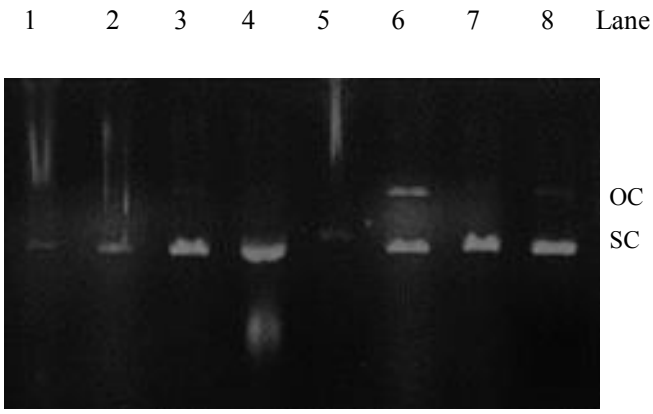

Fig. 2. Electrophoretic behavior of the circular DNA (pBluescript KS(+)) on agarose gel electrophoresis when treated by the organic extracts of some isolates, for example. lane 1; DNA intercalation, lane 2; DNA intercalation, lane 3; no obvious structural change, lane 4; a slight DSBs, lane 5; DNA intercalation, lane 6; SSB, lane 7; no obvious structural change and lane 8; control (circular plasmid DNA). These results are typical examples of experiments repeated many times.

total of 39 isolates in details. As listed in Table 1, the organic extracts affect DNA structure in a rather complicated manner. In some cases, a distinct organic extract induces different DNA structural changes. For example, in ethyl acetate organic extract obtained from isolate 71 supernatant, the DNA intercalation and DSBs were occurred in the presence of Ni^{2+} and H_2O_2, respectively. However, no structural changes observed in the plasmid DNA treated with Cu^{2+}. The same trend happened for the ethyl acetate organic extract obtained from isolate 101 biomass, isolate 562 supernatant, isolate 131 biomass, isolate 134 supernatant, isolate 013 biomass, the methanol organic extract of isolate 024 biomass, and so on (See Table 1). Nonetheless, some organic extracts affect DNA *via* just one mediator, which indicates the specific mechanism involved. For example, ethyl acetate organic extracts obtained from the biomass of isolates 033, 43, 71, and the supernatant of isolates 012, 45, 91 have suitable potential to be introduced as selective drugs of cancer in future. The isolates which produce effective compounds were selected and their genus identification were performed via 16S rDNA sequencing.

Phylogenetic Characterization

Thirty-nine isolates were identified as producers of bioactive secondary metabolite and the genus identification were done using 16S rDNA sequence homology analysis. Figs. 2a and 2b indicate the phylogenic tree of the isolates. The majority of isolates (69%), n = 27, belong to the genus *Bacillus*. Three isolates (7%): *Kocuria sp.RP44, Brachybacterium sp. RP45,* and *Microbacterium sp. RP581* were identified as members of actinomycetes superfamily and the rest (24%) belong to *staphylococcus* and other proteobacteria. Although, the *Bacillus* isolates are evolutionary close relationship (Fig. 2a), the respective organic extracts show significant differences in their bioactivities and can be concluded that probably various gene expression patterns have occurred. Furthermore, as previously described by Janson et al, our results revealed that secondary metabolite production is a species-specific process and each isolate shows its specific pattern [43].

Antibacterial Potential Study

The antibacterial potential of each one of the organic extracts was studied against both Gram-positive pathogen, *Staphylococcus aureus* ATCC 25923 and Gram-negative pathogens, *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Fig. 3). Three isolates produced effective antibiotics in methanolic extract of the biomass against *S. aureus* including *Bacillus* sp. RP137, *Bacillus* sp. RP041, *Virgibacillus* sp. RP072. Moreover, the ethyl acetate extracts of supernatants from culturing of *Paracoccus* sp. RP0264 and *Bacillus* sp. RP073 had also inhibitory effect on *S. areous* growth. Methanolic extract of the biomass in *Virgibacillus* sp. RP042 showed inhibition of growth in *P. aerouginosa*. In total point of view, 15.4% of total isolates could produce antibacterial secondary metabolites against tested pathogens. There are some reports about screening other marine ecosystems for existence of antibacterial agents' producers. 4% and 13% of bacterial pool isolated from Arabian sea of Pakistan and global ocean surface water and swamp samples could inhibit growth of different pathogens, respectively [44,45]. In comparison with the Arabian Sea, the Persian Gulf short line presents a rich and prone ecosystem for bioactive secondary metabolite producers. Furthermore, the most majority population of antibacterial agent producers were belonging to genus *Bacillus* with five isolates out of six frequencies (Fig. 4).

