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Original Article

Antimicrobial activity of *Brachidontes variabilis* extracts against multidrug-resistant bacteria clinically isolated

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Abstract

Introduction: Resistance to pathogens against kinds of traditional medicines is a public health problem, which calls for the continuous search for new compounds that are effective in eliminating diseases caused by these pathogens. Marine Animals come on top of animals that contain natural treasures of active substances that have not yet been discovered.

Methodology: In this study, two organic extracts (methanol and ethyl acetate) were prepared from the soft tissue of a marine organism (*Brachidontes variabilis*). Then, the antioxidant activity and phenol content were determined in these two extracts. Finally, their biological activities were studied toward drug-resistant microbes isolated from Syrian hospitals.

Results: The results showed that the IC50, the concentration required to reduce DPPH radicals by 50%, for methanol and ethyl acetate extracts were 179.286 and 73.676 mg/mL, respectively. The total phenolic contents of the methanol and ethyl acetate extracts were found to be 1.84 mg/g and 1.7 mg/g of the Gallic acid equivalent, respectively. The two extracts had an inhibitory effect on the growth of the following resistant bacterial species isolates: *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa*, at a concentration of 5 mg/mL, with inhibitory zones ranging from 5 mm to 20 mm. The ethyl acetate extract has an inhibitory effect on the growth of the fungal pathogen *Aspergillus fumigatus*, which is resistant to fungal antibiotics.

Conclusions: The results of this study are promising in finding new compounds with antimicrobial effects against resistant pathogens.

Key words: Molluscs; *Brachidontes variabilis;* antimicrobial; antifungal.

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Introduction

Oceans and seas occupy two-thirds of the Earth's surface; they are the largest habitats on Earth and contain the oldest forms of life. These marine environments provide a large portion of human requirements [1]. Due to characterize these habitats extreme physical, chemical, and geological factors, such as temperature, light intensity, salinity, and pressure, Marine organisms have adapted to these diverse factors by developing a wide range of forms, functions, and survival strategies. One of these adaptations is the production of secondary metabolites, such as enzymes, antibiotics, pigments, and biopolymers [2]. Recognizing the importance of marine natural products and the development of marine biotechnology, the European Union has introduced marine biotechnology as a source of biopharmaceutical and industrial applications, environmental treatment, and food production to ensure the purposeful and sustainable investment of marine resources [3]. Studies have shown that marine biodiversity is a major underutilized resource in various fields that serve humans [4]. At the beginning of this century, the interest in producing marine chemical products from marine organisms increased, focusing on isolating and identifying the chemical structures of these secondary metabolites [5,6]. Marine invertebrates are an important source of bioactive secondary products, which are used in medicine and pharmacy. For example, Trabectedin: a tumor antagonist, Vidarabine: an antiviral agent, and Cytarabine: an anti-leukemia agent, were isolated from marine sponges. Additionally, Ziconotide is a painkiller

that has been obtained from a cone snail. In addition to that, the studies have shown that a wide range of effective natural chemical compounds are produced by invertebrates [7]. *Brachidontes variabilis* is a bivalve mollusk that migrated from the Red Sea to the Mediterranean through the Suez Canal. It becomes a common species in Syrian marine waters, but it harms biodiversity and the coastal marine environment. It is a pollutant-tolerant species and it is used as a bioindicator [8]. This species attaches to rocks and all hard substrates on the Syrian coast and is abundant in shallow waters, with relatively high biomass in those areas. It is brownish-purple on the outside and purple on the inside, with a white belly area. *Brachidontes variabilis* feeds on phytoplankton by filtration, including the species *Pseudonitschia spp*, which is the main source of biotoxin production [9]. These characteristics make this organism produce varying concentrations of domoic acid, which vary between individuals depending on the location and time of collection. Thankfully, this concentration was below toxic levels in the latest local study conducted [10]. The spread of antimicrobial resistance has become a serious problem worldwide and needs to be controlled. Finding alternative natural active compounds could be useful to face this problem and prevent the spread of resistance strains [11]. We aim to study the organic extracts of this species of bivalve and chemically characterize it, and assess its biological effects, for us to be able to determine its medical benefits as a natural antimicrobial because it is a common species in the coastal waters of Latakia city.

