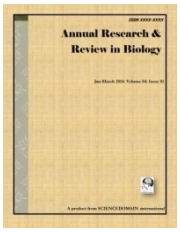

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Antibacterial Activity of *Lythrum salicaria* against Multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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Authors' contributions

Author EG made microbiological tests, wrote the first draft of the manuscript and managed the literature searches. Author HG collect plants and prepare the exctract in laboratory. Authors MZ and OK designed the study, wrote the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are extremely problematic pathogens. Treatment of hospital infections caused by these pathogens is very difficult. In this study, we aimed to investigate the antibacterial efficacy of a traditional medicinal plant, *Lythrum salicaria* against these problematic bacteria.
Methodology: Liquid extract of *L. salicaria* was prepared in methyl alcohol. A total of 30 *A. baumannii* and 27 *P. aeruginosa* strains which had been isolated from hospitalized patients as a nosocomial pathogen was used. The antibacterial activity of test materials was assessed by agar well diffusion test method.
Results: Mean inhibition zone diameter against *P. aeruginosa* and *A. baumannii* were found 16.09 mm (minimum 12 mm, maximum 20 mm) and 18.3 mm (minimum 10 mm, maximum 25 mm), respectively.
Conclusion: *L. salicaria* extract showed good antibacterial activity against these pathogens. Further pharmacokinetic and pharmacodynamic studies are needed for topical and oral usage of this traditional plant as a drug against multidrug-resistant pathogens.

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Keywords: Traditional plant; Antibacterial activity; Lythrum salicaria; P. aeruginosa; A. baumannii.

1. INTRODUCTION

Antibiotic resistance is an important problem. This is a significant issue not only in developed countries, but also in developing countries where the policies of antibiotic resistance is still insufficient and tends to use broader spectrum antibiotics [1]. A very small group of bacteria that referred as “ESKAPE bugs” are caused to the most of hospital infections. ESKAPE bacteria are *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa*, and *Enterobacter* species [2]. The common features of these bacteria are that they are resistant to a relatively large number of antibiotic and they are caused to pan antibiotic resistant infections. Among highly antibiotic resistant gram-negative rods, the prevalance of multidrug-resistant (MDR) *Acinetobacter spp.* and *P. aeruginosa* is increasing all over the world. Moreover, there is only limited treatment options for these pathogens and doctors tend to use broad-spectrum antibiotics or old agents such as colistin [3].

Overuse or misuse of antibiotics leads to increase in antibiotic resistance and cost of treatment. Other negative outcomes are medication errors, drug toxicity and drug interactions [4]. Increasing antibiotic resistance raises the need for new antibiotics. However, there has been a continued decline in the number of newly approved drugs [5]. Probably returning to the preantibiotic era has become a reality in many parts of the world [6].

As a lack of production of new antibiotics and resistance towards antibiotics becomes more common, a greater need for alternative treatments arises. *Lythrum salicaria*, known as purple loosestrife (*Lythraceae*) and "Tibbi hevhulma" in Turkish is well known as a medicinal plant from ancient Greek and Roman times and it has been an important drug for centuries [7]. *L. salicaria* is spread throughout the world as in our country. Areas of natural distribution of this taxon which originated in Europe and Asia, starting with Spain in the west, all over Europe, and dates back to the north parts of China and Mongolia in Asia. Also, shows a wide distribution in the northern parts of America and Canada [8].

Liquid extract of *L. salicaria* which was obtained by boiling have been used internally for diarrhea and chronic intestinal catarrh in traditional medicine for years [7]. Scientific investigations of *L. salicaria* recently demonstrated anti-inflammatory, anti-hyperlipidemic, antiatherosclerotic, strong antioxidant, and low-level anti-diabetic effects [9,10]. Antibacterial properties of this natural substance have also been identified. It has strong antimicrobial activity against *Candida albicans* and *E. coli* and weak antimicrobial activity against *S. aureus* and Helicobacter pylori [8,11]. To our knowledge, in English literature there were not any study about the efficiency of *L. salicaria* against MDR *A. baumannii* and *P. aeruginosa*. Resistance level of our bacteria differs our study from previous studies. This article is the first study that investigating the antibacterial efficacy of a traditional medicinal plant, *L. salicaria* against MDR *A. baumannii* and *P. aeruginosa*.

2. METHODOLOGY

2.1 Collection of Plant Materials

The flowers and the leaves of *L. salicaria* were collected from Sakarya, Turkey in the months June and July. The plant materials were brought to the laboratory immediately. They were washed with tap water and then were allowed to dry at room temperature in shade for a week.

2.2 Extraction and Isolation

Dried plant materials (50 g) were thoroughly processed in 250 ml ethyl alcohol (EtOH) with a warring blender for 10 min. The resulting mixture was filtered after two days. EtOH was evaporated under reduced pressure from the filtrate. Concentrated ethanol extract was dissolved in methyl alcohol (MeOH) and filtrated. The filtrate was again evaporated and the residue (8.3 g) was subjected to column chromatography over silica gel using MeOH as the eluent. A total of 3.4 g extract was obtained. A stock solution (17 mg/ml) of the extract was prepared in MeOH.