Cell Viability Assay

All fractions that showed a type of binding to the circular DNA in gel electrophoresis study (See Table 1) were tested for evaluation of their cytotoxicity effect against Jurkat (T cell leukemia) and HepG2 (hepatocellular carcinoma) cell lines. The detailed results are shown in Tables 2 and 3. Approximately 23% and 45% (17 and 33 fractions) of the tested organic extracts have more than 30% toxicity effect on Jurkat and HepG2 cells at 2 mg ml^{-1} final concentration, respectively. Regarding to this, it seems that HepG2 cells are much more sensitive than Jurkat cell line. It is might be due to physiological behavior of liver cells which have potent metabolizing activity. Some compounds in the organic extracts could be metabolized to a toxic ligand and affect the cell growth in its new chemical format [46]. This type of compounds has been introduced as prodrugs which can show selective effect on cancerous cells. Hepatocarcinoma cells could activated prodrugs preferentially by their specific enzymes such as glutathione

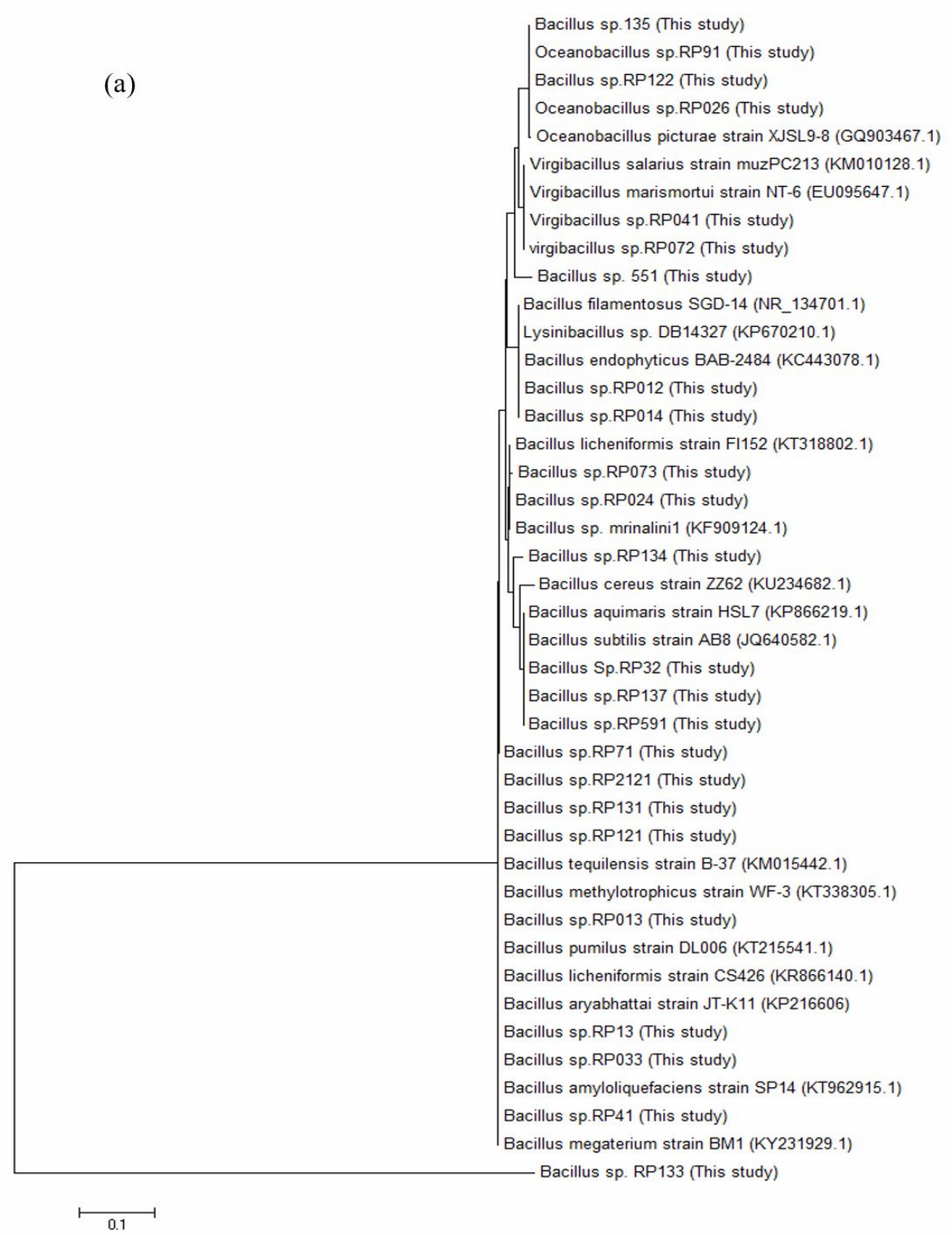

Fig. 3. Phylogenic tree of bacterial isolates based on their 16S rDNA sequence. a) *Bacillus* genus and b) the rest of isolates.

