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Short Communication

Study the Effect of Ethanol Extract of Achillea, Green Tea and Ajowan on Pseudomonas Aeruginosa

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ABSTRACT

Objective: Infections are increasing caused by antibiotic-resistant Pseudomonas aeruginosa day by day. For this reason, many researchers have tried to find new compounds as alternative antibiotics. The purpose of this study was to evaluate the effect of yarrow extract, green tea and Ajowan on Pseudomonas aeruginosa. **Methods:** Extraction of Achillea, green tea and Ajowan was done with rotary. For this study, 12 strains of Pseudomonas aeruginosa isolated from patients in the city of Zabol. Minimum inhibitory concentrations of the plant extract of Achillea, green tea and Ajowan was determined in different concentrations by dilution on bacteria with plate method. **Results:** Results from plant extracts showed the highest MIC (minimum inhibitory concentration) of green tea extract was concentration of 2.5 milligrams per milliliter for Pseudomonas aeruginosa that 8 strains were inhibited at this concentration. Also, The highest inhibitory concentration (MIC) of achiella extract against strains of Pseudomonas aeruginosa was 5 milligrams per milliliter that four strains were inhibited at this concentration, the lowest concentration of inhibitor was 62 milligrams per milliliter. **Discussion :** This study showed that Achiella, green tea and Ajowan have antibacterial effect against strains of Pseudomonas aeruginosa. However, the clinical use of these plants require more and wider research.

1.INTRODUCTION

Pseudomonas aeruginosa is a well known pathogen that causes nosocomial infections for the wide dissemination and antibiotic resistance, for these reasons treatment of infections caused by it is very difficult (1, 2). Achillea plants belonging to the Acteracee family that is native of Europe, North America, Australia and Southeast Asia (3). Achillea also used as a stimulant, sudorific and tonic. One of the properties of the antibacterial effects of Achiella are on a wide range of disease-causing agents in humans and animals (4). This plant are used as a tea for headaches, pain in the stomach, intestines, as well as ointments and wash skin inflammation, wounds and burns (5, 6). Ajowan is Aromaticseed that used generally for therapeutic purposes or as a digestive stimulant to treat liver disorders, thymol is the major component of

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Ajowan introduced as antimicrobial agents fungicides and Anti-disturbance (7). Tea (Camellia sinensis) is one of the most consumed drinks in the world, especially Iran which contains polyphenols such as Flavyn and Tyrabchyn. Polyphenols have anti-cancer effects. Catechin epithelium, epithelial Gallo catechin, catechin gallate epinephrine and epinephrine Gallo catechin-3-gallate four major green tea polyphenol (8, 9). The purpose of this study was to evaluate the effect of Achillea, green tea and Ajowan extract on Pseudomonas aeruginosa.

2.MATERIALS AND METHODS

2.1. Isolation of Pseudomonas aeruginosa

Pseudomonas aeruginosa strains used in this study was isolated from patient samples Zabol city. To identify the genus Pseudomonas aeruginosa tested by Gram stain, catalase test, oxidase, experiments sugars (Triple sugar iron) TSI and OF (Oxidation fermentation).

2.2. Half McFarland suspension:

For prepare Bacterial suspension, first, 24 hours prior testing, bacterial transferring from culturing stored medium to nutrient agar (Merck, Germany). After the growth of the bacteria, the culture medium was washed with normal saline solution and concentrated bacterial suspension was obtained. Then the amount of the bacterial suspension was poured into tubes containing sterile saline the turbidity was measured with a spectrophotometer at wavelength of 630 nm Until the turbidity of the solution equal with a solution of half McFarland turbidity, bacteria suspension was diluted with normal saline at a concentration of 1×10^8cfu / ml.

2.3. Test sensitivity of bacteria to antibiotics

Antibiotics were obtained from the company's anti-medicine Susceptibility of Pseudomonas aeruginosa to antibiotics was evaluated using disk diffusion Kirby-Bauer standard method. For this purpose, first of all strains were prepared on half of McFarland (1×10^8 cfu / ml) in Mueller-Hinton broth and were distributed and cultured on Mueller Hinton agar medium. Antibiotic discs on Mueller Hinton agar medium containing bacteria were placed near the edge of the plate, Plates are incubated for 24 h at 37 °C. And the diameter of inhibition were measured to determine sensitivity and resistance to antibiotics of strains and the results were compared with the NCCLS standard table.

2.4. Create the extract

For Create the extract of soaking method was used. So, after crushing the leaves collected from the plains of Sistan and balouchestan province, 50 grams of samples soaked for 48 hours in 96% ethanol and stored. The extract obtained with smooth filter paper was condensed using rotary.

