The antibacterial activity of hemolymph of spider, *Agelena labyrinthica* (Araneae: Agelenidae)

Nazife YİĞİT[1], *Mehlika BENLİ[2]
[1]Kirikkale University, Faculty of Science and Arts, Dept. of Biology, Kirikkale, Turkey
[2]Ankara University, Faculty of Science, Dept. of Biology, Ankara, Turkey,
*Sorumlu yazar: mehlikabenli_76@hotmail.com

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Abstract
Since the number of microorganisms which are resistant to antibiotics have been increasing steadily, the need for treating these pathogens requires novel and efficacious antimicrobial agents, both in medicine and in agriculture. In our study, the hemolymph of *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae) which is considered as an alternative resource for antibiotics, was tested against ten bacterial strains, and it was found that five out of ten strains were sensitive to hemolymph. The sensitive bacterial strains were *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Shigella sp.*, *Escherichia coli*, *Staphylococcus aureus*. Our results showed that spider hemolymph had antibacterial effects. This is the first study about antibacterial effects of *A. labyrinthica's* hemolymph.
Key words: Spider, *Agelena labyrinthica*, Hemolymph, Antibacterial activity

Agelena labyrinthica (Araneae: Agelenidae) Örümcek Hemolenfinin Antibakteriyal Aktivitesi

Özet
Antibiyotiklere dirençli mikroorganizmaların sayısı her geçen gün artığından dolayı, tarımda ve tıpta bu dirençli mikroorganizmalarla mücadele etmek için yeni ve etkili antimikrobiyal ajanlara ihtiyaç duyulmaktadır. Antibiyotikler için alternatif bir kaynak olarak düşünülen *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae)'nın hemolenfi 10 bakteri suşuna karşı test edilmiş ve 10 suştan 5 tanesi hemolenfe duyarlı olarak bulunmuştur. Duyarlı bakteri suşları: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Shigella sp.*, *Escherichia coli*, *Staphylococcus aureus*'dur. Sonuçlar, örümcek hemolenfinin antibakteriyal etkiye sahip olduğunu göstermiştir. Bu çalışma *A. labyrinthica* hemolenfinin antibakteriyal etkisi üzerine yapılan ilk çalışmadır.
Anahtar Kelimeler: Örümcek, *Agelena labyrinthica*, Hemolenf, Antibakteriyal aktivite

Introduction

Prolonged use of broad-spectrum antibiotics has led to the emergence of drug-resistant pathogens, both in medicine and in agriculture. New threats such as biological control have increased the need for novel and efficacious antimicrobial agents. Due to the development of antibiotic-resistant bacteria, antibacterial peptides have attracted the attention in recent years, in order to find new therapeutic agents. The peptides with antibacterial activity have been found in plants and the whole animal kingdom, from bacteria and different insect orders to amphibians, mammals and humans (Boman, 1991; Haeberli *et al*., 2000). It is interesting that the diversity, complexity and variety of the natural compounds that exhibited antimicrobial activity seem to be much wider than suspected.

The first antibacterial peptides were identified from the moth *Hyalophora cecropia*, the cecropins (Steiner *et al*., 1981). Several antimicrobial peptides were isolated from the venom and hemolymph of venomous arthropods such as scorpions, and spiders (Kuhn-Nentwig, 2003). Gomesin was the first antimicrobial peptide, which was isolated from spider blood cells (Mandard *et al.*, 2002). This peptide shows marked sequence similarities to antimicrobial peptides from other arthropods such as tachyplesin and polyphemusin from horseshoe crabs and androctonin from scorpions. Interestingly, it also shows sequence similarities to protegrins, antimicrobial peptides from porcine leukocytes. Gomesin strongly affects bacterial growth, as well as the development of filamentous fungi and yeast. In addition, it was showed that gomesin affects the viability

of the parasites such as *Leishmania* and *Plasmodium* (Silva *et al.*, 2000; Moreira *et al*., 2007).