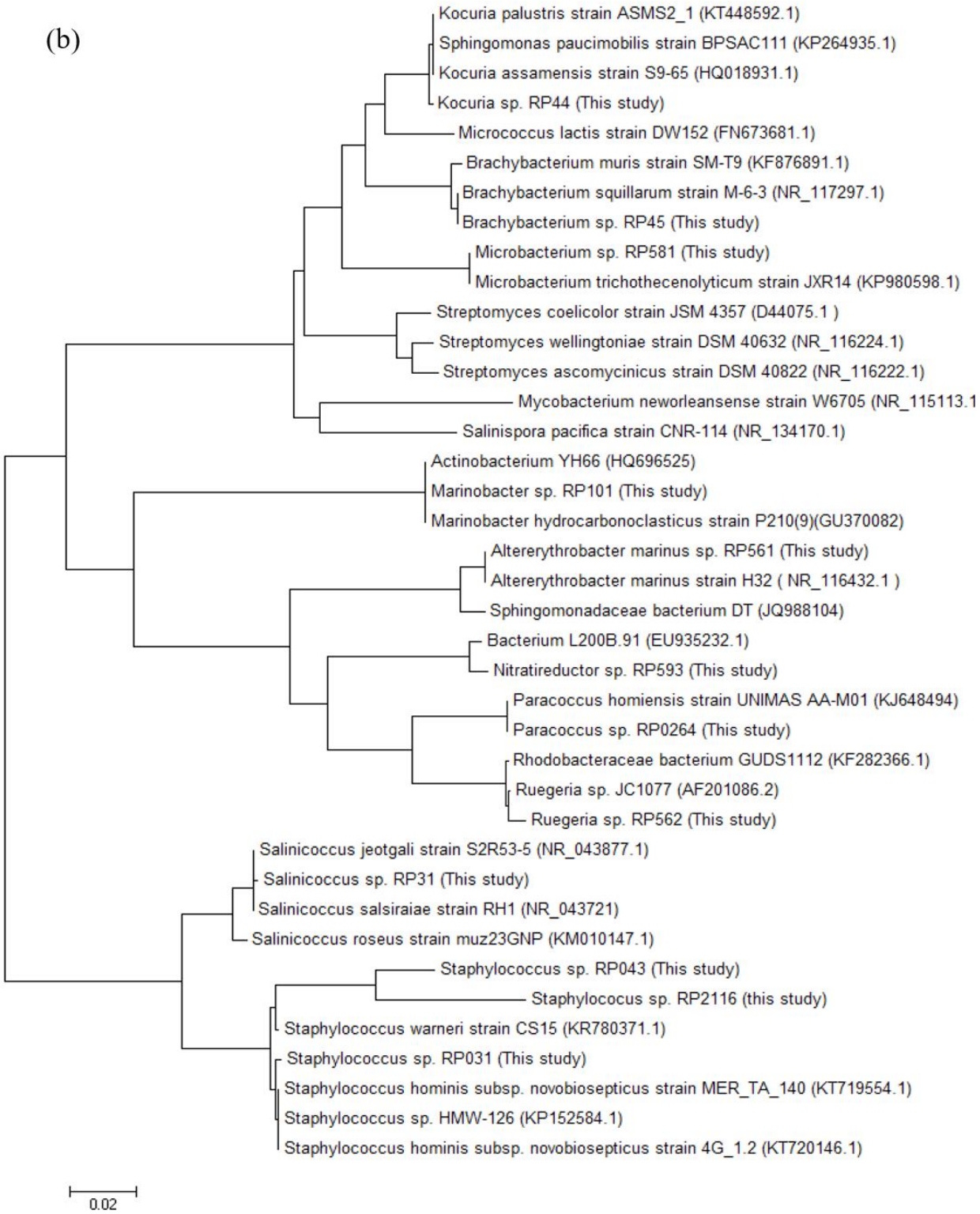

Fig. 3. Continued.

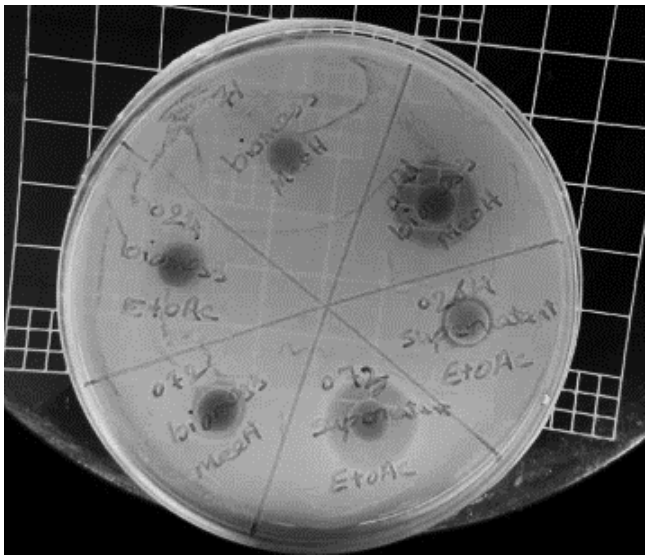

Fig. 4. Disc diffusion assay and determination of antibiotics in the organic extracts against *S. aureus*. Fractions of 041 and 072 biomasses, 073 and 0264 supernatants show antibacterial effect, however the extracts of 024 and 71 biomasses indicated no effect. These results are typical examples of experiments repeated many times.

Table 2. Cell Viability of Jurkat Cell Lines after Treatment with 2 mg ml^{-1} of the Effective Organic Extracts in a 48-hour Period of Time with Respect to their Fraction and Solvent Used for

Isolate	Fraction type	Solvent used	Cell viability (%)
Bacillus sp. RP13	Biomass	MeOH	59 ± 2.7
