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Study the antimicrobial effects of *Thyme*, *Aloe vera* and *Zararia multiflora* extracts against antibiotics resistant *Pseudomonas aeruginosa*

Ozra Hassanshahian[1*], Fereshteh Javadian[2]
[1]*Horticulture Dept., Shiraz University, Shiraz, I.R. Iran;* [2]*Microbiology Dept., Zabol University of Medical Sciences, Zabol, I.R. Iran.*
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ABSTRACT

Background and aims: Resistance of bacteria against antibiotics has always been a prevalent problem in medicine. So, finding new antimicrobial compounds with least side effects seems to be necessary. This paper aims to explore the antimicrobial effects of *Thyme*, and *Aloe vera* and *Zararia multiflora* extracts against *Pseudomonas aeruginosa resistant* to antibiotics.
Methods: *Thyme*, and Aloe vera and *Z. Multiflora* extracts were obtained by using rotary devices. Twenty samples of *P. aeruginosa* were collected from the patients being cured in Zabol hospitals. The least hindering and killing concentrations of the samples were calculated by lowering their density in sinks. Sensitivity of *P. aeruginosa* to the different antibiotics prepared by Padtan Teb Co was evaluated by the diffusion disk standard method proposed by Kirby Bauer. Data were analyzed statistically by determination of significant difference using analysis of variance (ANOVA) test. All tests were analyzed at the significance level $P<0.05$.
Results: Our results have shown that *Aloe vera* hinders the growth of bacteria in various concentrations. Despite the relative resistance of most of the samples in the used concentrations, the highest sensitivity was observed in 10 and 20 mg/l. Approximately, Aloe vera extract has shown the highest hindrance effect in 5 mg/l. Further, the highest sensitivity to *Z. Multiflora* was in 10 and 20 mg/l in which 100% of the bacteria were killed. Approximately, *Z. Multiflora* of 5mg/l had the highest resistive effect.
Conclusion: *Thyme*, and *Aloe vera* and *Z. Multiflora* extracts have considerable antimicrobial effect on the samples of the *P. aeruginosa* resistant to antibiotics.

Keywords: Antimicrobial effects, Antibiotic resistant, Pathogens, Plant extract.

Original article

INTRODUCTION

P. aeruginosa is the most well-known variety of *Pseudomonas*. It has the bluish or green bluish color often seen on surgery clothes. The color was noticed in 1860 even before discovery of its origin. Like the other Gram positive bacteria, *Pseudomonas* has lipopolysaccharides in its cellular walls with O antioxidant and antigen activities.[1] It is not as strong as the intestinal basil's endotoxins. Some unimportant toxins are

[*]**Corresponding author:** Ozra Hassanshahian. Horticulture Dept., Shiraz University, Shiraz, I.R. Iran, Tel: 00989136245981, E-mail: ohasanshahi@gmail.com

also produced by *P. aeruginosa*. leukocidin which is effective against poly-morph nuclear leukocyte and is produced by several samples. Almost half of the clinical samples give hemolysis on blood agar. Extra-cellular heat-resistant hemolysis is glycolipids; and the heat-sensitive ones are phospholipid C. there are other cytotoxic proteins that their importance was explained but their features were not mentioned.[2]

Zataria multiflora (*Z. multiflora*), belongs to the family of *Labiatae* and it is a native plant in Iran, Pakistan, and Afghanistan. It is traditionally used as antiseptic, anesthetic, and antispasmodic. *Z. multiflora* has also shown to have analgesic and anti-inflammatory effects.[1,2] The immune stimulatory effects of *Z. multiflora* essential oils in common carps have been recently reported.[3]

Z. multiflora includes multiyear and mono-year grasses and a few trees. Its leaves are parallel or adjoining and often lack earring. The main herbal drugs of *Zataria flora* explained below. *Z. multiflora* is a one-year plant whose seeds are used to cure chest diseases, cough and cold. Its flowers are used to cure normal diarrhea. The cold herbal medications sold in market use this plant's flowers. This plant has more than 60 varieties grown in the tropical countries of western and eastern hemispheres.[3]

Sabre Zard with the scientific name *Aloe vera* is a meat, multiyear and perennial plant. Its origins have been reported to be in the tropical Africa area.[4]

Anthraquinone glycosides such as aloin, amodin, alinosides, and alopine are among the most effective compounds found in *Aloe vera*.[4] Its gel and its derivatives are used in producing creams, shampoos, soaps, third-degree burns lotions; covering fruits and increasing their sustainability, improving kidney action, and enhancing immunity against and treatment of AIDS.[4]

This paper aims to explore the antimicrobial effects of *Thyme*, and *Aloevera* and *Z. multiflora* extracts on the samples of the *P. aeruginosa* resistant to antibiotics.