2.3 Strains and Growth Conditions

A total of 30 A. baumannii and 27 P. aeruginosa strains were obtained from our Infectious Diseases and Clinical Microbiology laboratory stocks. These stock strains were isolated from various clinics' hospitalized patients' clinical specimens, such as urine, sputum, and pus, which were sent for laboratory identification. The antibiotic susceptibility profiles of all isolates were assessed by Kirby Bauer's disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI)[12].

The isolates were stored at -20^{o} C in skimmed milk agar until used in this study. Prior to the study, each strain was subcultured on 5% blood agar at 37^{o} C for two consecutive days. From *A.baumannii* and *P. aeruginosa* strains obtained in the second passage, bacterial suspensions were prepared in tryptone soya broth (TSB) (Oxoid, Basingstoke, UK), and adjusted to a turbidity equal to McFarland 0.5 (1.5 x 10^{8} cfu/ml) (DIN EN 1040, 2005). All strains were studied with an agar well diffusion method [13].

2.4 Agar Well Diffusion Method

Each Mueller-Hinton agar plate was inoculated with the microorganism by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60^{o} each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. Once the agar was solidified, it was punched with eight millimeter diameter wells and filled with 50 µL of the test material. The concentration of the plant extracts employed was 17 mg/ml. They are incubated at 37ºC for 18 to 24 h. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) was measured with a ruler to the nearest millimeter. Each test was run in triplicate for every bacterial strain. MeOH was used as a negative control in each test. IZDs were reported as an average of the replicates showing antimicrobial activity.

3. RESULTS AND DISCUSSION

A total of 30 *A. baumannii and 27 P. aeruginosa* strains' susceptibility against *L. salicaria* was investigated in this study. All strains were MDR. Antibiotic resistance profiles of *A. baumanni* and *P. aeruginosa* strains are shown in Tables 1 and 2, respectively.

Table 1. The results of the disk sensitivity test and the inhibition zone of *L. salicaria* against *A. baumannii*

***A. baumannii* strains**	**The Inhibition Zone of *L. salicaria* against *A. baumannii* (mm) *mean ± std***	**CAZ**	**CIP**	**IMP**	**AK**	**CN**	**TZP**	**SAM**
Strain 1	16.7±4	0	0	0	10	20	0	0
Strain 2	19.3±4	0	0	0	0	20	0	0
Strain 3	18.7±1.15	0	0	15	0	0	0	0
Strain 4	22.3	0	14	0	0	0	0	0
Strain 5	15±1.73	0	0	15	0	0	0	0
Strain 6	16±1.15	0	0	16	0	0	0	0
Strain 7	18±2.88	0	0	14	0	0	0	0
Strain 8	22±2.88	0	0	15	20	15	0	0
Strain 9	19.3±1.15	0	0	10	0	0	0	0
Strain 10	17.3	0	0	0	10	20	0	0
Strain 11	19	0	0	0	0	0	0	0
Strain 12	18.3±1.15	0	0	0	10	20	0	0
Strain 13	18.7±5.77	0	0	0	0	15	0	0
Strain 14	19±4	0	0	0	0	20	0	0
Strain 15	19.7±5.77	0	0	0	0	17	0	0
Strain 16	22.7±2.88	0	0	0	0	15	0	0
Strain 17	21.3±1.15	0	0	0	0	17	0	0
Strain 18	20	0	0	0	0	20	0	0
Strain 19	17±1.73	0	0	0	0	10	0	0
Strain 20	20.7±1.15	0	0	0	10	25	0	0
Strain 21	18.3±2.88	0	0	0	0	20	0	0
Strain 22	16.7±2.88	0	0	0	0	10	0	0
Strain 23	19.3±1.15	0	0	0	0	0	0	0
Strain 24	22	0	0	0	0	0	0	0
Strain 25	15	0	0	0	0	0	0	0
Strain 26	16.3±1.15	0	0	0	0	10	0	0
Strain 27	16.7±5.77	15	12	20	12	17	15	0
Strain 28	14.7±4	0	0	0	0	0	0	0
Strain 29	16.7±5.77	12	0	22	15	0	20	0
Strain 30	13.3±2.88	0	0	0	0	0	0	0

CAZ: Ceftazidime, CIP: Ciprofloxacin, IMP: Imipenem, AK: Amikacin, CN: Gentamicin TZP: Piperacillin/Tazobactam, SAM: Ampicillin/sulbactam

MeOH was used for the extraction of the flowers and the leaves of *L. salicaria*. After extraction, mixture of 17-mg/ml *L. salicaria's* antimicrobial activity was assessed with the agar well diffusion method. In the present investigation, the MeOH extracts of *L. salicaria* showed varied levels of antibacterial activity against the bacterial strains tested. The zones of inhibition produced by the test material ranged between 10 and 25 mm for *A. baumannii*

and 12 to 20 for *P. aeruginosa* strains tested. Mean IZDs against *P. aeruginosa* and *A. baumannii* were 16,09 mm and 18.3 mm, respectively. Zone of inhibition did not occur (0 mm) in all tests with MeOH for both bacterial strain.

