



## ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF BLACK MUSSEL (*MYTILUS GALLOPROVINCIALIS*) FROM THE BLACK SEA, BULGARIA

Gabriela Tsankova<sup>1</sup>, Tatina Todorova<sup>1</sup>, Neli Ermenlieva<sup>1</sup>, Albena Merdzhanova<sup>2</sup>, Veselina Panayotova<sup>2</sup>, Diana Dobрева<sup>2</sup>, Katya Peytcheva<sup>2</sup>

1) Department of Microbiology and Virology, Faculty of Medicine, Medical University of Varna, Bulgaria

2) Department of Chemistry, Faculty of Pharmacy, Medical University of Varna, Bulgaria.

### ABSTRACT

**Background:** Over the past decade, there has been a growing interest in sea bivalves, which are an inexpensive and easily accessible source of high-quality proteins, lipids and secondary metabolites with antimicrobial and anti-fungal potential. Farmed Black Sea mussel (*M. galloprovincialis*) are promising objects for the study of their antimicrobial potential.

**Purpose:** The aim of this work is to determine the antibacterial activity of different extracts from the Black Sea mussel *Mytilus galloprovincialis* tissues by using the disc diffusion method with cultures of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

**Material/Methods:** Extraction of mussel tissues was done with different solvents: ethyl acetate (100%), methanol (100%), glycerol:water (50%, 1:1 v/v), ethanol (50%), acetone (70%), hot water. Antimicrobial activities of these extracts from *Mytilus galloprovincialis* was assessed by the disc-diffusion method.

**Results:** Testing antibacterial activity of black mussels revealed that ethyl acetate extract showed the highest activity against *Escherichia coli* (13 mm) and *Klebsiella pneumoniae* (11 mm) and no activity against *Staphylococcus aureus*. The glycerol: water extract showed growth inhibition effect against *Staphylococcus aureus* (11 mm) and *Escherichia coli* (10 mm), but no effect against *Klebsiella pneumoniae*.

**Conclusions:** The preliminary information presented in this study showed that the Black Sea farmed mussel could be an interesting source of antibacterial compounds. The glycerol-water extracts of *Mytilus galloprovincialis* had low antimicrobial activities against *Staphylococcus aureus* and more important against *Escherichia coli*.

**Keywords:** antibacterial activity, *Mytilus galloprovincialis*, disc-diffusion method,

### INTRODUCTION

Since 2008 Bulgaria is one of the important suppliers of Mediterranean mussels in the Black Sea region. Nowadays, Bulgarian mussel farms produced over than 1.5% of the cultivated mussels in the world [1]. Over the past decade, there has been a growing interest in sea bivalves, which are an inexpensive and easily accessible source of high-quality proteins, lipids and secondary metabolites. Marine invertebrates such as Black Sea mussels are a potential and promising source of structurally novel metabolites with anti-microbial, anti-fungal, anti-inflammatory and other pharmacological activities [2].

The current high prevalence of antibiotic-resistant bacteria demands the urgent need for new antimicrobial compounds. Mussels are promising objects for the study of their antimicrobial potential due to their specific habitats. Bivalves live in rich in bacteria and viruses environment and as filter-feeding species are exposed to different pathogens. As a result, their immune defence system must be based on non-specific, rapid cellular and humoral responses [3]. That is confirmed by the facts that in the recent year's various antimicrobial substances in molluscs tissues have been detected, extracted and characterized [4-6]. The mussel's antimicrobial activities are different and depend on the species of bacteria tested and solvents used for extraction. In addition, this functional property is species -specific. However, the information about the antibacterial activities of whole- body extracts of Black Sea mussel (*M. galloprovincialis*) from Bulgaria is not available. Having in mind all these facts, the aim of this work is to determine the antibacterial activity of different extracts from the Black Sea mussel *Mytilus galloprovincialis* tissues by using the disc diffusion method with cultures of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

### MATERIALS AND METHODS

#### Sampling

All samples were purchased in the summer (June-July) 2019 from a mussel farm, which is located in the

Northern part (Kavarna) of the Bulgarian Black Sea coast. The samples were stored in a freezer at -20°C. Average twenty-five specimens of black mussels from each month were used for analysis. The bivalves biometric characteristics were: mean weight – 11.5±0.5g and mean length – 4.5±0.5cm. All used solvents are HPLC grade.

#### Extraction

Extraction of mussel tissues was done with different solvents: ethyl acetate (100%), methanol (100%), glycerol: water (50%, 1:1 v/v), ethanol (50%), acetone (70%), hot water. Briefly, 3.0 g of mussel tissue were homogenized three times with 3 ml of solvent and shaken for 40 min at room temperature. After centrifugation (10 min, 3500 rpm) the extracts were filtered and combined. The crude extracts were stored at -20°C prior to analysis, but no longer than seven days.