METHODS

The samples of *P. aeruginosa* used in this study were taken from patients in Zabol/ Iran. The Gram staining, catalase, oxidase, triple sugar iron and oxidation fermentation tests were done to determine the species of *P. aeruginosa*.

To prepare microbial suspension, bacteria were transferred to the agar culture place (Merck of Germany). After growth of bacteria colonies, culture place surface was washed by saline solution and thick microbial suspension was obtained. Then, a little of the bacterial suspension was injected into sterile pipe containing normal saline and its darkness was measured by spectrophotometer with a wavelength of 630 nm. Density of suspension was decreased by using normal saline until darkness of solution with that of McFarland suspension. Finally, a bacterial suspension (108 cfu/ml) was obtained.

Sensitivity of *P. aeruginosa* to the different antibiotics prepared by Padtan Teb Co was evaluated by the diffusion disk standard method proposed by Kirby Bauer. Initially, all bacteria samples were prepared with McFarland ppm in Muller Hinton Broth (MHB) medium and were cultured on Muller Hinton Agar (MHA). Antibiotic disks were imposed on bacteria that growth on MHA medium. Plates were placed in incubator of 370C for 24 hours. Preventive clouds' diameters were measured to determine sensitivity of samples to antibiotics. Results were compared in NCCLS table.

Extraction of plant essential oil was done using distillation with steam and clonger machine. Approximately 200 g of the dry plant powder was poured in a 2L balloon and

two third of balloon volume was filled by water. Then, balloons were attached to Clonger machine to be distilled for 4 hours. After essence extraction, dewatering was performed and the obtained essence was stored in a dark contained in refrigerator.

Plant extracts were prepared using Maceration method (drenching). To this end, the seeds gathered from Sistan and Baluchistan fields were dried and 50g of the dried seed was drenched and stored in methanol for 48 hours. Then, the extract was condensed using filter paper using distillation machine in vacuum rotary.

Sensitivity of bacterial samples with multiple resistances to *Thyme*, and *Aloe vera* and *Z. multiflora* were measured using dilution in sink. Seven sinks with plate micro-titers were added 100 ml of the nutritive fluid Moller Hinton. 100 ml of the extract was added to the first sink and was stirred. The, the sample was transferred to the second sink and this process continued to the final sink. 100 ml of the sample from the last sink was extracted and 100 ml of the microbial suspension with half McFarland ppm was added to sinks. The obtained sample was stored for 24 hours in incubator of 370 C. The first sink that hindered the growth of bacteria after incubation was considered as MIC. MIC was removed from 10ml light sinks and was transferred to the Moller Hinton agar medium. After 24 hours, the first ppm that could hinder 99% of bacteria growth was considered as the minimum concentration.

All experiments were conducted in triplicates. Data were analyzed statistically by determination of significant difference using SPSS version 18.0 for Windows and compared using analysis of variance (ANOVA) test. All tests were analyzed at the significance level $P<0.05$.

RESULTS

The obtained results revealed that *Thyme* essential oil with various concentrations could inhibit the growth of bacteria. The results were presented in Table 1. As shown in this table the minimum inhibitory concentration was 1.8mg/ml in which one bacteria strains was inhibited. The maximum inhibitory concentration was 30 mg/ml in which three bacteria strains were inhibited. The least toxicity was 3.75 mg/ml in which one bacterial strain was completely destroyed.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Thyme* extracts against *P. aeruginosa*

Bacterial Strain NO.	MIC/MBC (mg/l)	Bacterial Strain NO.	MIC/MBC (mg/l)
1	15/30	11	1.87/3.75
2	30/30	12	7.5/15
3	15/30	13	30/30
4	3.75/7.5	14	15/30
5	7.5/15	15	7.5/15
6	15/30	16	30/30
7	3.75/7.5	17	15/30
8	15/30	18	7.5/15
9	7.5/15	19	15/30
10	15/30	20	3.75/7.5
Control (*P. aeruginosa* ATCC17865)	15/30		

Further, *Aloe vera* can inhibited bacteria growth in various concentration. Despite resistance of most strains in various concentrations, the most sensitivity was found in 10 and 20 mg/ml concentration ($P<0.05$). It showed the highest inhibitory effect on 5 mg/ml (Table 2).