Figure 1. Photograph of the invasive mollusk in the form of large clusters attached to rocks in the area from which it was collected.

Methodology

Collection of Samples

Samples of *Brachidontes molluscs* were identified variably during the spring period on 5/29/2022 from a specific location in the area of the Higher Institute for Marine Research located on the Blue Beach in Latakia Governorate, where hydrological measurements of marine mussels were made in a selected group area using a research measuring device of the type (Milwaukee mi 306) including values as follows: temperature 24.3 ºC and dissolved oxygen ratio 8.9 mg/L, salinity 31.5%, and then it was transferred containing ice implicitly to the research laboratoryin the Department of Animal Biology at Damascus university, and was kept in the refrigerator at a temperature of -20 degrees Celsius until used.

The Method Used for Extract Preparation

Samples of the bivalve *Brachidontes variabilis* were collected from the rocky shore of the Higher Institute of Marine Research (Latakia City) (Figure 1), chosen for its lack of industrial activities and absence of sewage discharge to ensure the absence of pollutants within the tissues of the bivalve. According to a previous study conducted at the Higher Institute of Marine Research, it was found that the studied species contained the highest percentage of biotoxins at the mentioned location during the spring, while the bivalve did not contain these toxins during the summer. Therefore, samples were collected at the end of the spring. Figure 1 shows a picture of the gaseous bivalve in large clusters in the area from which samples were collected, while Figure 2 represents a picture of the bivalve tissue after opening the valves of the organism. Then, the bivalve samples were thoroughly washed

Figure 2. Photograph of mollusk tissue after splitting the creature.

with water to remove sand. The valves were opened, and the samples were placed in distilled water to remove sand from within the bivalve tissue. The soft tissue mass was removed from inside the valves and then washed thoroughly with distilled water to remove salts, and the moist tissue mass was adequately dried using filter paper. The collected bivalve tissue, amounting to 50 g, was extracted from 1 kg of the entire organism and subsequently weighed.

The collected bivalve tissue, amounting to 50 g, was extracted from 1 kg of the entire organism and subsequently weighed. Then the quantity was divided into two parts to prepare two organic extracts with different polar solvents. In the next step, each 25g of the bivalve tissue was mixed with 10 times the amount of the selected organic solvents separately (highly polar methanol and weakly polar ethyl acetate). Each sample was ground using a blender, then the appropriate solvent was added and mixed well for ten minutes. The resulting mixture was poured into 500 mL glass bottles. These bottles were placed on a shaker to continue shaking at a speed of 200 rpm inside a refrigerator at a temperature of 4°C overnight. The next day, the samples were filtered, and the filtrate from each mixture was taken. The solvent was removed using a rotary evaporator, and the extracts were dried until a constant weight was achieved. And the weight of the methanolic extract after complete drying was 480 mg, while the ethanolic extract weighed 362 mg. These extracts were used in both the chemical and biological studies [12].

Chemical Study to Determine the Content of Biologically Active Substances in the Extracts

Evaluation of Free Radical Scavenging Activity (DPPH Test)

This is a colorimetric test that assesses the antioxidant activity of organic extracts to determine their medical efficacy due to their ability to inhibit free radicals, which are implicated in numerous diseases. This test is one of the most important and commonly used tests for determining the antioxidant capacity of substances by assessing their ability to inhibit free radicals. It involves a stable purple-colored free radical that turns yellow upon neutralization. The decrease in absorbance values of the reaction mixture at a wavelength of 515 nm indicates an increased ability of the extract to inhibit free radicals [13]. For the ethyl acetate extract, 100 µL of the methanol-diluted extract (initial concentration 100 mg/mL) was placed in test tubes to achieve the following concentrations (20, 30, 50, 60, 80, 100 mg/mL). As for the methanolic extract, 100 µL of the methanol-diluted extract (initial concentration 200 mg/mL) was also placed in test tubes to achieve the following concentrations (40, 60, 100, 120, 160, 200 mg/mL). Then, 1 mL of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (45 µg/mL) was added to each tube, and the tubes were placed in a dark place at room temperature for 30 minutes. Subsequently, the absorbance was measured at a wavelength of 515 nm, and the results were compared with a standard series of ascorbic acid (vitamin C) with an initial concentration of 0.02 mg/mL, which serves as a reference compound for the free radical scavenging test due to its high reductive capacity. This was done by adding 100 µL of the ascorbic acid solution (diluted in methanol) to achieve the following concentrations (0.004, 0.008, 0.01, 0.012, 0.016, 0.02 mg/mL) along with 1 mL of the previously mentioned DPPH solution. Then, the absorbance values for the samples were recorded, and the following equation was used to calculate the percentage of inhibition by the ethyl acetate extract [14]:

$$\text{I DPPH \%} = [(\text{A Control} - \text{A Sample})/\text{A Control}] \times 100$$

Total Phenolic Content Determination

The Folin method was utilized to determine the total phenolic content. This method is a colorimetric assay that employs Folin-Ciocalteu reagent (FCR), modified from Folin-Denis's reagent (FDR). Phenolics react with phosphomolybdic-phosphotungstic acid in an alkaline medium to produce a blue-colored solution, the absorbance of which is measured at a wavelength of 765 nm. This reaction involves a series of electron transfer reactions, resulting in the formation of blue-colored compounds [15]. Initially, the previously prepared extracts dissolved in methanol at an initial concentration of 200 mg/mL were diluted with acetone at a ratio of 1:5 to remove lipids. Thus, the concentration used in this experiment became 40 mg/mL. 20 µL of the extract was mixed with 750 µL of 6% Folin reagent, and after waiting for 3 minutes, 250 µL of 6% sodium carbonate solution was added. The mixture was thoroughly mixed and left in the dark at room temperature for an hour [16]. Afterward, the absorbance was measured at a wavelength of 765nm. The total phenolic content was determined by constructing a linear calibration curve for gallic acid extended in methanol at various concentrations starting from an initial concentration of 0.5 mg/mL. Table 4 represents the concentrations of gallic acid and their absorbance. The absorbance was plotted against the concentrations. The total phenolic content in each extract was estimated by substituting its absorbance value into the equation of the straight line for gallic acid.

Biological study

Organisms used in the research

Bacteria: Local isolates from university hospitals in Damascus were isolated. *Staphylococcus aureus, Streptococcus spp, Enterococcus spp, Klebsiella pneumoniae,* and *E. coli* are isolated from septicemia. *Pseudomonas aeruginosa* isolated from urinary tract infection.

Fungi: A local isolate of *Aspergillus fumigatus* was isolated from a pulmonary infection of a patient in Al-Mouwasat University Hospital in Damascus Governorate.

The biological activity against antibiotic-resistant pathogenic bacteria

Initially, several bacterial species were selected, both Gram-positive and Gram-negative (isolated and characterized in a previous study). They were activated in a sterilized Nutrient Broth medium by autoclaving and dispensed into 5 mL tubes. Before culturing the Bacteria, the tubes were then incubated for 24 hours to confirm the success of the sterilization process. Subsequently, samples were cultured to obtain pure bacterial colonies using a Blood agar medium, aiming to start the study of the biological effect of the extracts on them. Mueller Hinton agar medium was used to study the effect of both ethyl acetate and methanol extracts on the bacteria, and a sensitivity test was conducted on the same medium.

A bacterial suspension was prepared using nutrient broth medium, incubated for half an hour in the incubator, and then the turbidity was adjusted to 0.5 McFarland. Subsequently, a quantity of the suspension was spread onto Mueller-Hinton agar plates using sterilized cotton swabs in all directions. The plates were then inverted and left for 10 minutes inside the safety cabinet chamber. Afterward, antibiotic discs were placed onto the bacterial plates using pre-sterilized forceps and flame-sterilized. The same steps were repeated to test the efficacy of the extracts, but wells were punched in the bacterial plates, and 20 µL of each extract at a concentration of 50 mg/mL, extended with DMSO, was added to each well. The plates were then inverted and incubated in the incubator for 24 hours at 37°C. After 24 hours, the results were read by measuring the diameter of the inhibition zone formed around each well containing either antibiotic discs (Vancomycin, Ampicillin, Amikacin) (30µg, 10µg, 30µg) respectively (Thermo fisher®) or organic extracts, measured in millimeters.

