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Original Article

ANTIBACTERIAL AND SYNERGISTIC ACTIVITY OF ETHANOLIC AJWAIN (TRACHYSPERMUM AMMI) EXTRACT ON ESBL AND MBL PRODUCING UROPATHOGENS

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ABSTRACT

Objective: The worldwide problem of emerging antibiotic resistance has created a need to explore alternative approaches of treatment. One such approach is based on evaluating herbal compounds for their activity against pathogens causing infections.

Methods: In the current study, the effect of ethanolic extracts of ajwain was studied on 7 Metallo β-lactamase and 50 Extended Spectrum β-lactamase producing uropathogens which were identified and characterized previously in our laboratory. The bioactive components from ajwain were extracted at 60°C for 6-8h with the help of soxhlet apparatus using ethanol as solvent. This extract was concentrated at 40°C on water bath to obtain a semisolid mass which was used to perform qualitative and quantitative analysis.

Results: Bioassay of ethanolic extracts from ajwain showed 14mm-21mm zones of inhibition for ESBL and MBL producers. Minimum Bactericidal Concentrations (MBC) of ajwain extracts were found to be in the range of 0.5-10mg/ml. Ajwain extracts also showed synergistic activity with ampicillin by lowering the MBC of ampicillin from 10mg/ml to 200-400µg/ml. Time kill analysis of ajwain extracts showed complete loss of viability of test cultures after 2h incubation at 37°C at MBC concentrations and significant decrease in viability after 4h incubation at 37°C at ½MBC concentrations. Gas Chromatography Mass Spectroscopic analysis of the ajwain extracts showed the presence of iso-thymol (carvacrol) as its major constituent. Other components like cymene, terpinene, terpineol, emersol, ethyl ester etc were also detected.

Conclusion: These results collectively indicate the possible use of ajwain extracts in combination therapy to treat infectious diseases caused by multiple drug resistant pathogens.

Keywords: ESBL, MBL, Ajwain, Antibiotic resistance, Bioassay.

INTRODUCTION

Urinary tract infections (UTIs) are the most frequent bacterial infections encountered in community settings [1, 2]. It is estimated that a person is infected with UTI at-least once in his/her lifetime [3]. The most commonly encountered gram negative uropathogens are *E.coli, K.pneumoniae*, *Citrobacter* spp, *P.aeruginosa* and *Proteus* spp [4]. Of late, treatment of these common infectious diseases has become difficult due to the emergence of antibiotic resistant strains like ESBL (Extended spectrum β-lactamase) and MBL (Metallo- β-lactamase) producers. Extended spectrum β-lactamases (ESBLs) are enzymes produced by pathogenic bacteria that are capable of hydrolyzing oxyimino-cephalosporins, and are inhibited by β-lactamase inhibitors [5].

MBLs are bacterial zinc enzymes that are able to hydrolyze most β-lactam antibiotics [6, 7]. In addition, they also show a high degree of resistance to other groups of antibiotics [8]. The emergence of ever-increasing Multiple Drug Resistant (MDR) microbial strains has become a severe health threat to human-kind, and one of the biggest challenges to global drug discovery programs [9, 10]. In order to control such highly infectious cases, medical practitioners are left with no choice but to use higher doses of antibiotics, combination therapy etc. To avoid the present scenario of extreme drug resistance, the focus is gradually shifting towards using natural compounds from plants for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites like tannins, terpenoids and alkaloids that have been found to have antibacterial properties in vitro [11]. Herbal medicines have always been a rich source of drug discovery programs, and many plant derived compounds have shown promising activity against MDR pathogens [12, 13]. Several studies have reported antibacterial activities of *Eriobotrya japonica, Zataria multiflora* and *Terminalia chebula* on ESBL and MBL producers respectively [11, 14, 15]. Plants have been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistance-modifying agent [16, 17, 18]. The enhancement of antimicrobial activity or the reversal of antibiotic resistance by natural or synthetic non-conventional antibiotics has led to the classification of these compounds as enhancers of antibiotic activity [19]. A recent study has shown synergistic activity of Pomegranate pericarp extracts with ciprofloxacin on ESBL and MBL producers [20]. *Trachyspermum ammi*, commonly known as ajowan or ajwain, is commonly used as a spice. It is a highly valued and medicinally important seed-spice of the family *Apiaceae* grown in Iran, India, Pakistan and Egypt. It has been used as culinary spice worldwide and resembles thyme. It was traditionally used as a food flavouring agent and as a digestive stimulant [21].