Bacillus sp. RP41	Supernatant	EtOAc	57 ± 3.2
Bacillus sp. RP41	Biomass	EtOAc	64 ±4.9
Bacillus sp. RP43	Supernatant	EtOAc	57 ± 7.6
Bacillus sp. RP43	Biomass	EtOAc	57 ± 3.3
Kocuria sp. RP44	Supernatant	EtOAc	67 ± 2.4
Brachybacterium sp. RP45	Supernatant	EtOAc	49 ±1.5
Bacillus sp. RP551	Supernatant	EtOAc	53 ± 0.9
Ruegeria sp. RP562	Supernatant	EtOAc	60 ± 4.6
Bacillus sp. RP71	Supernatant	EtOAc	48 ± 2.4
Bacillus sp. RP71	Biomass	EtOAc	48 ± 3.7
Bacillus sp. RP71	Biomass	MeOH	56 ± 2.1
Bacillus sp. RP134	Biomass	EtOAc	52 ± 1.1
Paracoccus sp.0264	Biomass	EtOAc	46 ± 4.6
Paracoccus sp.0264	Biomass	MeOH	57 ± 8.9
Staphylococcus sp.2116	Biomass	EtOAc	55 ± 3.1
Bacillus sp. RP2121	Biomass	EtOAc	61 ± 2.3