Biological Activity Against Pathogenic Fungi

The fungal isolate studied was *Aspergillus fumigatus*, which is the causative agent of aspergillosis, a respiratory disease that threatens the lives of individuals with an immune deficiency. It was isolated from Al-Mawasat Hospital and demonstrated 100% resistance to both antifungal agents (Flucinazole, Abtek 25µg, Amphotericin B, Abtek 20µg) according to the Kirby Bauer method.

Initially, the fungal isolate was cultured on Sabouraud agar medium containing peptone, glucose, and agar, with a pH of approximately 5.6, to obtain pure isolated colonies. A fungal suspension was prepared using Sabouraud broth by inoculating a pure colony into 5mL of liquid Sabouraud broth, followed by incubation at 37°C for 30 minutes to adjust the suspension density to 0.5 McFarland. Then, a quantity of the suspension was spread on a Mueller-Hinton agar plate modified with 2% glucose and 5 µg/mL of methylene blue (GM-MH Agar). Antifungal disks were placed in the center of the plate to assess their sensitivity.

The same procedure was repeated to test the efficacy of the extracts, with the addition of two wells dug in the agar plate. Each well was filled with 20 µL of each extract at a concentration of 50 mg/mL diluted in DMSO. The plates were then inverted and incubated

Figure 3. Free Radical Scavenging Activity (DPPH Test) of the ethyl acetate extract. The IC50 was found to be 73.676 mg/mL.

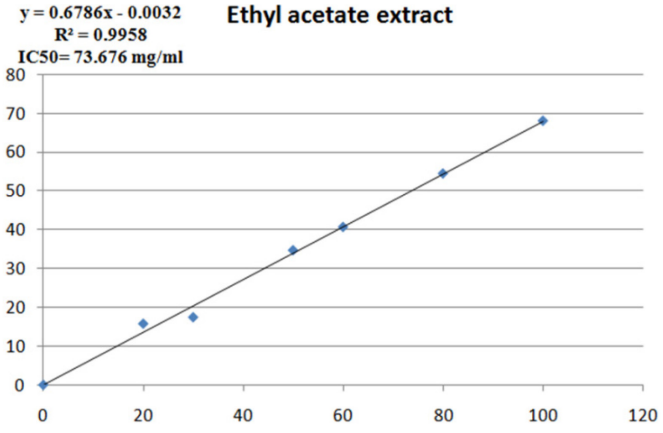


Table 1. Absorbance values for concentrations of the ethyl acetate extract and their inhibition percentages.

Ethyl acetate extract concentrations	20 mg/mL	30 mg/mL	50 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
Absorbency	0.410	0.402	0.318	0.289	0.222	0.156
Inhibition percentage I%	15.741%	17.385%	34.648%	40.608%	54.377%	67.940%

The value of A Control was 0.4866. The inhibition percentage was plotted against the concentrations using Excel software, and y = 50 was substituted into the equation of the straight line.

Table 2. Absorbance values for Methanol extract concentrations and their inhibition percentages.

Methanol extract concentrations	40 mg/mL	60 mg/mL	100 mg/mL	120 mg/mL	160 mg/mL	200 mg/mL
Absorbency	0.422	0.375	0.330	0.294	0.264	0.205
Inhibition percentage I%	8.380%	18.584%	28.354%	36.170%	42.683%	55.492%

A Control being 0.4606. Similarly, the IC50 was calculated and found to be 179.286 mg/mL (Figure 6).

Table 3. Absorbance values for Ascorbic acid concentrations and their inhibition percentages.