The sensitivity of isolated bacteria to different concentration of *Z. multiflora* essential oil was shown in Table 2. As shown in this table *Z. multiflora* essential oil with various concentrations could inhibit the growth of resistant bacteria. Despite resistance of most strains in various concentrations, the most sensitivity was found in 10 and 20 mg/ml concentration. It showed that highest inhibitory effect take place at 5 mg/ml.

Table 2: The sensitivity of *P. aeruginosa* strains to different concentration of *Aloe vera* and Z. *multiflora* and (mg/ml)

Concentration (mg/ml)	0.62	1.25	2.5	5	20	10
Sensitivity of bacteria after *Aloe vera* extract treatment (%)	0	0	5.88	23.52	100	94.11
Sensitivity of bacteria after Z. multiflora extract treatment (%)	0	0	21.15	70.35	100	100
Control (Ethanol as solvent)	0	0	0	0	0	0

DISCUSSION

Thyme is a plant from mint origin. Most of its aerial parts especially its flowers are used and have antimicrobial effects.[5] Tymidil is a phenolic compound and the main component of *Thyme*. The other effective component is cracodole which is well dissolved in alcohol and water-based solvents. These compounds are stored in young leaves during plant growth. Its alcoholic extract has antimicrobial and expectorant effects.[6,7]

Sadeqzad et al conducted a photochemical analysis which revealed the presence of Tymol (52.4%) and cracrole (6.2%) in *thyme* essence, which result in antimicrobial effects. Also, presence of 0. 2.5 and 5% of *thyme* extract can create hindrance clouds of 0, 9.3 and 15.6 mm in *S. paratyphi* A and 8, 8.6 and 21.6 mm in *S. saratyphi* B.[8]

Osman Sangelik reported that *thyme* extract has hindering effect against bacteria such as *P. aeruginosa*, and S. *aureus*.[9] The obtained results of Moghaddam et al showed that *thyme* alcoholic extracts of 1.64 and 1.32 hinder the growth of DNaseenzyme.[10] The study by Singh and Singh showed that *thyme* extract had hindering effect on anti-hemorrhagic. Thymoland Cracol is the most effective hindering factors. They have effects on warm positive and negative bacteria.[11]

Goudarzi et al revealed that specific concentrations of the alcoholic *thyme* extract showed considerable antibacterial effect. Having a ppm of 0.078 mg/ml, it showed hindering and removing effect against all bacteria samples. The effect of the extract decreased with concentration of the extract in the sinks. Liquid extract was not effective on the clinical samples in no concentration.[12]

The study of Fallah et al shown that *Z. multiflora* and *C. copticum* extracts had a high antibacterial effect on regular and IMP-producing *P. aeruginosa* strains in 6.25 mg/ml concentration.[13] Previous researches reported the antibacterial effect of Z. multifloraessential oil against various bacteria such as *S.typhimurium, S.aureus and B. cereus.*[14-16]

Saeidi et al studiedthe potential antibacterial activity of ethyl acetate and aqueous extracts of *Menthalongifolia* and hydro-alcoholic extract of *Z.multiflora* against important human pathogens. Theirresults show that the lowest inhibitory concentration and minimum bactericidal concentration values for *K. pneumonia* and *P. aeruginosa* was 1.25 and 2.5 mg/ml.[17]

Aloe vera is a type of aqueous plant originating from North Africa. It has medical uses and has been used for making creams and tranquilizing medicines. Yet, few studies have emphasized over safety of *Aloe vera* as a medicine.[18,19] Herbal plants are now widely used as sources of medicine around the world. Approximately 60 to 85 % of people around the world are now using traditional herbal plants as medicine. Irshad et al reported that aqueous and ethanolic extracts of *Aloe vera* extracts can create zone of inhibition (2 and 8 cm) against *E.coli* and *B.subtillus*, respectively.[20]

CONCLUSION

As the attempt for the production of herbal medicines is in progress around the world, the current study will assist the isolation of new products/medicines. Finally, it can be claimed that active chemical compounds prevailing in plant extracts should be used in medication of different bacterial infections and these herbs must be studied more comprehensively to find their potentiality in the medication of infectious diseases.

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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