The objective of the present study is to test the antibacterial activity of the hemolymph of *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae). *A. labyrinthica* is a common funnel web spider which builds a knockdown web in low vegetation (Preston-Mafham and Preston-Mafham, 1984). The spider typically waits in the funnel entrance until prey arrives on a sheet in front of the funnel. This spider is cosmopolitan species, and they can be seen all over Anatolia. *A. labyrinthica* is known as venomous spider in Turkey (Bayram *et al*., 2007). It causes some epidemiological problems in terms of fatal bites and allergic reactions, especially among children and old people.

Materials and Methods

Spiders

Adult individuals of both sexes of 500 spiders, *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae) were collected from Ankara, Kırıkkale and Mersin in Turkey from June 2003 to 2006. They were reared in special cages and fed with *Oncopeltus fasciatus* (Hemiptera) in the Biology Department of Ankara University.

Spider Hemolymph Applications

After the spiders were narcotized with ether, the fourth walking legs were separated from the coxa. The hemolymph flowed outward was collected with micropipettes. The amount of hemolymph was obtained from each spider was nearly 10 µl. The hemolymph was diluted in the range of 1/1 with insect saline solution (insect saline solution: 1.80 g NaCl, 1.88 g KCl, 0.16 g $CaCl_2$, 0.004 g $NaHCO_3$ 100 ml distilled water), and tested against ten bacterial strains.

Test Microorganisms

Antibacterial studies were carried out on a series of ten bacterial strains. Six Gram-positive bacteria (*Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 7644, *Enterococcus gallinarium* CDC-NJ-4, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* RSHE) and four Gram-negative (*Escherichia coli* ATCC 25922, *Shigella* RSHE, *Escherichia coli* RSHE, *Pseudomonas aeruginosa* ATCC 27853) were used for this study. These microorganisms were obtained from Microbiology Laboratory Culture Collection of Refik Saydam Hifzissihha Institute (RSHI). The strains were inoculated on nutrient broth (Merck) and incubated for 24 h at +37 ºC ± 0.1.

Determination of Antibacterial Activity

Cultures of microorganisms grown for 24 h were used and diluted (10^{-1}) with sterile physiological saline solution (0.85 % NaCl) (X 10^7 cfu/ml). 100µl of test microorganisms were inoculated on the surface of solid medium on plates (Muller Hinton Agar/Merck). Three drops of 1/1 diluted hemolymph (10 µl) were put on each agar plate containing microorganisms. Agar plates containing strains were incubated at +37 º ± 0.1 ºC for 24 h. After incubation, all plates were observed for inhibition zones, and the diameters were measured in millimeters. All experiments were carried out three times. Amikacin (30 µg/ml) (Eczacibasi), vancomycin (30 µg/ml) (Mayne), penicillin (10 U/ml) (I.E.Ulagay), gentamicin (10 µg/disc) (I.E.Ulagay), rifamicin (5 µg/ml) (Aventis), tetracycline (30 µg/ml) (SIGMA), ampicillin (10 µg/ml) (SELVA), chloramphenicol (30 µg/ml) (SIGMA), erythromycin (15 µg/ml) (SIGMA) were used as positive controls. For positive control these antibiotics were tested on the same microorganisms under the same conditions. The stock antibiotics mentioned above were prepared in appropriate amounts (µg/ml) and 20 µl were absorbed onto discs (6 mm), and for negative control, insect saline solution was used.

Results and Discussion

A. labyrinthica which is commonly distributed through Turkey is venomous spider species. The female of *A. labyrinthica* is nearly 22 mm and male is nearly 12 mm (excluding the legs) (Figure 1). The hemolymph of both sexes of adult spiders (*A. labyrinthica*) were collected and freshly used

in this study to determine antibacterial activity against the test bacterial strains. It was found that five out of ten strains were susceptible to hemolymph. The sensitive bacterial strains were *B. subtilis* RSHE, *P. aeruginosa* ATCC 27853, *Shigella* RSHE, *E. coli* RSHE, *S. aureus* ATCC 29213. Our results showed that spider hemolymph had antibacterial effects (Table 1). A similar study was performed by Freitas *et al.* (2007). They found that the hemolymph of spider *Acanthoscurria parahybana* showed a broad spectrum of antimicrobial activity, no significant toxicity.

