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Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial

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Abstract

The aim of this study was to evaluate the antimicrobial activities of *Trachyspermum ammi***,** essential oil against different kinds of microorganisms by microtiter plate method. All 36 isolates of (12 *Klebsiella pneumoniae*, 12 *E.coli* and 12 *Staphylococcus aureus*) isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections were evaluated. In this study, the essential oil of *Trachyspermum ammi* obtained by hydrodistillation for 2.5-3 h using a Clevenger-type apparatus and the minimum inhibitory concentrations were determined to characterize the antimicrobial activities of this essential oil. The results showed *E. coli* isolates were resistance to 4 of the antibiotics including ceftazidime (50%) cefixime (41.6%), tetracyclin(75%), erythromycin(58.3%). However *k. pneumonia* isolates were resistance to 3 of the agent including ceftazidime(33.3%) ,cefixime(58.3%), erythromycin(75%) and *S.aureus* isolates were resistance to 6 of the agent including cefixime (33.3%), trimethoprim-sulfamethoxazol(41.66%), penicillin(50%), oxacillin(3.3%), ceftazidime(66.6%) and vancomycin(8.3%) and the MIC value was also determined against all the tested bacteria. The highest MIC values of essential oil were determined 100ppm against *E. coli* and highest MIC value for *K.pneumoniae* was 250ppm. In conclusion, it seems that *Trachyspermum ammi* essential oil could inhibit the growth of all of the tested bacteria.

Key words: Antibacterial activity, Human pathogen, *Trachyspermum ammi*

Introduction

One of the more alarming recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Nature has served as a rich source of medicinal plants for thousands of years and an impressive number of moderndrugs has been isolated from natural antimicrobial agents with plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (an antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. *Trachysper ammi*, commonly referred as Bishop's weed, Carom seed (English names) and

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ajowan or ajwain or omum in Indian languages, is an erect annual herb with striate stem originated in Iran and India(Wadikar et al., 2012).Some biological effects of ajowan such as antiviral (Hussein et al, 2000), anti- inflammatory (Thangam et al., 2003), antifungal (Rasooli et al., 2008), antipyretic (Anis et al., 1986),antifilarial (Mathew et al, 2008), analgesic (Kaar et al., 2009; Dashti-Rahmatabadi et al., 2007), anti-nociceptive (Hejazian et al., 2008)and antioxidant activity (Bera et al., 2004) have been confirmed. The aim of this study was to evaluate the antimicrobial activities of *Trachyspermum ammi***,** oil against different kinds of bacteria.

Material and Method

Isolation of bacteria: All 36 isolates (12 *Klebsiella pneumoniae* and 12 *E.coli* and 12 *Staphylococcus aureus*) isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during deleted 2011- 2012 were evaluated. Isolated bacteria were identified by Gram stain and standard biochemical tests. Biochemical test employed were urease production, citrate

utilization and fermentation of sugars. Sugar fermentation tests performed were sucrose, glucose, mannitol, lactose, adonitol, dulcitol, melibiose and esculin. Indole test and H2S

production on TSI agar, oxidase, catalase and nitrate were also carried out. Besides these tests, motility and growth of organism in potassium cyanide were also checked. For

biochemical tests standard procedures were used(Cruickshank., 1980).

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. *S. aureus* , *Ecoli* and *K. pneumoniae* isolateds deleted were grown overnight on blood agar, Nutrient agar and colony of bacteria was prepared using the sterile serum physiology 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 antibiogrammed with different antibiotics deleted. ceftazidim(30 µg), tetracyclin (30 µg), erythromycin (15 µg), ceftazidime (30 µg), trimethoprim-sulfamethoxazol (1.25+23.15 µg), penicillin (10µg), oxacillin (30µg) and vancomycin (10 µg).

Plant materials*:*

The seed Ajowan**,** was collected in the region of Iran(Sistan , south-eastern, Iran) and plant in Zabol university herbarium received approval and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Distillation of essential oil

The seed Ajowan**,** was ground prior to the operation and then 300 g of ground rosemary was submitted to water distillation for 4 h using a Clevenger- type apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C.

Determination of Minimum Inhibitory Concentration (MIC) of essential oils

The broth micro dilution method was used to determine MIC. 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 250ppm, 100ppm, 50ppm and 10ppm. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) 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 a 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 incubator at 37°C for 18–24 hours. Turbidity as bacterial growth 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 values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The bacteria growth was indicated by turbidity (Bokaeian et al., 2013).

Results

Antibiotic susceptibility

Antibiotic susceptibility of *E. coli* isolates was evaluated for 4 antibiotics .However, overall, *E. coli* isolates were resistance to 4 of the agent including ceftazidime(50%)cefixime(41.6%), tetracyclin(75%), erythromycin(58.3%)(Table1), Antibiotic susceptibility of *K.pneumoniae* isolates was evaluated for 3 antimicrobial. However *k. pneumonia* were resistance to 3 of the antibiotics including ceftazidime(33.3%),cefixime(58.3%), erythromycin(75%)(Table 2) and *S.aureus* were resistance to 6 antimicrobial , cefixime(33.3%), trimethoprim-sulfamethoxazol (41.66%), penicillin(50%), oxacillin(83.3%), ceftazidime(66.6%) and vancomycin(8.3%)(Table 3).

