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The Effects of the Ethanol Extract of Dracocephalum Moldavica (Badrashbu) Against Strains of Antibiotic-Resistant *Escherichia coli* and *Klebsiella pneumonia*

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Abstract

Background: Today, due to the increased drug resistance, plants are used for treatment. The aim of this study was to investigate the effect of ethanol extract of Badrashbu (*Dracocephalum moldavica*) against strains of antibiotic-resistant *Escherichia coli* and *Klebsiella pneumonia*.
Methods: Badrashbu plant extracts were prepared using a rotary device. *Escherichia coli* and *Klebsiella pneumonia* were isolated from nosocomial infection patients at hospitals of Zabol. The MIC and MBC of Badrashbu extracts in different concentrations of bacteria were determined by disk diffusion method.
Results: The results of plant extracts showed that the highest MIC (minimum inhibitory concentration) for *E. coli* isolates was 20 milligrams per milliliter, and 6 strains were inhibited at this concentration. The highest MIC for *K. pneumonia* was 40 milligrams per milliliter, and only one strain was inhibited at the concentration. This result can be used to treat the disease.
Conclusions: The results of this study showed that Badrashbu extract inhibits *Escherichia coli* and *Klebsiella pneumonia*; so it can be used to reduce the plant disease.

Keywords: Plant Extract, Antibacterial Activity, Antibiotic Resistant

1. Background

Medicinal plants are now being widely used as a good source for treating diseases in different countries. About 60 to 85% of people in different countries use traditional treatments for common diseases. *Dracocephalum moldavica* is an aromatic plant from Lamiaceae family native to central Asia and eastern and central Europe (1). It is used as a tea or medicinal plant for curing deficiencies of stomach, kidney, and lung. Its antimicrobial effects have been widely studied. The plant has been used for controlling the growth of *Escherichia coli* and *Salmonella* (2-5). The plant's extract also has anti-helicobacter activities (6). It has been used as pain-killer, anti-inflation (7, 8), antimicrobial (9), and antifungal agent (10).

E. coli is Gram-negative basil and an important bacterium from Enterobacteriaceae family. It causes infections such as sepsis or blood infection, gastroenteritis, infant meningitis, wound infection, pneumonia, peritonitis, and urinal infections. It is also a cause of kidney disorders in children (11, 12).

Klebsiella pneumoniae is a gram-negative intestinal bacterium and a part of the natural microflora of the body. About one-third of people have this bacterium. The bacterium causes a wide range of diseases including bacteremic infections, pneumonia, and urinal infections. *Klebsiella pneumonia* covers a small number of pneumonia cases but causes a high mortality rate of 90%. This microbe is more likely to cause necrosis than pneumococcal pneumonia. This paper aims at exploring the effects of ethanol extract of *Dracocephalum moldavica* (Badrashbu) against strains of antibiotic-resistant Escherichia coli and *Klebsiella pneumonia*.

2. Methods

The seed of *Dracocephalum moldavica* was purchased from Pakan Bazr company of Isfahan and cultivated in the

Zabol University's greenhouse. Plants were gathered and the leaves were dried away from sunlight.

Maceration method was used for preparing the extract. To this end, leaves were cut and 50 g of each time sample was filtered by filter paper. After extraction, the obtained extracts were condensed by the rotary device (distilling in a vacuum) at 40 and 50°C and dried during 2 days at the same temperature.

2.1. Determining the Dry Weight of the Extract

First, a pipe was weighed and then charged with 1 mL of the extracts. Afterwards, the pipe content was dried at room temperature. After drying up of the extracts, the pipe was weighed once again. The difference of the pipe weights showed the dry weight of the extracts.

The various samples of *E. coli* were gathered from the positive samples of the patients hospitalized in Buali, Payambar Akram, and Ali bin Abitalib hospitals of Zahedan. The samples were cultured on nitrite agar medium. IMVIC test was used to separate *E. coli* from other gram-negative bacteria. The gathered isolates of *K. pneumonia* were cultured on blood agar and Mc Canki media. The grown colonies, which were similar to *K. pneumonia*, were detected by using biochemical tests such as growth in Klinger agar media, Simon Citrate, SIM, and Urea agar.

2.2. Antimicrobial Test of the Extract

The sensitivity of bacterial isolates to plant extracts was examined by using dilution method in broth. 100 mL of MHB was added to seven broths of microtiter plates. To the first broth, 100 mL of the diluted fluid of the extract was added. After mixing, 100 mL of the first broth was added to the second broth and the same was done until the last broth. 100 mL of the last broth was replaced with 100 mL of the microbial suspension containing half McFarland (10^7 units in mL) and was kept 24 hours in an incubator at 37°C. The first broth inhibiting the growth of bacteria in the incubator was regarded as MIC (13).

