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Antimicrobial Effects of Ethanol, Methanol and Ethyl Acetate *Teucrium polium* and *Citrullus colocynthis* extract on *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a common pathogen in a wide range of infections, from urinary tract infections to bacteremia. Intrinsic resistance to antimicrobial agents in this bacterium worsens the condition of the treatment of infections caused by it. The aim of this study was to investigate the antimicrobial effects of ethanol, methanol, and ethyl acetate *Teucrium polium* Extract and *Citrullus colocynthis* on a *p. aeruginosa* isolated from Zabol hospital. *P. aeruginosa* specimens were collected from Zabol city. Plant extracts were prepared using a rotary machine and the minimum inhibitory concentration and minimum trace concentration were determined by microdilution method. The lowest inhibitory concentration of methanolic extract of *C. colocynthis* against *p. aeruginosa* was 1.25 mg / ml while the lowest inhibitory concentration of ethyl acetate extract of *C. colocynthis* was 0.62 mg / ml, two strains were inhibited in this concentration and the highest inhibitory concentration was 5 mg, 6 strains of bacteria in this concentration has been restrained. The results of this study showed that watermelon extract of *T. polium* Extract and *C. colocynthis* have a significant antibacterial effect. Extract from this plant can be used to deal with specific pathogenic bacteria.

1. Introduction

Hospital infections are one of the major medical problems in developed and developing countries' Development, that promotes the spread of infectious diseases in the community[1]. In recent years, attention has been paid to hospital infections because only in 1995, eighty-eight thousand deaths from hospital infections have been reported in the world [2].

P. aeruginosa is a common microorganism that can be infected with various virulence factors in people with burns, and cystic fibrosis, and complication of abnormal reduction of neutrophils; and in patients with immune deficiency. The resulting microorganism occurs both in the community and in the hospital environment, but its prevalence in hospitals leads to chronic infections, especially in patients with reduced immune system and cystic fibrosis [3]. Antibiotic resistance of this bacterium has increased, which has caused many problems in their treatment [4].

Antimicrobial compounds derived from plants with different mechanisms of antibiotics eliminate bacteria that are involved in treatment of the infections caused by resistant microbial strains are clinically important [5-7].

From the botanical point of view, Teucrium is from the family *Lamiaceae* Mint. Of this genus, more than 340 species are known in the world, and in Iran there are 13 species and 4 sub-species, 3 of which are native to Iran. *T. polium* L is a herbaceous, persistent, branched, dense and crusty plant. In the

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middle of the first century AD, the plant was boiled as a diuretic to eliminate water contamination, urinary excretion, and in fresh leaves to treat the wounds [8-10].

The floral branches of this plant have a mildly powerful effect on the stomach, fever and antiseptic, and its effective ingredients relieve the ability of the digestive system and the liver to function properly. For more than 2000 years, various species of Teucrium have been known as medicinal herbs and have proven to be anti-seizure, anti-inflammatory, analgesic, anti-fever, and having other healing properties. This plant is widely distributed in different parts of north, west, south and center of Iran, the Alborz region, around Tehran, especially in most semi-arid mountains [8, 11].

The Abu Jahl watermelon fruit that is known as Hamas is rich in laxatives. Administration of high doses of this herb causes diarrhea, heartburn and, at higher doses, causes bloody diarrhea in humans [8]. The fruit's shell of this plant contains a bitter mixture called colosintin and colosintin and other compounds including glycosylated phytostrols, pitch, pectin, albuminides, etc. [12, 13].

The aim of this study was to investigate the antimicrobial effects of ethanol, methanol, ethyl acetate *T. polium* extract and *C. colocynthis* on *P. aeruginosa* .

2. Methods

2.1 Isolation of *P. aeruginosa*

Different strains of *P. aeruginosa* used in this study were collected and isolated from patients admitted to Amir al-Momenin hospital in Zabol City. To identify the genus *P. aeruginosa*; hot curing, catalase, oxidase, and confirmation tests of sugars were used. Extractin *T. polium* Extract and *C. colocynthis* in Zabol city were collected and dried (fig. 1). To prepare the ethanol extract, methanol and ethyl acetate, 10 grams of dried powder were placed in 100 ml of 100 ml ethanol 96% ethanol extracts.

Fig. 1. Characteristic of *Teucrium polium* (A) and *Citrullus colocynthis* (B)

The contents of the Arlenes were mixed at room temperature for 24 hours at room temperature with a Pars Azma-Iran shaker (130 ppm), and straightened with Watten No. 2 paper. Solvent separation from the extract

was performed by a rotary machine (Heidolph, Germany) and using a vacuum pump (vacuum distillation). The extract was weighed and then solved in DMSO solvent. The extract was stored in a refrigerator until use in antimicrobial experiments at 4 ° C.