2.5. Determination of the dry weight of the extract

The weight of a test tube determined and one ml of extract was transferred into it. Tube containing extract was dried at room temperature. The weight difference tube was equivalent one ml of the extract. Average three times, was calculated as weight dried extract. Then dissolved in the solvent DMSO and was maintained at 4 °C until used .

2.6. Antimicrobial testing of extraction

Susceptibility of bacterial isolates with multiple resistance to the plant extract of Achillea, green tea and Ajowan was investigated using the dilution plate. To seven micro-titter plates was added amount of the 100 ml nutrient broth Mueller Hinton Broth (MHB). The first well was added 100 ml of the diluted solution of the extract, after mixing, 100 ml of the first plate is removed and added to the second plate, this work was done in this way until the last plate. Remove the end plate 100 ml of culture medium containing 10^7 units per ml, 100 ml of bacterial suspension equivalent to 0. 5 McFarland added to all wells and incubated at 37 °C for 24 h.

The first well that prevent the growth of bacteria is considered as MIC and to ensure 10 ml of the clear plate was transfered to the Mueller Hinton agar medium. After 24 hours the first dilution that could kill 99.9% of bacteria as shown Minimum lethal concentration.

3. RESULTS

Results from plant extracts showed the highest MIC (minimum inhibitory concentration) of green tea extract was concentration of 2.5 milligrams per milliliter for Pseudomonas aeruginosa that 8 strains were inhibited at this concentration While the lowest MIC of green tea extract for Pseudomonas aeruginosa was concentration of 1.25 milligrams per milliliter which was inhibited from growth of four strains of bacteria (Table 1). The highest inhibitory concentration (MIC) of achiella extract against strains of Pseudomonas aeruginosa was 5 milligrams per milliliter that four strains were inhibited at this concentration, the lowest concentration of inhibitor was 62 milligrams per milliliter which one strain has been inhibited in this concentration. The results of ethanol extract of Ajowan showed that three strains of Psedomonas in none of the concentrations of the extracts have the ability to grow and be completely gone.

Table 1: Minimum Inhibitory Concentration (MIC), Achillea, green tea and Ajowan extract against strains of Pseudomonas aeruginosa

Bacteria	Green Tea	Achiella	Carum copticum	Bacteria	Green Tea	Achiella	Carum copticum
1	2/5	5	5	7	2/5	5	1/25
2	2/5	2/5	2/5	8	2/5	0/62	1/25
3	2/5	2/5	2/5	9	1/25	2/5	1/25
4	2/5	5	No growth	10	1/25	No growth	1/25
5	2/5	5	5	11	1/25	2/5	No growth
6	2/5	2/5	1/25	12	1/25	1/25	2/5

4. DISCUSSION

Ajowan fruit flavored whose value varies depending on the location of growth, have been reported rate of between 2 and 9 (V/W). Essence is known by Ajowan oil name. This essence have colorless or brown apparent and smell like thymol. Compounds been reported thymol, Karakrvl, Alfa and beta-pinene, terpinene, parasymn (10). In general, the more components of the essence are phenolic compounds that have antioxidant and anti-microbial properties and thus have potential use as a preservative in food. This essence have a variety of applications in the medical and pharmaceutical industries (11).

Murthy and colleagues reported the results showed that the extract of Ajowan a high antimicrobial activity against pathogens in foods at a dose of 2.5 mg per liter.

Ajowan extract has high antimicrobial activity against Bacillus cereus with diameter of inhibition zone (mm48) while on bacteria such as Streptococcus, Ecoli, Pseudomonas aeruginosa inhibitory effect is low (12). In studies of Yaaghoob zadeh and colleagues showed that Ajowan essense has high antimicrobial activity against strains of Ecoli isolated from stool buffalo with diameter of inhibition zone (mm23 / 28) (13). Studies Tokme chi and colleagues showed that the essense Thyme and Ajowan is able to inhibit the growth of bacteria, Aeromonas hydrophila, in this study, the minimum inhibitory concentration (MIC) 310 and 1250 micrograms per milliliter respectively (14).

Abroumand and colleagues reported results of chromatography Ajowan essense showed 11 compound that the main component contains gamma Trpynn, Para-Simon and thymol. Another important compound reported is Karakrvl, this is a potent antioxidant and have high antibacterial effects, The results showed that in 500 ppm concentration of essense observed no growth in E.coli strain (15). Achillea plant has long been one of the plants is used in the treatment of wounds, digestive problems and infectious and is effective in reducing blood fat (16). Among the materials that can be found in Achillea Chamazulene, Karyvfylyn, 1 & 8-cineol and flavonoids such as epinephrine and routine Zhenin noted (16). The study results showed that the essence Bvmadarn Issabeagloo et combination of α-pinene, Camphene, β-pinene, Limonene, γ-Terpinen, β-caryophyllene, α-Humulene, Germacrene and Cadinene the diameter of inhibition zone of 30% of oil Achillea against bacteria S.aureus, S.intermedius, S.hyicus, S.epidermidis, S.saprophyticus, S.aureus ssp.anaerobious, S.capare, S.gallinarum, S.arlettae, S.lentus, S. equorum, S.simulans, S.delphin and S.chromogenes respectively 10.14, 10.30, 10.37, 9.49, 9.76, 9.98, 9.08, 9.73, 9.84, 10.12, 10.35, 9.87, 9.80, 9.54, respectively (17).