3. Results

The study results showed that *E. coli* strains were resistant to antibiotics such as Ceftazidime (55%), Erythromycin (61.2%), cefixime (48%), and tetracycline (79.1%). Further, *K. pneumonia* isolates were resistant to antibiotics such as Ceftazidime (35%) and cefixime (61.2%). The extracts had inhibitory effects against most of the isolates. About 50% of the *E. coli* isolates showed the MIC of 20 and 10 mg/mL while 8.3% and 33.3% of the *K. pneumonia* isolates showed the MIC of 40 and 10 mg/mL. On the other hand, the results of plant extracts revealed that the highest MIC of *E. coli* isolates were 20 mg/mL in which 6 isolates were inhibited while the MIC for *Klebsiella pneumonia* was 40 mg/mL in which only one isolate was inhibited (Table 1).

Table 1. The MIC of Extracts Against *E. coli* and *Klebsiella pneumonia*

Bacterial Sample *E. coli*	MIC, mg/mL	Bacterial Sample *K. pneumonia*	MIC, mg/mL
1	20	1	20
2	20	2	20
3	20	3	20
4	20	4	20
5	20	5	40
6	20	6	10
7	10	7	20
8	10	8	20
9	10	9	10
10	10	10	10
11	10	11	10
12	10	12	10

4. Discussion

The results showed that *E. coli* strains were resistant to antibiotics such as Ceftazidime (55%), Erythromycin (61.2%), Cefixime (48%), and tetracycline (79.1%). The results obtained by Muhammadi et al. (14) also showed that *E. coli* isolates were resistant to antibiotics such as Gentamicin (27.77%), Ampicillin (80.55%), Nitrofurantoin (13.88%), Ceftazidime (63.89%), and Sephtriacson (63.88%). Madani et al. (15) also revealed that *E. coli* strains were most resistant to antibiotics such as ampicillin (91.4%), Cotrimoxazole (61.1%), Cefixime (46.8%), and Gentamicin (43.3%).

On the other hand, *Klebsiella pneumonia* isolates were resistant to antibiotics such as Ceftazidime (35%), and Cefixime (61.2%). In the study conducted in Besat hospital, *Klebsiella pneumonia* isolates were resistant to antibiotics such as Gentamicin (46.7%), ampicillin (30%), Nitrofurantoin (26.7%), and Ceftazidime (13.3%) (16). The results of this study are in accordance with the results of the current study. The results of the present study showed that *Dracocephalum moldavica* extract could inhibit *E. coli* and *K. pneumonia* at various concentrations. The antimicrobial effects of *D. moldavica* essential oil were measured against 4 gram-positive and 2 gram-negative bacteria and factors such as quantitative and qualitative activities related to inhibitory including cloud, cloud diameter, and MIC rate were evaluated. MIC was shown for bacteria, at the scope of 26 - 36

and 30 - 38 mm at concentrations of 0.4 - 0.8 microgram per disk. The two types of bacteria were equally sensitive to *D. moldavica* essential oil. Therefore, the inhibitory effect of the essential oil is dependent on its concentration (17). The mentioned essential oil has shown anti-fungal effects against *M. heimalis*, *P. notatum*, *A. niger*, and *F. oxysporum*. The essential oil with concentrations of 0.02, 0.04, 0.08, and 0.12 mg/mL could inhibit the growth of a famous fungus called Aspergillus niger with concentrations of 32, 53, 77, 100, and 100, respectively. The essential oil also had similar effects against *P. notatum*, *M. heimalis*, *notatum*, and *F. oxysporum*. *D. moldavica* essential oil could inhibit them at a concentration of 0.08 mg/mL. The antifungal effect of *D. moldavica* oil was higher so that it could inhibit all isolates with a concentration of 0.08 (MIC) and all fungi with the same concentration. There seems to be a relationship between the chemical compounds of the oil and anti-fungal activity. Many of the essential oil components show antimicrobial effects and many others are structurally considered as ketones, alcohols, or phenolic monoterpenes (18, 19).

In the study of Kamali, the highest antibacterial index was observed for ethyl acetate extract on *Bacillus cereus* in both disc diffusion (12 mm) and MIC-MBC methods and the MIC value for extracts ranged between 0.781 and 25 mg/mL. The results of this investigation indicated that *Dracocephalum kotschyi* was found to possess moderate antibacterial activities. Further research is required to identify the active photochemical compounds responsible for these biological activities (20).

The extract of *Dracocephalum moldavica* contained polar compounds such as luteolin-7-O-glucoside, rosmarinic acid, luteolin and apigenin and this extract demonstrated antioxidant activity in all the antioxidant assays but it was not as potent as the positive control (21). The most active compounds of this genus are flavonoids, such as Luteolin, quercetin, apigenin and these flavonoids appear to play an important role in the biological activities of Dracocephalum species (22). In addition, these flavonoids possess anti-bacterial properties (23).

4.1. Conclusion

The study results revealed the antimicrobial effects of *Dracocephalum moldavica* against bacteria relating to urinal infections. These effects should be more carefully studied and their supporting components should be detected. In this way, they can be used in medicine formula for treating infections.

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Footnotes

Conflict of Interests: None declared conflicts of interest.

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