Ascorbic acid concentrations	0.004 mg/mL	0.008 mg/mL	0.010 mg/mL	0.012 mg/mL	0.016 mg/mL	0.020 mg/mL
Absorbency	0.517	0.447	0.420	0.401	0.329	0.210
Inhibition percentage I%	4.081%	17.068%	22.077%	25.602%	38.961%	61.038%

A Control being 0.539. The IC50 was calculated and found to be 0.019 mg/mL (Figure 7).

for 24 hours at 37°C. After 24 hours, the results are read by measuring the diameter of the inhibition zone formed around each well containing the extracts, as well as around the antifungal disks, measured in millimeters.

Due to the significance of the results for the ethyl acetate extract, the experiment was conducted in liquid Sabouraud medium in a 48-well plate. Gradient concentrations of the extract were prepared as follows: (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 mg/mL), and the inhibitory effect was studied by comparison with the untreated control. Then, 200 µL of each well was plated on GM-MH Agar medium, and the growth was controlled after 24 hours.

Results

Chemical Study to Determine the Content of Biologically Active Substances in the Extracts

Evaluation of Free Radical Scavenging Activity (DPPH Test)

The free radical scavenger activity was calculated by measuring the absorbance of the ethyl acetate extract at 515nm. The inhibition percentage ranged between 15.741% and 67.940% at different concentrations ranging between 20-100 mg/mL, with IC50:73.676 mg/mL as described in Table 1 and Figure 3. For the methanolic extract, the inhibition percentage ranged between 8.380% and 55.492% at different concentrations ranging from 40 to 200 mg/mL with IC50: 179.286 mg/mL as described in Table 2 and Figure 4.

The results of ascorbic acid showed that the inhibition percentage ranged from 4.081% to 61.038% at different concentrations between 0.004 mg/mL and -0.020 mg/mL with IC50: 0.019 mg/mL as described in Table 3 and Figure 5.

Figure 5. Free Radical Scavenging Activity (DPPH Test) of the ascorbic acid. The IC50 was found to be 0.019 mg/mL.

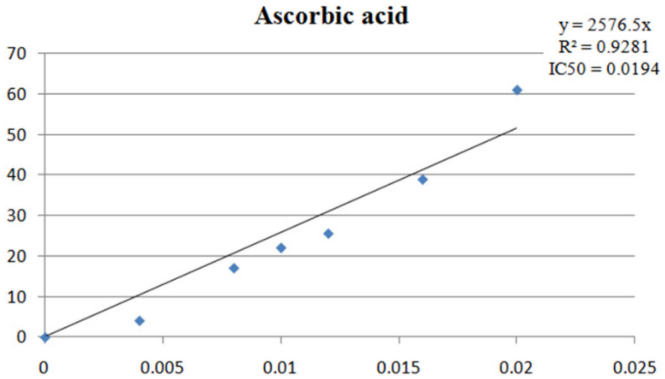


Figure 4. Free Radical Scavenging Activity (DPPH Test) of the methanolic extract. The IC50 was found to be 179.286 mg/mL.

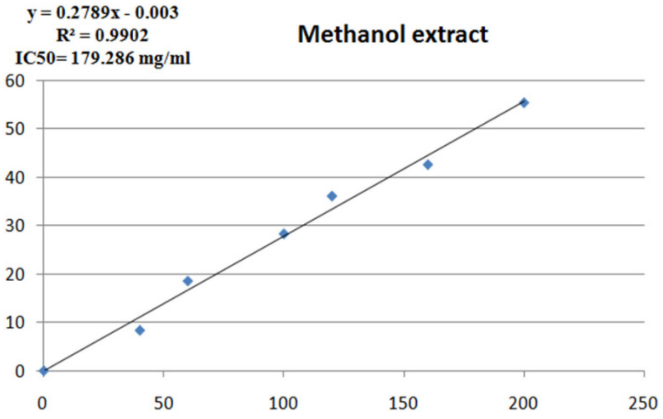


Figure 6. The figure shows the absorbance of Gallic acid at different concentrations.