Table 2. The results of the disk sensitivity test and the inhibition zone of *L. salicaria* against *P. aeruginosa*

***P. aeruginosa* strains**	**The Inhibition Zone of *L. salicaria* against *P. aeruginosa* strains (mm)*mean ± std***	**CAZ**	**CIP**	**IMP**	**AK**	**CN**	**TZP**	**SAM**
Strain 1	15	0	0	0	0	0	16	0
Strain 2	14±2,1	0	0	10	10	0	0	0
Strain 3	19,3±1,4	0	0	0	10	0	12	0
Strain 4	17,7±2,1	0	0	0	16	0	0	0
Strain 5	15,3±0,7	0	0	10	10	0	10	0
Strain 6	16,3±0,7	0	0	10	16	0	10	0
Strain 7	15	0	0	8	16	0	20	0
Strain 8	15	0	0	18	17	0	20	0
Strain 9	15,3±0,7	0	0	12	17	0	20	0
Strain 10	15	0	0	10	10	0	20	0
Strain 11	15,7±1,4	0	0	10	12	0	17	0
Strain 12	16,7±0,7	0	0	0	10	0	20	0
Strain 13	16	0	0	0	10	0	17	0
Strain 14	15	0	0	12	12	0	20	0
Strain 15	15,7±1,4	0	0	12	10	0	20	0
Strain 16	15,3±0,7	0	0	10	12	0	12	0
Strain 17	16,7±1,4	0	0	10	0	0	0	0
Strain 18	16	0	0	10	0	0	20	0
Strain 19	14	0	0	10	0	0	0	0
Strain 20	18	0	0	0	0	0	16	0
Strain 21	17,3±2,1	0	0	0	10	0	0	0
Strain 22	17,3±2,1	0	0	10	0	0	12	0
Strain 23	16,3±2,1	0	0	0	10	0	0	0
Strain 24	17±2,1	0	0	12	0	0	16	0
Strain 25	16,7±0,7	0	0	0	12	0	0	0
Strain 26	16,3±0,7	0	0	10	0	0	18	0
Strain 27	16,7±0,7	0	0	0	10	0	15	0

CAZ: Ceftazidime, CIP: Ciprofloxacin, IMP: Imipenem, AK: Amikacin, CN: Gentamicin TZP: Piperacillin/Tazobactam, SAM: Ampicillin/sulbactam

We have first time shown that certain *L. salicaria* compounds have significant effects against both MDR *A. baumannii and MDR pseudomonas aeruginosa*. We attach importance to this effect because we have very few antibiotics in the treatment of serious infections such as pneumonia, skin-soft tissue infections, and bacteremia caused by *A. baumannii* and *P. aeruginosa*. Moreover, developing resistance to antibiotics, including carbapenems, decreased the number of antibiotics that can be used. In this instance, polymyxin, sulbactam or netilmicin can be reasonable options. But serious side effects such as nephrotoxicity of this drugs and insufficient impact of them when they used in monotherapy limits their usefulness [14]. Therefore, many infections occurring in intensive care units can be accommodated helpless. In our opinion, *L. salicaria* solve this problem.

Becker et al.[15] identified that the active principle of the antibacterial compound of *L. salicaria* was hexahydroxydiphenoyl ester vescalagin. Borchardt et al.[16] reported that seed extracts of *Lythrum salicaria* had inhibition zones of 8, 11, 17 and 22 mm against *E. coli*, *P. aeruginosa*, *S.aureus* and *C. albicans*, respectively. In another study, Dulger et al. [17] found that leaves of *L. salicaria* produced inhibition zones of 12, 10, 10, and 8 mm against *S. aureus*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Micrococcus luteus*, respectively. In the present study mean inhibition zones against *A. baumannii and P. aeruginosa* were 18.3 mm and 16.09 mm, respectively. The present study differs from other studies with the tested bacteria. *In this study, A. baumannii and P. aeruginosa* strains were obtained from hospitalized patients' clinical specimens. Also, all of them were MDR. So, these strains were extremely problematic bacteria and add an additional value to this work.

The extract of *L. salicaria* can be used in serious infections caused by *A. baumannii* and *P. aeruginosa* orally. There is no doubt that this substance should be tested with in vivo and in vitro assays before clinical use. These tests also should be confirmed in several clinical trials. However, we already know that this medicinal plant has been used orally in various indications in traditional medicine [7]. Therefore, use of this substance to obtain antibacterial effect in humans will be much easier than much synthetic material.

Its topical form can be used as an antiseptic in skin and soft tissue infections, burn wound infections, diabetic foot infections and decubitus wound infections due to *A. baumannii* and *P. aeruginosa,* too. Further studies focused on the safety of topical application and its dermal direct absorption should be done.

4. CONCLUSION

As a conclusion, *L. salicaria* has an antibacterial effect against MDR *A. baumannii* and MDR *P. aeruginosa.* However, further pharmacokinetic and pharmacodynamic tests are needed for routine clinical use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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