The study E.salvagnini showed Achiella extract alone and in combination with Phenova, Imidazolidinyl and Nipagin / Nipasol inhibitory from growth of Bacillus subtilis (18).

The study of Orujyan, results showed that the essensel of Achillea contain camphor (65/28%), 1, 8 cineol (95/26%), camphene (98/5%), beta-pinene (8/4%), alpha-pinene (2 / 4%) and borneol (4%), and the minimum inhibitory concentration is obtained against Gram-positive bacteria between 15/0 to 75/0 mg mland against Gram-negative bacteria between 5.1 to 3 milligrams per milliliter . The most sensitive pathogens to this essense was S.aureus and the most resistant pathogene was Salmonella enteritidis (19).

Kermanshah and colleagues study, the minimum inhibitory concentration of Achiella extract for Streptococcus mutans was 50 micrograms per milliliter and for lactobacilli rhamnosus 5.12 micrograms per milliliter and Actinomyces viscosus was 50 micrograms per ml (20).

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Antimicrobial Activities of Teucrium Polium Against *Salmonella Typhimurium*

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ABSTRACT

Objective: Teucrium polium L. (family Lamiaceae) is a wild-growing flowering plant, found abundantly in South-Western Asia, Europe and North Africa. Traditionally, T. polium has been used for different pathological conditions such as gastrointestinal disorders, inflammations, diabetes and rheumatism. The purpose of this study was to evaluate the antimicrobial effect of ethanol extract of Teucrium polium against Salmonella typhimurium isolates from poultry is resistant to penicillin. **Methods:** Teucrium polium extract using vacuum from the center (Rotary) were 12 strains of Salmonella typhimurium was isolated from poultry in the city of Zabol. Minimum inhibitory concentrations of MBC Teucrium polium extract in different concentrations by dilution in the wells was determined on bacteria. Susceptibility to several antibiotics by Kirby-Bauer disk diffusion standard were evaluated. **Results:** The result of herbal extraction showed the most MIC (the minimum inhibitory concentration) was 10mg/ml concentration that 2 strains of them were inhibited by this concentration Teucrium polium. The lowest MIC was 2/5 ppm concentration that two strain of *Salmonella* were inhibited. Although the clinical relevance of extracts and essential oils because of fewer side effects than other current treatments for common antibiotics, it seems valuable, but more research to clinical application of the mechanism of action of ethanol extract and purified effective composition of this plant is on microbial agents.

1.INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Belmekki et al., 2013; Farnsworth, 1989). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Bajpai et al., 2005). The genus *Teucrium*, which belongs to the family Labiatae, includes 300 species widespread all around the world (Bonnier, 1990; Coste, 1909). A large number of known medicinal species belonging to the genus *Teucrium* are used in folk medicine and pharmacy (Chang et al., 2006). The species of the genus *Teucrium* are very rich in phenolic compounds with very strong biological activity (Acar and Goldstein, 1996). Among the species, *T. polium* (L.) is widely used in folk medicine for many treatment interventions (Ali Shtayeh et al., 2000). This plant is well known for its antinociceptive (Abdollahi et al., 2003), antioxidant (Couladis et al., 2003), hypolipidemic (Rasekh et al., 2001), antiinflammatory, anti-rheumatoid, and hypoglycemic (Gharaibeh et al., 1988) properties.The purpose of this study was to evaluate the antimicrobial effect of ethanol extract of Teucrium polium against Salmonella

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typhimurium isolates from poultry is resistant to penicillin.

2. MATERIAL AND METHOD

2.1. Plant materials

The leaf T. polium was collected in the region of Iran dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

2.2. Preparation of extracts

Plant was properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman No. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator.

2.3. Bacterial strains

All strains were isolated at different times during 2013-2014 from contaminated bird. Samples were diluted and/or homogenized in TSB medium, and isolates obtained by *Salmonella* selective enrichment in Rappaport–Vassiliadis (RV) medium after 24 h incubation at 43°C.