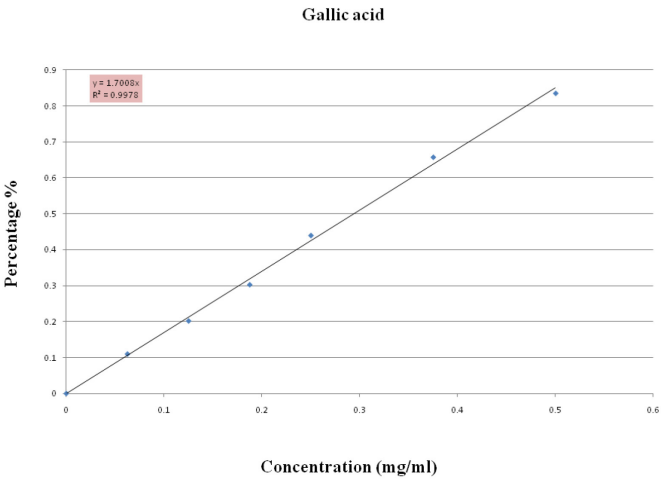


Table 4. Absorbance of Gallic acid concentrations at different concentrations at 760nm.

Gallic acid concentrations	0.0625 mg/mL	0.125 mg/mL	0.1875 mg/mL	0.25 mg/mL	0.375 mg/mL	0.5 mg/mL
Absorbency	0.110	0.202	0.303	0.440	0.658	0.836

Total Phenolic Content Determination

After the reaction period was over, the absorbance was measured at a wavelength of 765nm. It was found that the absorbance of the ethyl acetate extract was 0.5595, and the absorbance of the methanolic extract was 0.246.

Then, the absorbance of gallic acid was plotted in terms of concentrations as shown in Figure 6, where Table 4 represents the concentrations and absorbances of gallic acid. Then, the total content of phenols in each extract was estimated by substituting its absorbance value in the straight-line equation for Gallic acid. The total phenolic contents of the ethyl acetate and methanolic extracts were found to be 1.84 mg/g and 1.7 mg/g of the Gallic acid equivalent, respectively.

Microbiological study

The anti-bacterial activity of the methanolic and ethyl acetate extracts against antibiotic-resistant pathogenic bacteria

Different strains of Gram-positive and Gram-negative bacteria were used in this study. These isolates showed a different antibiotic-resistant pattern, which is described in Table 5. The antibacterial activity of methanolic and ethyl acetate extracts was verified on solid medium at a concentration of 50 mg/mL against 6 different bacterial isolates (*Staphylococcus aureus*, *Streptococcus spp, Enterococcus spp., Klebsiella pneumonia, E. coli, Pseudomonas aeruginosa*), and the inhibition zones were measured after 24 hours of incubation at 37°C. The diameters of inhibition zones ranged from 5-25mm (Table 6, Figure 7).

These results indicate that both extracts have an inhibitory effect on the growth of isolates of the following species: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa*. The methanolic extract had a greater effect on the first and third isolates, while the ethyl acetate extract had a greater effect on the second and fourth isolates, noting that the studied isolates are resistant to at least one antibiotic.

Anti-fungal activity

The antifungal activity of the methanolic and ethyl acetate extracts was studied against *Aspergillus fumigatus* resistant to Amphotericin B and fluconazole (Table 7).

Table 5. Diameter of inhibition zones formed around the antibiotic discs for each of the bacteria used.

Bacteria	Antibiotics		
	Vancomycine	Ampicilline	Amkcine
Staphylococcus aureus	20-10mm	-	\
Streptococcus sp.	40-20mm	-	\
Enterococcus sp.	-	-	-
Klebsiella pneumonia	\	-	10mm
E. coli	\	-	20-10mm
Pseudomonas aeruginosa	\	-	10-5mm

(\) An antibiotic should not be used with this type of germs, (-) There is no inhibition aura.

Table 6. Inhibition zone of methanolic and ethyl acetate extract at 50 mg/mL against different Gram-positive and Gram-negative bacteria.

