

Synergistic Antibacterial Activity of *Trachyspermum ammi* (Ajwain) and Metal Salts against Pathogenic Bacteria

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ABSTRACT

Medicinal plants are used as an alternative of drugs and therapeutic agents all over the world from ancient era due to facing the disaster of resistivity against antimicrobial drugs by microorganisms and also due to its lesser side effects. The aim of this study was to evaluate the antimicrobial activity of methanolic and ethanolic extracts of *Trachyspermum ammi* (ajwain) against seven isolated bacterial spp. *in vitro*, *P.aeruginosa*, *B.subtilis*, *S.aureus*, *Proteus sp.*, *Klebsiella pneumoniae*, *S.typhi* and *E.coli* by well diffusion and disc diffusion techniques. In addition, enhancement of antimicrobial activity of ajwain by adding metal salt was also observed. The result demonstrated that both extract of ajwain give greater effectivity against all 7 bacterial spp, but zinc sulphate and calcium sulphate did not enhanced the antibacterial activity when added to ajwain extract while copper sulphate, magnesium sulphate and sodium sulphate enhanced the antibacterial activity when added to ajwain extract against all the bacterial spp. with same concentrations. Significant results were obtained in ajwain metal combination as compared to seed extracts alone that suggests its future appliance in food and pharmaceuticals.

Key words: Ajwain, Antibacterial, Ethanol, Metal Salts, Methanol,

INTRODUCTION

Estimated that 258,650 higher plants found in the world, approximately 10% of them are reported as medicinal plants used for cure and prevention for some minor and major diseases in disease acquiring community (Shinwari, 2010). Now a day's some pharmaceutical companies producing many kind of antibiotics due to ability of organisms to get resistivity against previously discovered many antibiotics which are active against fungi, yeast and both gram positive and negative bacteria (Hawrelak and Myers, 2004; Cohen, 1992) this resistivity is mainly due to misuse of antimicrobial agents in food of animals, human and as a anti pesticide in herbs and plants, and amount of

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these antimicrobial agents directly related to resistivity (Shakya *et al.*, 2013; Hammerum and Heuer, 2009). Many of them cause severe harm to person's vital organs like liver, kidneys, pancreas and spleen and also interfere in functions of immune system due to overuse or misuse of doses, resulted to avoid this harm in many countries researchers work on natural alternatives which are environmental and human friendly and not related to any side effect by its use called as medicinal plants, they use different forms like oil extract, powder and ethanolic and methanolic extracts etc, and have many constituents which shows effectivity against antibiotic resistive and non resistive organisms and also reduce the harmful effect towards their consumers (Giamarellou, 2006). Variety of antimicrobial agents (active

against bacteria, both gram-positive and gram-negative, fungi, yeast, viruses and protozoa) are found in medicinal plants like tannins, terpenoids, alkaloids, flavonoides, phenolic and polyphenolic, quinines, emodin, oligopeptide systemin, abscisic acid, Eugenol, protoanemonin, anethemic acid nordihydroguaiaretic acid, colchicine etc and many more (Leon *et al.*, 2001; Cowan. 1999; Dorman, 2000).

Trachyspermum ammi commonly known as ajwain in Pakistan and it is arises from medicinal herbs of Egypt and found in all Asian countries (Pakistan, India, west Bengal), Iran, Iraq and Afghanistan (Mondal *et al.*, 2008), it has both nutritional as well as medicinal uses (Hussein *et al.*, 2000), grow in salty soil, it is profusely branched annual herb, 60-90 cm in length, stem is striated with 16 white color actinomorphic mail or bisexual flowers, fruit is aromatic, brown, cordate and oval in shape. It is used against many digestive tract infections and diseases as it possess antiviral anti inflammatory, anti bacterial and antifungal, analgesic, antiparasitic, antiplatelet-aggregatory, anti oxidative, anti spasmodic, germicidal. Ajwain contains small amounts of phytochemicals such as pinene, cymene, limonene and terpinene. The presence of terpenes, glycosides and sterols in plant has been found to possess anti-inflammatory properties. The phenolic constituents of ajwain are mainly responsible for the antiseptic and antitussive properties (Jeet *et al.*, 2012)

The trace metals in herbal plants play a vital function as structural and working elements of metal proteins and enzymes in a living cell (Nagajyoti *et al.*, 2010). These metal ions either enhance or diminish the biological activity of some therapeutics. Thus, the control of heavy metals in medicinal plants and their wares should be such that the safety and efficacy of herbal products are ensured (Hifsa *et al.*, 2009).

