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Antibacterial And Hemolytic Activities Of *Amsonia orientalis* (Syn. *Rhazya orientalis*) Decne. Against *Legionella pneumophila* Strains

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ABSTRACT

Legionella pneumophila, a causative agent of Legionnaires' disease, can colonize the water systems easily. Despite that, disinfection of the systems is difficult and expensive. Since many of the commercially available disinfectants are harmful for staff responsible for disinfection, and civilians who may inhale to aerosols from treated systems, the search of new antibacterial compound is gaining more importance. In the current study, the antibacterial and hemolytic activity of the *A. orientalis* extracts was investigated against eleven different *L. pneumophila* strains isolated from water system in Istanbul. It was determined that the ethanol-leaf extract was the most effective on the bacteria. However, considering antibacterial and hemolytic activities, the stem+leaf chloroform extract have been the most suitable antimicrobial agent agains *L. pneumophila*.

I.INTRODUCTION

Legionella pneumophila is an aerobic, flagellated, filamentous Gram-negative bacteria. The bacteria may have been in the natural and man-made water systems (lakes, pools, air condition systems, etc.) where they can use amoeba for reproduction and survive against chlorinated compounds. *L. pneumophila*, acquired by droplet inhalation of water contaminated by bacteria, is the causative agent of Legionnaires' disease (LD) and pontiac fever. The bacteria presence in the water systems may have been associated with outbreaks of Legionnaires' disease. Therefore, it should be very important to take counter measures against outbreaks of *Legionella*.

The control of *Legionella* bacteria in water systems is provided with the proper use of the biocidal products. However, the resistance in *L. pneumophila* bacteria has been developed owing to the indiscriminate use of commercial antimicrobial biocides commonly used in the water systems. Additionally these chemicals, in some cases, are particularly dangerous for personnel who make applications. The scientists try to solve the problem with studying on new natural antimicrobial agents from various sources, such as medicinal plants.

The screening of plants for antimicrobial activity has shown that plants can be a potential source of new antiseptic and disinfectant formulations as well as in the treatment of diseases (Olukoya et al., 1993). When the relevant literature was examined, it was shown that antimicrobial agents extracted from a variety of plants have recently been evaluated for their antibacterial properties against *Legionella* and various bacteria (Çete et al., 2005; Changa et al., 2008; Shimizu et al., 2009). However, up to now, only grapefruit seed extract has been tested against *Legionella* under warm water situation (Furuhata et al., 2003).

Amsonia orientalis Decne. belonging to Apocynaceae family is a medicinally important plant which has very restricted distribution on the world only in Turkey and Greece (Davis, 1978). When literatures were researched on *A. orientalis*, which is critically endangered in nature according to IUCN categories, it was shown to be extremely limited number of studies on the antibacterial activity of this plant (Akyalcin et al., 2006). However, antibacterial activity against *L. pneumophila* has not been extensively investigated.

Therefore, the objectives of this research were to investigate antibacterial and hemolytic activities of deionized water, ethanol, methanol, acetone, chloroform and n-hexane extracts obtained from the different parts of *A. orientalis* against eleven different *L. pneumophila* strains isolated from water system in Istanbul.

II. MATERIALS AND METHODS

A. orientalis samples which is identified by Dr. Osman Erol, Department of Botany were stored at the Herbarium, Faculty of Science, Istanbul University (Herbarium no: 40081 ISTF). The different parts (stem and leaf, leaf+stem) of *A. orientalis* were extracted with deionized water, ethanol, methanol, aceton, chloroform, n-hexane.

Antibacterial activity of these extracts against *L. pneumophila* strains were carried out according to the agar diffusion method and was determined by measuring the diameter of the inhibition zone formed around the disc. In the antibacterial activity tests, late log phase cultures of *L. pneumophila* were used. The bacteria cultures were suspended in aseptic tap water to get a density of 0.3 at OD600. After the 0.1 mL suspensions were spread on Buffered Charcoal Yeast Extract Agar (BCYE) Plates, the discs, which absorbed 20 µL plant

extract and dried, were placed. At the end of the three-day waiting period in 37 ºC, 85% humidity and 5% CO_2 the diameter of inhibition zones were recorded.

Hemolytic activity of the plant extracts was determined using microdilution method based on hemolysis against human red blood cells. Zero hemolysis (blank) and 100% hemolysis controls were determined using erythrocytes suspended in PBS buffer alone and 0.003% saponin suspension in sterile distilled water. All experiment all experiments were performed in triplicate.

III. RESULTS AND DISCUSSION

The resistance of bacteria against antimicrobial agents has an important problem nowadays. Due to multiple drug resistant (MDR) bacteria outbreaks, the scientists have been seeking new antimicrobial agents from different origins. The plants have been potential sources because of their effective, safe, reachable and cheap natures (Ruttoh et al. 2009). The medical importance of Apocynaceae family members as species of *Nerium*, *Adenium*, *Trachomitium*, *Apocynum* genus has known for years (Suffredini et al. 2002). *Amsonia* genus has been also known therapeutic plants (Kimiran Erdem et al. 2013). Therefor in the present study antibacterial potential of *A. orientalis* on *L. pneumophila* was investigated.

The results obtained in the present study suggest that *A. orientalis* extracts had the ability to inhibit the growth of *L. pneumophila* and the antibacterial activity differs with the applied extraction method (Table 1, 2).