Used Germs	Abstracts	
	Ethyl acetate extract, 50mg/mL	Methanol extract, 50mg/mL
Staphylococcus aureus	15mm	25mm
Streptococcus sp.	-	-
Enterococcus sp.	-	-
Klebsiella pneumonia	15mm	10 mm
E. coli	5 mm	20 mm
Pseudomonas aeruginosa	15-20mm	10 mm

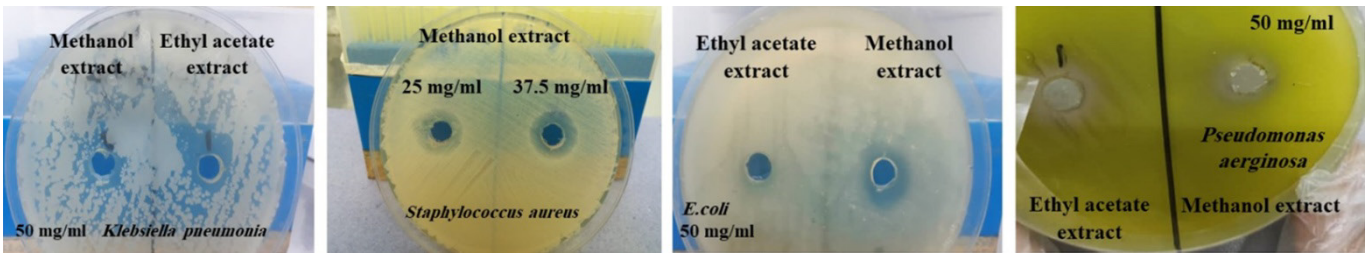
Table 7. The results for antifungals, where the fungi grew on all plates containing the antifungals used. The isolated fungal species represents 100% resistance to both antibiotics used.

Antifungal	Amphotericin B	Flucinazole
Aspergillus fumigatus	-	-

Table 8. Inhibition zone of methanolic and ethyl acetate extract against *Aspergillus fumigatus* fungal strains.

Organic extract	Ethyl acetate extract	Methanol extract
Aspergillus fumigatus	40mm	15mm

Figure 7. Inhibition zone representing the antibacterial activity of methanolic and ethyl acetate extract against different bacterial strains.



The antifungal activity of the methanolic and ethyl acetate extracts was studied against *Aspergillus fumigatus* on the solid medium Sabouraud agar. By using 50 mg/mL. The concentration of the inhibition zone was 15 mm for methanolic extract and 40 mn for ethyl acetate extract (Table 8).

Finally, the both extracts have a significant antimicrobial activity against bacterial and fungal isolates resistant against antimicrobial agent.

Discussion

The aim of this study was to test the effectiveness of mollusk extracts *Brachidontes variabilis*, which is an invasive species for the shores of the Syrian coast. The effectiveness of its extracts has not previously been studied locally and has not been studied globally. Therefore, the discussion was directed to compare with global studies of marine bivalves of mollusk of various types. For the chemical study of Crude extracts prepared from the entire mollusk tissue of Brachidontes *variabilis* by using methanol and ethyl acetate as an organic solvent and we obtained a good quantities of phenols. The results of this study are consistent with the results of the study of [17-20] in which the results of the chemical analysis of the raw methanol extract of one of the closely studied species, *P. viridis*, using a thin layer chromatography (TLC) method, showed the presence of quantities of alkaloids, saponins, terpenoids, sterols, and polyphenols, The results of biochemical examination of water extract, methanol and ethanol for *P. viridis* showed the presence of alkaloids, phenolic compounds and saponins. The presence of these types of compounds in the prepared extracts of these types can be explained by the diet of this organism, which depends on the method of filtration and feeds on phytoplankton, which contain in its chemical composition polyphenols, alkaloids, saponins, terpenoids, and sterols.

The study showed the ability of both extracts to inhibit free radicals using the DPPH reagent, and these results are consistent with the results of the study of Karaulova *et al.* Conducted on the raw aqueous extract of a bivalve species (*C. japonica*) [21], the results of the DPPH test showed the ability of the extract to curb free radicals, and this result was interpreted according to the results of chemical analyses of the extracts of the clamshell tissue used, where it was found that the amount of proteins and peptides with a low molecular weight is closely and positively related to the effectiveness of radical braking, while the amount of proteins with a high molecular weight is negatively linked to the effectiveness of radical scavenging, and in the study, The methanol extract of *P. viridis* showed a better antioxidant ability than other extracts used in the DPPH test. In our study, the ethyl acetate extract was more effective than the methanol extract in curbing free radicals. It is worth mentioning that the organic solvent ethyl acetate is known to extract hydrophobic compounds more efficiently than other solvents [20].