The objective of the present study was to

evaluate the antibacterial activity of ajwain against different pathogenic bacteria and also the enhancement of this activity by addition of metal salts.

MATERIALS AND METHODS

Bacterial Culture: Total seven bacterial spp. were used for the study. Five were gram negative (*Klebsiellpneumoniae*, *Pseudomonas aerogenosa*, *Proteus sp.*, *E. coli*, *S.typhi*) and two were gram positive (*S.aureus*, *B.subtilis*)

Preparation of bacterial inoculum for antimicrobial activity testing: Take loop full of bacterial culture and inoculate in nutrient broth media, Incubate at 37 degree centigrade temperature for 24 hours. After 24 hours of incubation, match this fresh bacterial culture tube with tube number 5 (0.5 ml BaCl₂ and 9.5 ml of H₂SO₄) of MacFarland and use it as inoculums for antimicrobial testing.

Preparation of extract: 500 gm of ajwain seeds were purchased from local market of North Karachi. Make extract of ajwain seeds in two different solvents ethanol and methanol.

1. **Ethanol extract of ajwain seeds (EEAS):** Take 20 gm of ajwain seeds wash with distilled water and then 95% alcohol, Air dry in sun light for 2 days, then in oven at 40 degree centigrade for 2 hours. Make fine powder of seeds by using grinder, Place in 100 ml E-flask and add 50 ml ethanol, Cover with silver foil, and place in dark area for 7 days, after seven days filter this extract with watt's man filter paper. Concentrate by using rotary evaporator.
2. **Methanolic extract of ajwain seeds (MEAS):** Take 20 gm of ajwain seeds wash with distilled water and then 95% alcohol, Air dry in sun light for 2 days, then in oven at 40 degree centigrade for 2 hours. Make fine powder of seeds by using grinder,

Place in 100 ml E-flask and add 50 ml methanol, Cover with silver foil, and place in dark area for 7 days, after seven days filter this extract with watt's man filter paper Concentrate by using rotary evaporator.

Preparation of ajwain-metals extracts (AME):

Take 1 gm salts powder (Mg.SO₄, CaSO₄, CuSO₄, FeSO₄ and ZnSO₄). Add in 100 ml distilled water mix it well.

Antimicrobial activity by well diffusion

technique: Antimicrobial activity is examined by well diffusion method. 24 hours fresh culture of bacterial inoculums used. Lawn is prepared in MHA plate for each of 7 bacterium culture two sets of 3 plates one set for EEAS and one for MEAS, one for positive control (plate:1) one for negative control (plate:2) and one for sensitivity testing (plate:3) against ajwain. Make well by using 8 mm borer in the center of plate. Add 50 micro liters of EEAS and MEAS in well of plate: 3 of each set, add 50 micro liter of cephalosporin antibiotic suspension in plate: 1, add 50 micro liter in well of plate: 2. Incubate for 24 hours at 37 degree centigrade in incubator. After 24 hours measure the zone of inhibition by mm scale.

Antimicrobial activity by disc diffusion

technique: 7 individual discs for each extract of EEAS and MEAS were prepared using filter paper. On MHA plates make lawn of each organism. Place disc without extract as a negative control, disc of cephalosporin as a positive control and prepared disc with EEAS for sensitivity testing. Repeat same steps for disc of MEAS. Incubate all plates at 37 degree temperature for 24 hours.

Antimicrobial activity of ajwain-metal extracts (AME) combination:

For this purpose take sterile test tubes and make AME of each metal salt. 1:5 dilution of AME is prepared by mixing 100% solution of metals and pure extract of ajwain seeds (1 ml of salt and 5 ml of ajwain extract). Lawn of fresh culture of bacteria is prepared in MHA plate. Make 3 wells in a single plate one for negative control, one for negative control and one for sensitivity tasting, mark as 1, 2 and 3 respectively. Well 1 filled with antibiotic solution (cephalosporin), in well no. 2 add distilled water and in well no 3 add MAE solution 50 micro-liters each. Incubate for 24 hours at 37 degree centigrade. After 24 hours of incubation measure the zone of inhibition by using mm scale.