Table 3. Cell Viability of HepG2 Cell Lines after Treatment with 2 mg ml^{-1} of Different Organic Extracts in a 48-hour Period of Time with Respect to their Fraction and Solvent Used for

Isolate	Fraction type	Solvent used	Cell viability (%)
Bacillus sp. RP41	Supernatant	EtOAc	65 ± 5.3
Bacillus sp. RP41	Biomass	EtOAc	68 ± 0.5
Kocuria sp. RP44	Supernatant	EtOAc	61 ± 7.7
Brachybacterium sp. RP45	Supernatant	EtOAc	19 ± 4.3
Brachybacterium sp. RP45	Biomass	MeOH	60 ± 7.6
Bacillus sp. RP71	Supernatant	EtOAc	55 ± 7.1
Bacillus sp. RP71	Biomass	EtOAc	12 ± 0.7
Bacillus sp. RP71	Biomass	MeOH	19 ± 9.2
Marino bacter sp. RP101	Biomass	EtOAc	14 ± 0.3
Ruegeria sp. RP562	Supernatant	EtOAc	53 ± 7.8
Alterythrobacter sp.561	Supernatant	EtOAc	23 ± 3.4
Bacillus sp. RP591	Biomass	EtOAc	74 ± 7.5
Bacillus sp. RP121	Supernatant	EtOAc	18 ± 3.1
Bacillus sp. RP133	Biomass	EtOAc	13 ± 0.9
Bacillus sp. RP137	Biomass	EtOAc	11 ± 1.1
Bacillus sp. RP013	Biomass	MeOH	9 ± 0.7
Bacillus sp. RP013	Supernatant	EtOAc	15 ± 0.9
Bacillus sp. RP013	Biomass	EtOAc	18 ± 1.4
Bacillus sp. RP024	Supernatant	EtOAc	75 ± 6.5
Oceonobacillus sp. RP026	Supernatant	EtOAc	27 ± 8.9
Staphylococcus sp.031	Supernatant	EtOAc	18 ± 0.9
Bacillus sp. RP033	Biomass	EtOAc	61 ± 7.9
Bacillus sp. RP033	Biomass	MeOH	10 ± 0.3
Virgibacillus sp. RP042	Supernatant	EtOAc	19 ± 0.5
Virgibacillus sp. RP042	Biomass	EtOAc	13 ± 1.6
Virgibacillus sp. RP042	Biomass	MeOH	77 ± 3.0
Bacillus sp. RP013	Biomass	MeOH	28 ± 2.3
Virgibacillus sp. RP072	Supernatant	EtOAc	50 ± 8.3
Bacillus sp. RP073	Supernatant	EtOAc	80 ± 7.1
Bacillus sp. RP073	Biomass	EtOAc	16 ± 2.5
Bacillus sp. RP073	Biomass	MeOH	45 ± 2.9
Bacillus sp. RP2121	Biomass	MeOH	51 ± 6.1
Paracoccus sp.0264	Biomass	EtOAc	74 ± 3.7

Table 4. Membrane Protection (%) of Human Erythrocyte Treated by the Organic Extracts Obtained from Biomass of Bacterial Isolates in a Final Concentration of 10 mg ml^{-1}

Isolates	Solvent used	RBC membrane protection (%)
Dexamethasone	-	47.4 ± 3.0
Bacillus sp. RP133	EtOAc	85.0 ± 2.4
Bacillus sp. RP133	MeOH	71.7 ± 5.4
Bacillus sp. RP135	MeOH	60.0 ± 8.3
Bacillus sp. RP137	EtOAc	88.9 ± 3.4
Bacillus sp. RP137	MeOH	50.0 ± 9.9
Bacillus sp. RP551	MeOH	81.7 ± 5.0
Alterythrobacter sp.561	EtOAc	41.7 ± 1.4
Microbacterium sp. RP581	MeOH	90.0 ± 5.3
Microbacterium sp. RP581	EtOAc	75.0 ± 5.7
Bacillus sp. RP591	EtOAc	90.0 ± 2.5
Nitratireductor sp.RP593	EtOAc	60.0 ± 3.2
Bacillus sp. RP013	EtOAc	41.7 ± 6.3
Bacillus sp. RP013	MeOH	65.0 ± 2.1
Bacillus sp. RP014	EtOAc	50.0 ± 4.6
Oceonobacillus sp. RP026	EtOAc	71.7 ± 7.8
Staphylococcus sp.031	MeOH	55.1 ± 3.6
Bacillus sp. RP055	EtOAc	45.5 ± 12.2
Bacillus sp. RP055	MeOH	86.7 ± 1.4
Bacillus sp. RP073	EtOAc	50.0 ± 8.9
Bacillus sp. RP073	MeOH	90.0 ± 7.9
Paracoccus sp. RP0264	MeOH	83.3 ± 2.4
Staphylococcus sp.2116	EtOAc	83.3 ± 3.7