For the results of testing the effectiveness of extracts on bacteria, the effectiveness of the methanol extract was greater on both bacterial types (mm25) *Staphylococcus aureus and E. Coli* (20mm) and this finding for *Staphylococcus aureus* is consistent with a study that showed that all the tested bacteria are sensitive to *Perna viridis* mollusk methanol extract, but the most inhibitory effects were against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. As for the ethyl acetate extract, its efficacy was higher on the two species (20mm) *Pseudomonas aerginosa and Klebsiella pneumoni*(15mm)*, and* this result corresponds to the study of Tsankova *et al.* [19]. For *Klebsiella pneumoniae* and contrary to *it for Escherichia coli* and *Staphylococcus aureus* where antibacterial efficacy tests for *Mytilus galloprovincialis* extracts showed that ethyl acetate extract was the highest efficacy against *Escherichia coli* (13 mm) and *Klebsiella pneumoniae* (11 mm) and had no efficacy against *Staphylococcus aureus* [20].

Another local study of two class of mollusks from the bivalve row, methanol extract showed a higher inhibitory effect on the growth of bacterial isolates than ethyl acetate extracts [21,22]. Turning to the pathogenic fungus in our study, *Aspergillus fumigatus* was selected, which showed 100% resistance to the fungal antibiotics used. When applying the organic extracts of methanol and ethyl acetate, it was found that both extracts have vital effectiveness on *Aspergillus fumigatus*, but the effect of ethyl acetate extract was higher, as its effectiveness lasted for more than 48 hours, whether by studying the effectiveness by measuring the diameter of the inhibition aura (40 mm) or by applying the extract in a liquid medium and calculating the value of mic, which was estimated to be 12.5 mg/mL. Compared to another study on ethanol crude extracts (ECEs) prepared from two types of bivalves (clams), which can survive in harsh environmental conditions and surrounded by microbes of different types, namely Corbicula *fluminea* and *Geloina expansa*. The results showed antifungal effectiveness against *Candida albicans* and *Aspergillus niger*. The inhibition aura of *Corbicula fluminea* extract and *Geloina expansa* extract was between (8.3mm-12.2mm) [23]. Unlike our results, another study showed

that the methanolic extract fractionated form Loligo duvauceli Orbingy and Doryteuthis sibogae have no activity against the *Candida sp*, *Rhizopus sp*, *Aspergillus flavus* and *Aspergillus fumigates* [24].

It can be said that the effect of the pathogenic fungus on organic extracts varies according to several conditions, including the type of organism used and its location, the method of extraction and the type of organic locus chosen, as well as the environmental conditions to which the organism is exposed. In the study the efficacy of *Chara vulgaris* extracts, a type of green algae, towards the flagellar phase of tropical leishmania cutis, was studied and it was found that ethyl acetate extract is better than water extract, as the inhibitory value of half was approximately µg/mL 62.5 after 24 hours of treatment [25]. In conclusion, it can be said that the organic extracts of *Brachidontes variabilis* have vital effectiveness towards microbes (bacteria, fungi), and can be a potential source of chemical compounds with unique antimicrobial properties that will replace traditional medicines that are currently used and that are losing their effectiveness in treatment with the development of resistant pathogens.

Conclusions

This research represents the first attempt to leverage the abundant Mediterranean mussel, *Brachidontes variabilis*, found along the Syrian coast, by studying the impact of its tissue contents, specifically bioactive substances, on drug-resistant pathogens (including bacteria and fungi) prevalent in Syria. The effectiveness of organic extracts prepared from the muscle tissue was studied and tested against various drug-resistant microbes, including several strains of both gram-positive and gram-negative bacteria resistant to antibiotics, as well as a fungus strain exhibiting 100% resistance to antifungal agents. From a chemical perspective, these organic extracts demonstrated their ability to inhibit free radicals and were found to contain phenolics, highlighting their significance as antioxidants.

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Conflict of interest

No conflict of interest is declared.

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