#### Determination of antibacterial activity by disc diffusion susceptibility test

Bacterial strains tested were clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* obtained from the Laboratory of Microbiology, St. Marina University Hospital, Varna.

The surface of Mueller-Hinton or Mueller-Hinton Blood agar was inoculated with a bacterial suspension of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* with the density of 5x10<sup>5</sup>cfu/ml. Sterile filter paper disks were pre-impregnated with 50 µL varied concentration of tested extracts and were placed on the surface of the agar, using a needle. The plates were incubated at 36±1°C for 24h. Using a ruler, the zone of complete growth inhibition around each of the disks was carefully measured to within the nearest millimeter. The diameter of the disk was included in the measurement.

**RESULTS AND DISCUSSION**  
Antimicrobial activities of different extracts from *Mytilus galloprovincialis* are presented in Table 1 and Figure 1.

#### RESULTS AND DISCUSSION

Antimicrobial activities of different extracts from *Mytilus galloprovincialis* are presented in Table 1 and Figure 1.

**Table 1.** Antibacterial activities of different extracts of *Mytilus galloprovincialis*

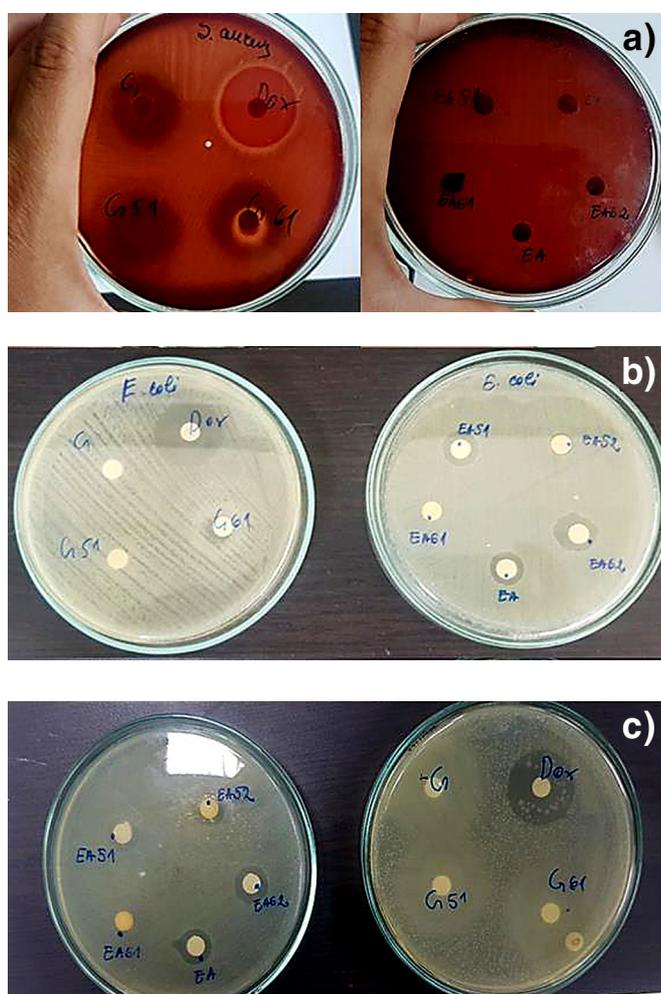
EA-1; M-1; GW-1; EW-1; AW-1; W-1 – black mussel extracts from June 2019 in the corresponding solvents;

| Extracts   | Antibacterial activities (mm of the zone of inhibition) |                              |                              |
|--|---|------------------------------|------------------------------|
|  | Gram-negative   |                              | Gram-positive                |
|  | <i>Escherichiacoli</i>                                  | <i>Klebsiella pneumoniae</i> | <i>Staphylococcus aureus</i> |
| <b>Ethyl acetate</b> (negative control)          | 11 mm   | 11 mm                        | -                            |
| EA-1   | 11 mm   | 11 mm                        | -                            |
| EA-2   | 13 mm   | 11 mm                        | -                            |
| <b>Methanol</b> (negative control)               | -   | -                            | -                            |
| M-1  | -   | -                            | -                            |
| M-2  | -   | -                            | -                            |
| <b>Glycerol: water</b> (1:1) (negative control)  | -   | -                            | 7 mm                         |
| GW- 1  | -   | -                            | 8 mm                         |
| GW-2   | 10 mm   | -                            | 11 mm                        |
| <b>Ethanol: water</b> (1:1) (negative control)   | -   | -                            | -                            |
| EW-1   | -   | -                            | -                            |
| EW-2   | -   | -                            | -                            |
| <b>Acetone: water</b> (70:30) (negative control) | -   | -                            | -                            |
| AW-1   | -   | -                            | -                            |
| AW-2   | -   | -                            | -                            |
| <b>Hot water</b> (negative control)              | -   | -                            | -                            |
| W-1  | -   | -                            | -                            |
| W-2  | -   | -                            | -                            |
| Doxycycline (positive control)                   | 21 mm   | 22 mm                        | 24 mm                        |