RESULTS

Table I. Antibacterial activity of EEAS by disc diffusion

S. No	Organism	EEAS	Positive control	Negative control
1	<i>E.coli</i>	11mm	10 mm	0 mm
2	<i>Paerogenosa</i>	10 mm	12 mm	0 mm
3	<i>S.typhi</i>	19 mm	12 mm	0 mm
4	<i>Proteus</i>	16 mm	15 mm	0 mm
5	<i>Klebsiella</i>	17 mm	19 mm	0 mm
6	<i>S.aureus</i>	17 mm	18 mm	0 mm
7	<i>B.subtilis</i>	16 mm	5 mm	0 mm

Table II. Antibacterial activity of EEAS by well diffusion

S. No	Organism	EEAS	Positive control	Negative control
1	<i>E.coli</i>	17 mm	12 mm	0 mm
2	<i>P.aerogenosa</i>	14 mm	11 mm	0 mm
3	<i>S.typhi</i>	26 mm	18 mm	0 mm
4	<i>Proteus</i>	20 mm	15 mm	0 mm
5	<i>Klebsiella</i>	20 mm	20 mm	0 mm
6	<i>S.aureus</i>	20 mm	19 mm	0 mm
7	<i>B.subtilis</i>	26 mm	9 mm	0 mm

Table III. Antibacterial activity of MEAS by disc diffusion

S. No	Organism	MEAS	Positive control	Negative control
1	<i>E.coli</i>	7 mm	10 mm	0 mm
2	<i>P.aerogenosa</i>	9 mm	12 mm	0 mm
3	<i>S.typhi</i>	22 mm	12 mm	0 mm
4	<i>Proteus</i>	16 mm	15 mm	0 mm
5	<i>Klebsiella</i>	18 mm	19 mm	0 mm
6	<i>S.aureus</i>	16 mm	18 mm	0 mm
7	<i>B.subtilis</i>	18 mm	5 mm	0 mm

Table IV. Antibacterial activity of MEAS by well diffusion

S. No	Organism	MEAS	Positive control	Negative control
1	<i>E.coli</i>	16 mm	10 mm	0 mm
2	<i>P.aerogenosa</i>	15 mm	12 mm	0 mm
3	<i>S.typhi</i>	24 mm	12 mm	0 mm
4	<i>Proteus</i>	16 mm	15 mm	0 mm
5	<i>Klebsiella</i>	22 mm	19 mm	0 mm
6	<i>S.aureus</i>	18 mm	18 mm	0 mm
7	<i>B.subtilis</i>	21 mm	5 mm	0 mm

Table V. Antibacterial activity of ajwain metal extract/ AME (1:5) combination

S. No.	Organism	CuSO4	FeSO4	MgSO4	CaSO4	ZnSO4
1	<i>E.coli</i>	19 mm	15 mm	17 mm	10 mm	11 mm
2	<i>P.aerogenosa</i>	18 mm	17 mm	12 mm	2 mm	1 mm
3	<i>S.typhi</i>	29 mm	24 mm	22 mm	12 mm	11 mm
4	<i>Proteus</i>	20 mm	11 mm	16 mm	11 mm	14 mm
5	<i>Klebsiella</i>	15 mm	11 mm	21 mm	15 mm	16 mm
6	<i>S.aureus</i>	15 mm	10 mm	15 mm	4 mm	6 mm
7	<i>B.subtilis</i>	16 mm	17 mm	13 mm	13 mm	11 mm



Figure 1. Antibacterial activity of EEAS and MEAS against *S. typhi*, *E.coli* and *S. aureus*.