Determination of susceptibility of bacterial isolates to plant extracts was done by dilution method in wells. Six wells were created in a solid culture medium, and each well was added 100 µl of Nutrient Muller Hinton (MHB). Then, to the first well, 100 ml of dilute solution of the extracts of plants was added and after mixing, 100 µl of the first well was added to the second well, and this was done until the last well. From the final well, 100 µl of the medium was extracted, and the amount of 10 µl of the microbial suspension containing 107 µg / ml was equivalent to 0.5 McFarland and incubated at 37 ° C for 24 hours. The first well that prevented the growth of the bacteria after insertion in the incubator was considered as the minimum inhibitor concentration. In order to ensure transparent 10 µl wells, they were transferred to the Muller Hinton Agar medium, and after 24 hours the first crop that was able to kill 99.9% of the bacteria was shown as the least lethal concentration.

3. Results

The results of this study showed that the lowest inhibitory concentration of ethanolic extract of *C. colocynthis* against *P. aeruginosa* was 2.5 mg/ml, one strain of which was inhibited in this concentration and the highest concentration of methanol extract was 40 mg/ml.

The lowest inhibitory concentration of methanolic extract of *C. colocynthis* against *p. aeruginosa* was 1.25 mg / ml while the lowest inhibitory concentration of ethyl acetate extract of *C. colocynthis* was 0.62 mg/ml, two strains were inhibited in this concentration and the highest inhibitory concentration was 5 mg, 6 strains of bacteria in this concentration has been restrained (Table 1).

The results of this study showed that the lowest inhibitory of *T. polium* concentration was 5 mg/ml, 2 bacterial strains were inhibited in this concentration and the highest inhibitory concentration was 40 mg/ml. Three strains of bacteria are inhibited in this concentration.

The lowest inhibitory concentration of methanolic extract of *T. polium* against *P. aeruginosa* was 2.5 mg/ml, which was a strain of bacteria inhibited and had the highest inhibitory concentration of 20 mg/ml, of which 3 strains of bacteria in this concentration has been restrained (Table2, fig. 2).

The lowest inhibitory concentration of the ethyl acetate extract of *T. polium* was 0.62 mg/ml, with the highest inhibitory concentration of 5 mg/ml, of which four bacteria were inhibited in this concentration.

Table1. Results of minimum inhibitory concentration and minimum concentration of *C. colocynthis* particles against *P. aeruginosa*

Bacterial code	*C.colocynthis* (MIC/ MBC)		
	Ethanol	Methanol	Ethyl acetat
1	40/ 40	20 / 40	5/ 10
2	2.5 / 5	5/ 10	1.25/ 2.5
3	20/ 40	10/ 20	5/ 10
4	5/ 10	5 / 10	0.62/ 1.25
5	5/ 10	2.5 / 5	0.62/ 1.25
6	20 / 40	5/ 10	5/ 10
7	10/ 20	10/ 20	2.5/ 5
8	2.5/ 5	20/ 40	5/10
9	20 / 40	1.25 / 2.5	2.5 / 5
10	5 / 10	10/ 20	5/ 10
11	10 / 20	20/ 40	1.25/ 2.5
12	10/ 20	10 / 20	5 / 10

Table 2. Results of minimum inhibitory concentration and minimum concentration of *T. polium* particles against *P. aeruginosa*

Bacterial cod	*T. polium* (MIC/ MBC)		
	Eethanol	Methanol	Ethyl acetat
1	20/ 40	10/ 20	2.5 / 5
2	10 / 20	2.5/ 5	5/ 10
3	40 / 40	20 / 40	5/ 10
4	20/ 40	20 / 40	10/ 20
5	5/ 10	10 / 20	1.25/ 2.5
6	10 / 20	5/ 10	0.62 / 1.25
7	40 / 40	5/ 10	5/ 10
8	20/ 40	20 / 40	2.5/ 5
9	5 / 10	10 / 20	10/ 20
10	40 / 40	5/ 10	5/ 10
11	20/ 20	5/ 10	2.5/ 5
12	20/ 40	10/ 20	5/ 10

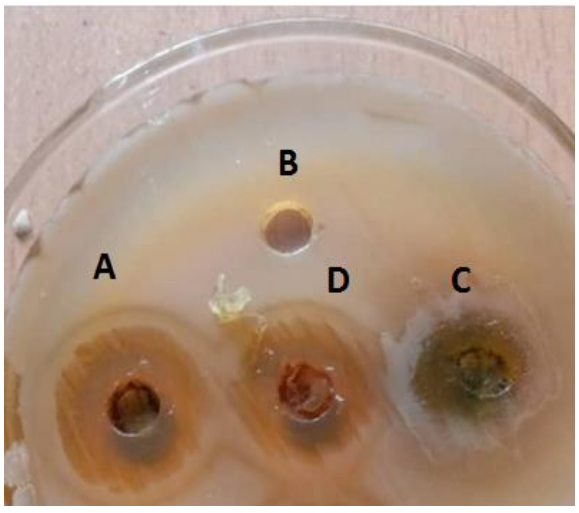

Fig. 2 The effects of Ethanol(A), Methanol (D) and Ethyl Acetate (C) OF *Teucrium polium* extract and control (DMSO)(B) on *Pseudomonas aeruginosa*

4. Discussion

P. aeruginosa is a potentially pathogenic gram-negative bacillus that has high potential for pathogenicity and infection in inpatients and those with defective immune systems. This organism can affect any member of the body and causes important clinical conditions, including respiratory tract infections, soft tissue, bones, urinary tract infection, and bacteremia[14]. Medicinal plants are used to treat infections caused by Pseudomonas.