Since ancient times, it has been used as a therapeutic agent against inflammatory diseases. It is known to have antibacterial [22], antifungal [23], antiseptic [24], anti-helminthic [25], hypolipidaemic [26], antioxidant [27], anti-inflammatory [28] and carminative [29] properties. It was mainly used by the practitioners of the ayurvedic and unani systems of medicine for the treatment of several disorders. It is still used as an expectorant [30], galactogogue [31], antitussive [32] and diurectic [33] agent in many developing countries. The active components of ajwain oil are phenols, mainly thymol and some carvacrol [21]. Thymol is a monopterone compound, which gives aromatic fragrance to seeds. It has local anesthetic, anti-bacterial and antifungal properties. Thymol and Carvacrol inhibit the peroxidation of liposome phospholipids in a concentration dependent manner [34]. It also provides antiviral, antihypertensive, antispasmodic, bronchodilator, and hepato protective properties in addition to the previously described benefits of ajwain [35].

In addition, ajwain also contains small amounts of other phyto-chemicals such as pinene, cymene, limonene and terpinene. The presence of terpenes, glycosides and sterols in plant has been found to possess anti-inflammatory properties [21]. The phenolic constituents of ajwain are mainly responsible for the antiseptic and antitussive properties [21]. In India, ajwain seeds are used to ease asthma [36].

The current study was carried out to investigate the bactericidal and synergistic effect of ajwain extracts with ampicillin on ESBL and MBL producing uropathogens.

MATERIALS AND METHODS

Test organisms

195 gram-negative uropathogens were collected from local pathological laboratories and hospitals situated in south Mumbai and were characterized for ESBL and MBL production in our laboratory in a previous study [37].

Fifty ESBL producing uropathogens that included 10 representative isolates of each of the following genera, i.e., *Klebsiella*, *Escherichia*, *Pseudomonas*, *Proteus* and *Citrobacter*, and 7 MBL producing uropathogens were used in the current study. They are listed in Table 1. These isolates were maintained on Luria-Bertani (LB) agar slants supplemented with 100µg/ml of ampicillin and stored at refrigerated conditions.

Table 1: Test Pathogens used in the study

ESBL Producers		MBL Producers	
Organism	**Total no.**	**Organism**	**Total no.**
E.coli	10	*E.coli* strain 1	1
Klebsiella pneumoniae	10	*E.coli* strain 2	1
Citrobacter amalonaticus	3	*E.coli* strain 3	1
Citrobacter diversus	7	*Klebsiella pneumoniae strain 1*	1
Pseudomonas aeruginosa	10	*Klebsiella pneumoniae strain 2*	1
Proteus mirabilis	5	*Citrobacter amalonaticus*	1
Proteus vulgaris	5	*Pseudomonas aeruginosa*	1
Total	**50**	**Total**	**07**

Processing of the Ajwain seeds

Ajwain seeds were purchased from the local market and authenticated by an expert botanist. It was then used for the extraction of its bioactive components. The dried seeds were crushed into fine powder with the help of a mechanical grinder and refrigerated in sealed vials until further use.

Preparation of the extract

Over 100g of the processed ajwain powder was extracted with 200ml of ethanol using soxhlet apparatus for a period of 6-8h. The extract was further concentrated at 40°C on a water bath to obtain a semisolid mass. This mass was re-suspended in ethanol to get the required concentration of the extract for carrying out further analysis. This concentrated extract was prepared in large volume and preserved at 4^0C in sealed vials until further use. This procedure avoids batch to batch variations.

Sterility testing of Plant extracts

The sterility of ajwain extract was checked by inoculating a loopfull of the extract on Nutrient Agar (NA) and Sabouraud's Agar (SAB) plates, and checking for growth of bacterial and fungal contaminants respectively after 1 week of incubation at room temperature [38].