Table 1. Comparison of antibacterial activity against *L. pneumophila* serogroup 1 strains according to the extraction methods of different parts of plant

Extracts	Bacteria				
	8	**9**	**10**	**11**	**12***
stem+leaf chloroform	0±0	0±0	13±1	22±1	10±0
stem+leaf methanol	0±0	0±0	15±1	23±1	11±1
leaf n-hexane	0±0	0±0	0±0	0±0	0±0

leaf acetone	0±0	0±0	18.7±1.5	23.3±0.6	14±1
leaf ethanol	19±0.6	10.3±0.6	14±0	30.3±0.6	0±0
leaf deionise water	14.3±0.6	10.3±0.6	0±0	18.3±0.6	0±0
stem deionise water	12.7±1	11.7±0.6	0±0	0±0	0±0

*: *L. pneumophila* ATCC 33152 standard strain

Table 2. Comparison of antibacterial activity against *L. pneumophila* serogroup 2-14 strains according to the extraction methods of different parts of plant

Extracts	**Bacteria**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
stem+leaf chloroform	0±0	0±0	26.7±0.6	17.7±1.5	0±0	21±1	20.7±0.6
stem+leaf methanol	0±0	0±0	17±1	16.7±0.6	9.3±0.6	15.3±0.6	15.3±0.6
leaf n-hexane	0±0	0±0	0±0	0±0	0±0	0±0	0±0
leaf acetone	0±0	0±0	24±1	18.7±1.5	0±0	22±1	36.7±1.2
leaf ethanol	0±0	0±0	29±1	19.3±0.6	9.3±0.6	33.7±0.6	22.3±0.6
leaf deionise water	0±0	0±0	13±0	20.3±0.6	0±0	16.3±0.6	0±0
stem deionise water	0±0	0±0	10.7±0.6	12.3±0.6	0±0	0±0	0±0

Although the bioactive components of *A.orientalis* (syn. Rhazya orientalis) was known (Itoh et al. 2002; Akyalçın et al. 2006; Zongo et al. 2009), there has been a limited study on the antibacterial activity of plants against *L. pneumophila* (Kimiran Erdem et al. 2013). In the current study, the ethanol leaf extract of *A. orientalis* was the most active against tested *L. pneumophila* strains (75%) when compared with acetone extract (58%). However, the hexane leaf extract did not show antibacterial activity against any of the *L. pneumophila* strains tested. On the other hand, the chloroform stem+leaf extract of *A. orientalis* stem+leaf showed less inhibitory activity against the tested bacteria than the methanol extract. Additionally, the antibacterial activities of extracts have shown differences according to serogroups. The ethanol leaf extract had the strongest activity against serogroup 1 bacteria, while the most powerful activity against serogroup 2-14 isolates was detected with stem+leaf methanol and leaf ethanol extracts. For all that, the highest inhibitory activity was seen against *L. pneumophila* serogroup 2-14 strain (zone of inhibition: 36.7±1.2mm) using the acetone leaf extract (Table 2).

In the present study, it was determined that chloroform stem+leaf extract, the hexane leaf extract and deionise water stem extract did not show hemolytic activity. The ethanol leaf extract exhibited only antibacterial activity with low hemolytic activity (≥5 mg/ml) (Figure 1).

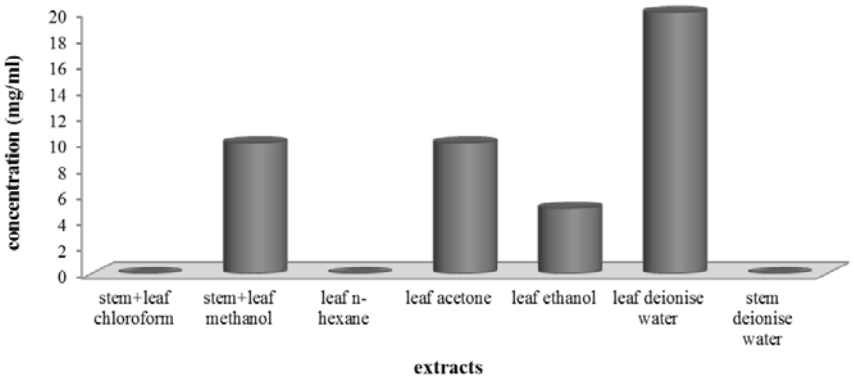

Figure 1. Hemolytic activity of extracts

When the results have been evaluated, it was seen that the hexane had not a good solvent for *A. orientalis*. On the other hands regarding to hemolytic activity of extracts the stem+leaf chloroform extract have been the most suitable antimicrobial agent agains *L. pneumophila*.

L. pneumophila outbreaks have been an important problem, due to mortality of bacteria on immunosuppressive people. After infection, the medical treatments are only started after diagnosis. Since the symptoms may be interpreted as cold by patients, beginning of treatments will be lately. These conditions have been caused to deaths. Because of this reason the more effective antibiotics against *L. pneumophila* should be developed. On the other hand, the best response is eradication of *Legionella* from water systems. Since the bacteria live in amoeba in water systems, it is able to survive against biocidal applications. For the disinfection of these systems some users apply too frequently or overdoses treatments. This situation is dangerous for public health and harmful for water systems. In the present study, the extract of *A. orientalis* has a potential for using treatment of Legionnaires' disease and disinfection of water systems. For this purpose, the purification of extracts that are both non-hemolytic and antibacterial has been aimed in the further studies.

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