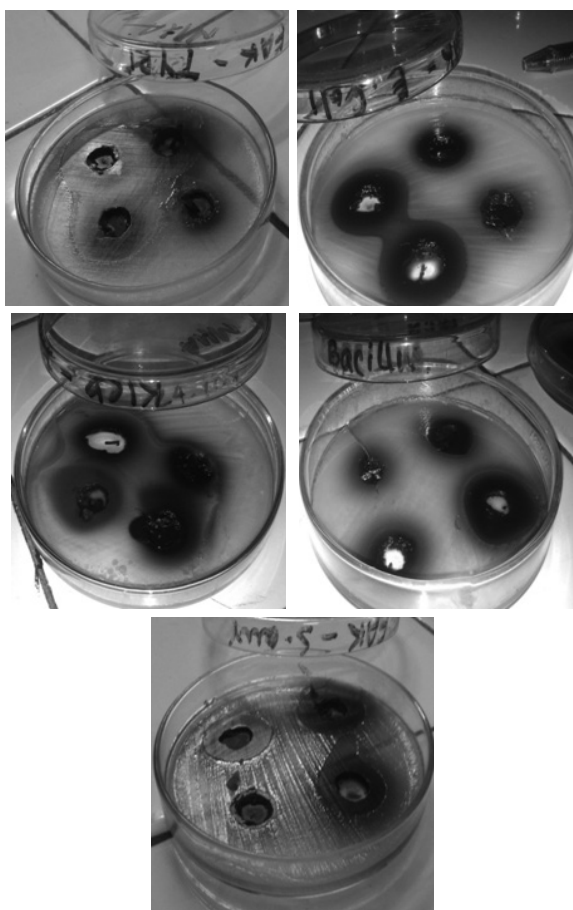


Figure 2. Antibacterial activity of AME combination against *S. typhi*, *E. coli*, *K. pneumoniae*, *B. subtilis* and *S. aureus*.

The antibacterial activity of the ajwain extract and extract-metal salt combinations was evaluated against a seven bacterial species. The

diameters of the inhibition zones obtained are presented in Table I – V. Among the screened extracts, both EEAS and MEAS were found to be effective against gram positive and gram negative bacteria.

DISCUSSION

In this study antimicrobial activity of ajwain in two solvents ethanol and methanol were examined against seven bacterial spp. *E. coli*, *P.aeruginosa*, *K.pneumoniae*, *Proteus sp.*, *S.typhi*, *S. aureus*, and *B. subtilis* and also observed the enhancement of this activity by adding trace metal salts CuSO_4 , FeSO_4 , MgSO_4 , ZnSO_4 , CaSO_4 on it.

The antibacterial activity of EEAS and MEAS was determine by disc diffusion and well diffusion methods and inhibitory zones diameter were measure in the presence and absence of each extract and on the basis of zone of inhibition obtained bacterial population divided in three categories resistant $>7\text{mm}$, intermediate $>12\text{mm}$, sensitive $<12\text{mm}$. In the present study, *B.subtilis*, *Proteus sp.*, *S.typhi*, *S.aureus*, *K.pneumoniae* were found to be sensitive with both EEAS and MEAS and by adding metal salt, CuSO_4 , FeSO_4 and MgSO_4 their zones of inhibition increased which means if we use AJWAIN with these salts, they enhance the antibacterial activity of ajwain and inhibit bacterial growth more significantly, but when

we add CaSO_4 and ZnSO_4 alongwith MEAS and EEAS their zone of inhibition decreased than that of EEAS and MEAS alone means these two salts give antagonistic effect with ajwain seeds when they used in combination. *Paerugenosa* and *E.coli* give smaller zones of inhibition against EEAS and MEAS as compared to other bacterial species.

Over all the extract possessed strong antimicrobial activity against gram positive bacteria (*S. aureus*, *B. subtilis*) and gram negative bacteria (*Paerugenosa*, *K. pneumoniae*, *Proteus sp.*, *S.typhi*, *E.coli*). In general few gram negative spp such as *E.coli* and *Paerugenosa* have been reported to be more resistant than others (Al-Zubaydi *et al.*, 2009). It may be due to presence of resistivity genes on their genetic material or may be due to in appropriate activity of ajwain extract on them.

In the present study comparing to metal salt combination with ajwain extract alone. The result showed that the activity of extract alone was enhanced by adding CuSO_4 , FeSO_4 , and MgSO_4 which means these three salts showed synergistic affect with extract, so when we use ajwain seeds with those food stuffs in which little or more amount of these salts or trace metals as a constituent are found, give natural support to our digestive tract against many food born diseases, while CaSO_4 and ZnSO_4 decreased the activity of EEAS and MEAS and showed antagonistic effect with these two salts may be due to inability of making combination of salts with extract. The current study and the report by Simon *et al.*, demonstrates that antimicrobial properties of natural products can be enhanced by the addition of metal ions, especially cupric and ferric salts.

The results of this experiment provide another justification in favor of affectivity of antimicrobial activity of medicinal plant and enhancement of this activity by adding

trace metals so it can use as bio-medicine and preservative.

CONCLUSION

This study emphasizes antibacterial properties of ajwain extract in two solvents, methanol and ethanol, and enhancement of this activity by adding trace metals salts. Our results suggested that the combination of trace metals CuSO_4 , FeSO_4 , MgSO_4 , with ajwain extracts can be utilized as an effective antibacterial source thus the study can be useful for further applications in food and pharmaceutical products.

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