2.4. Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (CLSI, 2002). The procedure followed is briefly described here. *Salmonella* isolated plates were grown overnight on Nutrient agar and colony suspension was prepared using the sterile salin water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ampicillin (10 µg) and penicillin (10 µg). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/mL. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/mL) was added to each well to achieve the concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37oC for 18- 24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

2.5. Statistical Analysis

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

3. RESULTS

The result of herbal extraction showed the most MIC (the minimum inhibitory concentration) was 50 ppm concentration that 5 strains of them were inhibited by this concentration. The lowest MIC was 25 ppm concentration that 7 strain of *Salmonella* were inhibited. The highest and lowest MBC value of extract were 100mg/ml respectively (Table1).

Tabel1: The minimum inhibitory concentration extract against *Salmonella*

Bacterial cod	MIC/MBC for extract plant(µ/ml)	Antibiotic resistant
1	25/ 50	AM- P
2	50/ 50	AM- P
3	50/ 100	AM- P
4	50/100	AM- P
5	25/50	AM- P
6	25/ 50	AM- P
7	25/ 50	AM- P
8	25/ 50	AM- P
9	50/ 100	AM- P
10	25/ 50	AM- P
11	25/ 50	AM- P
12	50/ 100	AM- P

AM= Ampicillin

P=Penicillin

4. DISCUSSION

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature (Mulligen et al. 1993, Davis 1994, Robin et al. 1998). The result show that most MIC (the minimum inhibitory concentration) was 10mg/ml concentration that 2 strains of them were inhibited by this concentration. The lowest MIC was 2/5 ppm concentration that two strain of *Salmonella* were inhibited. The study of Belmekki, the major compounds were germacrene D (25.81%), bicyclogermacrene (13%), ß-pinene (11.69%) and carvacrol (8.93%). Furthermore, the essential oil was tested against five bacteria (three Gram-positive and two Gram negative) and three fungi at different concentrations. Results showed that the oil exhibited moderate inhibitory effects on *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus*, with a minimum inhibitory concentrations of 3 to 5 µl/ml (Belmekki et al., 2013). The study of minimal inhibitory concentration of the taken extracts compared to the strains resistant to *Klebsiella pneumonia* showed that all the above extracts have antimicrobial properties and they were able to prevent the growth of strains resistant to *Klebsiella pneumonia*, whereas all 8-strain klebsiella in the study were resistant to the three (aqueous, ethanolic, ethyl acetate) extracts (Shakibaiee et al., 2000) . In the same study, species and sub-species of teucrium had certain anti-staphylococcus effect, most of which was on the *S. epidermidis*. This bacteria was not studied in our study, but all 3 *Staphylococcus aureus* samples were resistant to *Teucrium polium* extracts (Sarac and Ugur, 2007). The study results showed that the inhibitory zone diameter Sarac and Ugur TP ethanol extract against bacteria S.aureus ATCC25923, S.aureus MU 38, S.aureus MU44 and S.epidermidis MU30 with 9, 8, 8 and 11 mm) (Sarac and Ugur, 2007**.**

The study of Zerroug, extracts of T. polium gave zones of inhibition against Bacillus subtilis, Micrococcus luteus and Paracoccus paratrophus of 3.7, 2.0 and 2.0 mm**,** respectively. A. iva extract only inhibited the growth of Paracoccus paratrophus, giving a zone of inhibition of 3.0.mm (Zerroug et al., 2011). The hydroalcoholic extract of *T. polium* had a relatively satisfactory effect on Salmonella typhi. (Darabpour et al., 2010). The antibacterial activity of essential oil and methanolic extract of Teucrium polium was determined against Pseudomonas aeruginosa, Pantoea agglomerans, Brenneria nigrifluens, Rhizobium radiobacter, Rhizobium vitis, Streptomyces scabies, Ralstonia solanacearum, Xanthomonas campestris and Pectobacterium cartovorum by disc diffusion method. Our results indicate that both methanolic extract and essential oil did not show antibacterial activity against P. aeruginosa. Also the essential oil did not show antibacterial activity against P. cartovorum. In general, both methanolic extract and essential oil showed the same antibacterial activity against R. solanacearum, P. agglomerans, B. nigrifluens and S. scabies(Purnavab et al., 2015).

Antibacterial activity of the crude extract, as well as with four of the isolated metabolites, was observed with Staphylococcus aureus anti-biofilm activity in the low µMol range. Diverse sesquiterpene-skeleton structure and corresponding comprehensive enzyme capacity is discussed(Elmasri et al., 2014). The extract was effective against both yeast and carrageenin pyrexia in rats. It also exhibited a marked antibacterial action against both gram positive and gram negative organisms and was found to be non toxic in acute studies(Autore et al., 1984).

CONCLUSION

The results of this work show that the extract of *T. polium* possesses antimicrobial properties, which can be used as natural antimicrobial agents for human and infectious diseases

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Authors' Contributions

All the authors had equal roles in design and writing of the manuscript.

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