S-transferases (GSTs) particularly by GSTπ [47].

On the other hand, it is well understood that, although hydrophobic materials can transport through the cell membrane simply by diffusion, polar ones need specific transporters, channels or receptors to move into the cells [48]. The log P value of ethyl acetate and methanol solvents are 0.9 and 0.85, respectively which plays a critical role in the polarity of dissolved ligands. So, the methanol extracts assumed to be more hydrophilic than ethyl acetate ones and it is well known that, ligands with positive log P values are more lipophilic and can pass through the cell membrane easily. Making the point, as shown in Tables 2 and 3, the majority (more than 75%) of organic extracts having cytotoxicity effect towards cancerous cells were obtained from ethyl acetate extraction which probably refers to above mentioned issue. Moreover, it was shown that the severity of DNA structural changes either break or intercalation shows a good correlation with the cytotoxicity effect of the corresponding extracts. Based on the present data, similar trends have been observed which is probably due to DNA breaks ability of compounds that could increase the cytotoxicity efficiency on cancerous cell lines.

Anti-inflammatory Study

Anti-inflammatory potential of the organic extracts was examined via human Red blood cells (RBC) membrane stabilization analysis in vitro. Biomass organic extracts of 39 bacterial isolates tested in a 10 mg ml^{-1} final concentration and dexamethasone (2 mM) were used as a positive control (47.4% RBC membrane protection). Bacterial isolate's secondary metabolites with anti-inflammatory activity more than 40% on RBC membrane were listed in Table 4. The highest RBC membrane protection is approximately 90% in the case of treatment with methanolic extracts obtained from the bacterial isolates *Microbacterium* sp. RP581 and *Bacillus* sp. RP073 and the ethyl acetate organic extract of *Bacillus* sp. RP591. Sixteen isolates out of 39 was capable to produce potent anti-inflammatory metabolites under laboratory conditions. As shown in Table 4, RBC membrane protection observed in both ethyl acetate and methanol organic extracts of *Bacillus* sp. RP133, *Bacillus* sp. RP137, *Microbacterium* sp. RP581, *Bacillus* sp. RP013, *Bacillus* sp. RP055 and *Bacillus* sp. RP073.

CONCLUSIONS

All take into account, we persuade a study on searching for natural compounds produced by bacterial isolates from Persian Gulf with antibacterial, anti-inflammatory potentials and also could target DNA in a cancerous cell specific manner. We succeed to identify 6, 21 and 39 novel producers of the aimed compounds having antibacterial, anti-inflammatory, and DNA interaction potentials, respectively. However, there are things to take into consideration when it comes to cancer therapy, such as the polarity of desired compounds. The cell membrane is a semipermeable barrier and although hydrophobic compounds move in to the cell simply by diffusion, hydrophilic ones need specific transporters, channels or receptors to pass through cells. Our cell viability assay data (Tables 2 and 3) refer to the same trend. In the case of nonpolar crude extracts (ethyl acetate fractions), the cytotoxicity effect observed, but in the most cases, polar crude extracts have not shown any effects on the cell survival. In the present study, we show that the Persian Gulf short lines is a rich ecological pool and its microbial flora is a novel source for drug discovery research.

ACKNOWLEDGMENTS

The authors would like to thank the research affairs of Shiraz University through Grant number of 143077, Shiraz, Iran. This work was supported in part by a grant for Scientific Research from the Iran National Science foundation (INSF) under contract number 93035486.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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