Antibacterial activity

Inhibitory effects of essential oil from Ajowan**,** against *E.coli*, *K.pneumoniae* and *S. aureus* were demonstrated in tables1, 2, 3. The results in tables 4, 5, 6 showed that essential oil of Ajowan had inhibitory effect against *E.coli*, *K. pneumoniae* and *S. aureus*.The MIC value was also determined against all the tested bacteria. The highest MIC values of essential oil were found to be 100ppm against *E.coli* on of MIC value for *K. pneumoniae* was 250ppm (Tables 2, 3).

Discussion

In the study show that isolates of *E. coli* were resistance to 4 of the agent antimicrobial including ceftazidime(50%), cefixime(41.6%), tetracycline (75%), erythromycin(58.3%), *k. pneumonia* were resistance to 3 of the agent antimicrobial including ceftazidime(3.3%) ,cefixime(58.3%), erythromycin(75%) and *S.aureus* were resistance to 6 of antimicrobial cefixime(33.3%), trimethoprim-sulfamethoxazol(41.66%), penicillin(50%), oxacillin(83.3%), ceftazidime(66.6%) and vancomycin(8.3%). The MIC value was also determined against all the tested bacteria. The highest MIC values of essential oil were found to be 100ppm against *E.coli* and on of MIC value for *K.pneumoniae* was 250ppm. Natural preservatives are the chemical agents derived from plants that prevent the decomposition of products by any means (Dorman et al, 2000). The study of Usha, ethanol extract of Ajowanr revealed antibacterial activity against *Pseudomonas sp*, whereas acetone extract of spices exhibited highest activity against *Escherichia coli*. Acetone extract of Ajowan showed no activity against *Staphylococcus au*reus and *Bacillus subtilis* (Usha et al, 2012). Murthy show that, the extract was found to be highly effective for *B. cereus* with 48 mm zone of inhibition followed by *S. aureus*, *B. subtilis*, and *L. monocytogenes.* On the other hand, lesser inhibition was observed in *Streptococcus, Y. enterocolitica*, *E. coli*, and *P. aeruoginosa.* Most of the gram-positive bacteria,such as *B. cereus, B. subtilis*, *S. aureus*, *and L. monocytogenes*, showed good inhibition activity when compared to gram-negative bacteria (e.g. *E. coli and P. aeruginosa*)(Murthy et a., 2009). Kaure et al show that, the result show that ambint water, hot water and boiling water extract of ajowan revealed an antibacterial activity against *Entrococcus faecalis* and *Staphylococcus aureus* (Kaur et al, 2009). The study of Malekinejad, the result show *Trachyspermum copticum* essential oil also showed antibacterial effects with rather high MIC values of 1.25 mg/ml (Malekinejad et al, 2012).Chemical composition of Ajowan oil exhibited the presence of thymol, γ-terpinene and O-cymene without carvacrol as the main component of Ajowan oil. Antimicrobial activities

of oil is apparently attributable to high phenolic compounds such as thymol and carvacrol (Mahboudi et al, 2010) or p-cymene, the antimicrobial effect of thymol and carvacrol is due to damage in membrane integrity with change in pH hemostasis also equilibrium of inorganic ions, p-cymene does not have antimicrobial activity but it increases the antimicrobial activity of thymol or carvacrol (Delgado et al, 2004; Ultee et al, 2002).

Conclusion

In conclusion, plant essential oil tested in this study had potential antibacterial activities against the bacteria strains. Our results support the use of these plants in traditional medicine and suggest that some of the plant essential oil possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.

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Table 1: Antimicrobial susceptibility, MIC essential oil for Ecoli.

Bacterial cods	**MIC for Essential oil**	**Resistance pattern**
1	50ppm	E, CE, CF, TE
2	50ppm	E, CF
3	100ppm	CE,CF,TE
4	100ppm	E, CE, CF, TE
5	10ppm	E, CE, CF, TE
6	50ppm	-
7	10ppm	-
8	50ppm	TE
9	50ppm	E,TE
10	10ppm	E,TE
11	10ppm	TE
12	10ppm	E, CE, CF, TE

E= Erythromycin, CE= cefixime, CF= Ceftazidime, TE= Tetracyclin

Table 2: Antimicrobial susceptibility, MIC essential oil for *k. pneumoniae.*

Bacterial cods	**MIC for Essential oil**	**Resistance pattern**
1	10ppm	E,CF
2	10ppm	E,CF
3	250ppm	E
4	10ppm	-
5	10ppm	E
6	10ppm	E,CE,CF
7	10ppm	E,CE,CF
8	100ppm	E,CE,CF
9	100ppm	-
10	10ppm	E,CE,CF
11	100ppm	E
12	100ppm	CE

E= Erythromycin, CE= cefixime, CF= Ceftazidime

Table 3: Antimicrobial susceptibility, MIC essential oil for *S.aureus*.

Bacterial cods	**MIC for Essential oil**	**Resistance pattern**
1	50ppm	CE, SXT, V, CF, P, OX
2	50ppm	CE, SXT, CF, P, OX
3	50ppm	CE, SXT, CF, P, OX
4	50ppm	CF,OX
5	100ppm	CF,OX
6	100ppm	OX
7	50ppm	-
8	50ppm	-
9	50ppm	OX
10	100ppm	SXT,CF,P,OX
11	100ppm	CE, SXT, CF, P, OX
12	50ppm	CF,P,OX

CE=Cefixime, SXT= Trimethoprim-sulfamethoxazol, V=Vancomycin, CF=Ceftazidime, P=Penicillin, OX=Oxacillin