EA-2; M-2; GW-2; EW-2; AW-2; W-2 - black mussel extracts from July 2019 in the corresponding solvents;

Disc-diffusion method is preferred for rapid identification of bioactive metabolites and is usually used for initial screening of antimicrobial activity of different extracts [7]. Testing antibacterial activity, of *Mytilus galloprovincialis* revealed that ethyl acetate extract EA-2 showed the highest activity against *Escherichia coli* (13 mm) and *Klebsiella pneumoniae* (11 mm) and no activity against *Staphylococcus aureus*. However, this activity towards Gram-negative bacteria is comparable with the inhibition zone of the solvent (ethyl acetate). Moreover, one of the tested extracts EA-2 did not show any inhibition effect, and we could speculate that EA-2 even had protective activity against the ethyl acetate toxicity to the bacterial cells.

**Fig. 1.** Antibacterial activities of ethyl acetate (EA) and glycerol: water (GW) extracts of *Mytilus galloprovincialis* on cultures of *Staphylococcus aureus* (a), *Escherichia coli* (b) and *Klebsiella pneumoniae* (c)



The glycerol: water extract GW-2 showed growth inhibition effect against *Staphylococcus aureus* (11 mm) and *Escherichia coli* (10 mm), but no effect against *Klebsiella pneumoniae*. The zone of inhibition of the solvent (negative control) against *Staphylococcus aureus* was 7 mm, thus, the net effect against this Gram-positive bacterium is almost negligible. In contrast, the pure solvent is

not active against *E. coli*, and the measured negative growth effect of GW-2 could be considered as significant.

In this study among the three pathogens tested, both gram-negative strains were the more sensitive to ethyl acetate extracts, while gram-positive *S. aureus* was resistant in this case. These results suggest that gram-negative bacteria may be more susceptible than other investigated strain. One possible reason for the observed results is the specific structural differences in the outer layers of both gram-positive and negative bacteria. According to Shan et al. [8], gram-negative bacteria possess an outer membrane and a unique periplasmic space, which are not found in gram-positive bacteria. Moreover, the gram-negative bacteria resistance to antibacterial compounds is related to the hydrophilic surface of their outer membrane, which acts as a barrier to the percolation of different antibacterial substations. Kiran et al. [9] also supposed that more effective antimicrobial compounds are water-insoluble. This statement is confirmed by the fact that the ethyl acetate extracts showed higher antimicrobial potential compared to other extracts tested. Presented results in this study are similar to Nightingale et al. [10] who presented that ethyl acetate extract from slug have high activity against *Escherichia coli* and *Klebsiella pneumoniae*. Other studies reported that various solvent extracts from Black mussel *Mytilus galloprovincialis* [11], horse mussel *Modiolus modiolus* [4], crustacean [9] and bivalves [7] also affected a wide range of gram-positive and gram-negative pathogens significantly. Unfortunately, the data presented in different studies are difficult to compare due to the various test methods, the insufficient number of samples and diverse pathogens used. Observed discrepancy and differences in antibacterial activity reported from several researchers can be explained with a broad spectrum of bioactive compounds in the extracts and the specific extracting capacity of the solvents.

## CONCLUSION

The preliminary information presented in our study showed that the Black Sea farmed mussel could be an interesting source of antibacterial compounds. The glycerol-water extracts of *Mytilus galloprovincialis* had low antimicrobial activities against *Staphylococcus aureus* and more important against *Escherichia coli*. This basic research can help to develop antimicrobial drugs from marine natural products. Identification and isolation of specific bioactive compounds from marine mussel extracts from Bulgarian part of Black Sea need to be carried out in further studies.

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### Address for correspondence:

Gabriela Tsankova  
Department of Microbiology and Virology, Faculty of Medicine, Medical University of Varna, Bulgaria  
3, Bregalniza Str., 9002 Varna, Bulgaria.  
E-mail: [gabriela\\_sc@abv.bg](mailto:gabriela_sc@abv.bg)