Table 1. The inhibition zones were formed by spiders' hemolymph, negativecontrol and some standard antibiotics

Bacteria	The diameter of inhibition zone (mm)										
	1 /1 Hemolymph	Negative control	Amikacin	Vancomycin	Penicillin	Gentamicin	Rifocin	Tetracycline	Ampicilin	Chloramphenicol	Erythnomycin
Enterococcus gallinarum CDC-NJ-4	-	-	-	12	-	15	13	-	-	-	11
Enterococcus faecalis ATCC 29212	-	-	16	12	-	16	14	-	-	-	11
Bacillus subtilis RSHI	22	-	24	19	22	25	23	12	-	13	24
Escherichia coli RSHI	-	-	18	-	-	18	-	-	-	-	-
Shigella RSHI	10	-	20	-	-	19	-	-	-	-	-
Escherichia coli ATCC 25922	10	-	16	-	-	17	-	-	-	-	-
Streptococcus pyogenes ATCC 19615	-	-	13	12	-	16	15	-	-	-	12
Staphylococcus aureus ATCC 29213	10	-	17	15	19	17	27	12	-	-	18
Listeria monocytogenes ATCC 7644	-	-	25	16	-	27	39	-	-	-	19
Pseudomonas aeruginosa ATCC27853	15	-	17	-	-	15	-	-	-	-	-

The male of *A. labyrinthica* The female of *A. labyrinthica*

Figure 1. Overall dorsal view of male and female *A. labyrinthica*

Gomesin was isolated from hemolymph of tarantula spider (*Acanthoscurria gomesiana*) strongly affects bacterial growth such as *E. coli* and *Micrococcus luteus*, as well as the development of filamentous fungi and yeast (Silva *et al.*, 2000). Pereira *et al.* (2007) isolated antibacterial acylpolyamine, named mygalin, from the hemocytes of the same spider (*A. gomesiana*). The antimicrobial activity of mygalin was tested against three microorganisms *E. coli, M. luteus* and *Candida albicans*, and mygalin was active against only *E. coli.* In another study, the hemolymph spider *Acanthoscurria parahybana* showed a broad spectrum of antimicrobial activity (Freitas *et al.*, 2007).

In our studies, *A. labyrinthica* hemolymph exhibited antibacterial activity against five of the tested bacteria. *B. subtilis* and *S. aureus* which were found to be susceptible to hemolymph were also susceptible to antibiotics used in positive

controls. While *P. aeruginosa*, *Shigella* and *E. coli* are quite resistant against the antibiotics that were tested, they are susceptible to hemolymph. The prevention of the development of these susceptive bacterial strains by the hemolymph is a significant finding. The inhibition zone diameters of hemolymph and antibiotics have very close values (Table 1). This shows us that the hemolymph solution can be as effective as antibiotics. The revealing and development of the antibacterial compounds in the hemolymph will provide an opportunity for the production of new compounds with natural activities as an alternative to antibiotics.

Studies of antimicrobial peptides provide new insights into dynamic interactions between microorganisms and their host, and generate new paradigms for the pathogenesis. The antimicrobial peptides of higher eukaryotes differ structurally from conventional antibiotics produced by bacteria and fungi, and they are active against a large spectrum of microorganisms, including filamentous fungi in addition to protozoan and metazoan parasites (Vizioli and Salzet, 2002; Marshall and Arenas, 2003). Because of these, they offer novel templates for pharmaceutical compounds that could be effective against increasingly resistant microorganisms (Ganz and Lehrer, 1999).

Many infections acquired in hospitals are caused by potentially fatal bacteria, such as *S. aureus* that are resistant to many antibiotics such as methicillin (Vizioli and Salzet, 2002). Our study demonstrated that hemolymph of *A. labyrinthica* was effective against *S. aureus*. Consequently, to develop effective and new drugs against antibiotic-resistant micro-organisms, the hemolymph of spider may be used as a potentially new source of natural antimicrobial agents.

Conclusions

Our findings indicate that hemolymph of *A. labyrinthica* possesses antibacterial activity. By the findings and purification of the active agent that is present in hemolymph, it will be possible to discover new natural drugs serving as chemotherapeutic agents for treatment with pathogens and take these antibiotic-resistant bacteria under control. This study will be a base to our further investigations on advanced purification and effect mechanism of its active compounds.

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