In traditional medicine, tuberculosis is used in anti-diabetes cases, serum cholesterol and serum triglyceride, anti-appetite, anti-inflammatory, antioxidant, anti-fever, antimicrobial and analgesic drugs. The phytotoxic analysis of the tuberous essential oil showed that the plant contains alkaloids, terpenoids, flavonoids, glycosides, tannins, sterols and saponins [15].

Darabpour et al. investigated the effect of different extracts of the tuberola against the pathogenic bacteria. The inhalation hole diameter of ethanolic extract of tuberculosis in a concentration of 400 mg / ml against *B.anthracis, B.pumilus, B. Licheniformis, B.cereus, S.aureus, S.epidermidis, A.pyogenes, E.coli, S.typhi, P.mirabilis, b.bronchiseptica and Y.enterocolitica* is equal to 18-10-8-10-12-13 R-9-13 R-11 and 9 mm, respectively, while the inhibition zone of the methanolic extract at a concentration of 400 mg / ml against the same bacteria is 10-12- 10- 11-8-9 -R- R-14-R-22-10 mm, respectively [16].

Antibacterial effects of methanolic extract of clamp on Staphylococcus aureus and Salmonella typhimurium have been proven[17]. In another study, antibacterial activity and antioxidant activity of methanolic extract of the tuberous plant were investigated and tested using a disk diffusion method on a number of gram-positive and gram negative bacteria [18]. Antibacterial effects of the extracted hydroalcoholic extract of the pulpuratum with dilution of 1.8 by disc diffusion method have been shown on *Escherichia coli* and *Staphylococcus aureus*

[19]. In another study, the antibacterial effects of Tropicidae on pathogenic bacteria were investigated using Microtiter Plate method. The results showed that the most anti-bacterial activity of Tropicidae was against *P. aeruginosa* and *Bacillus cereus* bacteria [20].

In Turkey, the effect of peppermint oil on micronuclei was investigated on a number of bacteria, which had the highest effect on Bacillus cereus [21]. Antibacterial activity of ethanolic and methanolic extracts of pulpotum was studied by disk diffusion method. The results showed that the ethanolic extract had the highest effect on *Bacillus anthracis*, while the highest effect of methanolic extract on septic bronchospasm was observed [22].

In the other study antibacterial effects of ethanoic extract of pollen flowers were studied and antibacterial effects were significantly reported on gram-positive and gram negative bacteria and no toxic effects of this extract were observed [23].

C. colocynthis (L.) Schrad (Cucurbitaceae) (bitter cucumber), is an annual herbaceous plant. Stem is angular and rough, leaves are rough, 3 to 7 lobed, 5 to 10cm long; flowers are monoecious, solitary, peduncled, axilliary, corolla 5-lobed, and ovary is villous and a fruit commonly known for its bitterness, found in India, Sudan, Iraq and Iran [24].

The study of Eidi aimed to evaluate the antifungal activity of hydroalcoholic extract of *C. colocynthis* fruits against different *Candida* and *Aspergillus* strains. The growth inhibition value of the extract showed high antifungal activity against Aspergillus fumigatus and Aspergillus niger and a lower effect against Candida guilliermondii and Candida kreusei. The minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values ranged from 1.56 to 12.5mg/ml and 3.125 to 25mg/ml, respectively [25].

Gurudeeban et al. investigated the effect of ethanolic extract of Abujah watermelon on pathogenic bacteria, and the results showed that the inhibitory diameter of B.subtilis, S.aureus, S.faecalis, S.pyogenes, E. coli, K.pneumoniae, P.mirabilis, P.vulgaris, S.typhi and V.cholerae were 0.15 ± 11, 0.25 ± 7, 0.52 ± 3, 0.52 ± 11, 0.26 ± 3, NA, 0.65 ± 12 and NA mm, respectively [26].

It is related that the ethanol 50% extract of *C. colocynthis* which is active against gram positive bacteria i.e. *Bacillus pumilus and Staphylococcus aureus* whereas it is inactive against gram negative bacteria *Eschreschia coli and P. aeruginosa* [27].

Another study revealed that methanol, ethanol, acetone and distilled water extract have potent activity against *Escherichia coli* [28].

5. Conclusion

The results of this study showed that the extracts of ethanol, methanol, ethyl acetate *T. polium* Extract and *C. colocynthis* inhibit bacteria of *P. aeruginosa* at different concentrations and different solvents, so that the concentration of the extract increases the inhibitory level for bacteria. *P. aeruginosa* is a potentially pathogenic agent and its resistance is constantly increasing, which suggests that the plant can be used to treat *Pseudomonas* infection.

Conflict of Interest

All authors disclose any financial and personal relationships with other people or organizations and the authors declare that there are not any potential conflicts of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publications

The author read and proved the final manuscript for publication.

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