Qualitative evaluation of antibacterial efficacy of ajwain extract by agar well diffusion method

The effect of ethanolic extracts of ajwain on the test pathogens was assayed by agar well diffusion method [39]. A loopfull of each of the test isolates were inoculated in 10 ml of Brain Heart infusion (BHI) broth and incubated at 37°C for 24h in order to obtain actively growing log phase cultures. Sterile 20 ml of molten NA butt was cooled to around 40°C and then seeded with 0.4 ml test culture (0.1 O.D. at 540nm) and poured into sterile Petri plates. Using a sterile cork borer (8mm diameter), wells was punched in each plate after solidification of the medium. 50µl of the plant extract was then added to the wells, and incubated at 37°C for 24h to observe the zones of inhibition against the extract. Control wells were also set up using 50µl of ethanol (solvent) for each isolate. The experiment was carried out in triplicates, and the results were reported as mean ± Standard Deviation (SD).

Evaluation of MBC of ajwain and ampicillin by agar dilution method

The agar dilution method was used to determine the MBC of ajwain extract and ampicillin individually. Different concentrations of ethanolic extracts of ajwain (0.5-10 mg/ml with an interval of 0.5 mg/ml) or ampicillin (1-10mg/ml with an interval of 1mg/ml) were supplemented into molten NA butts cooled to around 40°C. After solidification of the medium, 5µl of test isolates were spot-inoculated on the plates, and incubated at 37°C for 24h. MBC was defined as the lowest concentration of plant extract/ampicillin that completely inhibited the growth of test cultures [40].

Determination of the synergistic action by agar dilution method

The agar dilution method was similarly used to determine the synergistic activity between ajwain extracts and ampicillin. It was done by incorporating sub-lethal (½MBC) concentrations of ajwain extracts into molten NA butt which were cooled to around 40°C along with 100-500 µg/ml of ampicillin with an interval of 100µg/ml [40].

Time Kill Analysis

A time kill analysis was carried out by performing a viable count of test pathogens in the presence of MBC and 1/2 MBC concentrations of ajwain extract in terms of CFU/ml. The surviving pathogens were estimated over a period of 4h. The time kill analysis was performed using method described by Carson *et al* [41]. The stock concentration of ajwain extract was prepared in nutrient broth while MBC and ½ MBC concentrations (50ml each) were prepared in the respective broth medium from the stock concentration. The final concentration of test microorganism in each tube was around 10^5 CFU/ml, which was confirmed by viable count method. The nutrient broth medium without ajwain extract was used as a control. 0.5ml of sample aliquots were removed at 0-4 h with an interval of 2h serially diluted and plated on NA plates. The plates were then incubated at 37°C for 24h to determine the effect of different concentrations of ajwain extracts on the viability of the test organisms.

GC-MS Analysis

The bioactive components from ajwain seed extract were analyzed by GC-MS HP 7890 system (Agilent technologies). The GC-system was equipped with capillary column with dimensions 30m X 0.25mm X 0.25µm. The program used for GC oven temperature was 5 min isothermal at 300ºC, followed by 90º-260ºC at a rate of 10ºC/min, then held at 260ºC for 5 min, The injection port temperature was 240º C [42]. The used was Joel, AccuTOF GCV MS system with time of flight analyzer was used. The entire analysis was carried out at IIT Bombay, Mumbai 400076. The compounds of the crude extract were identified by comparison of their retention indices (RI) and mass spectra fragmentation with those on the stored library available with IIT, Bombay.

RESULTS

Sterility testing of Plant extracts

Ethanolic extracts of ajwain was found to be free from bacterial and fungal contaminants as observed by streaking it on NA and SAB plates after 1 week of incubation at room temperature.

Qualitative evaluation of antibacterial efficacy of ajwain extract by agar well diffusion method

Figure 1 illustrates the antibacterial activity of ethanolic extracts of ajwain. The mean zones of inhibition were found to be in the range of 15-20 mm (Table 2) for all the tested ESBL and MBL producing isolates.

Evaluation of MBC of ajwain extracts and ampicillin individually and synergistically by agar dilution method

The MBC of ajwain extracts and ampicillin were estimated by agar dilution method. The MBC values are tabulated in Table 3. All the test pathogens showed very high MBC for ampicillin (>10mg/ml). The MBC of ajwain extracts was found in the range of 20-30mg/ml (Table 3).

However, in presence of sub-lethal concentrations of ajwain extract, the MBC of ampicillin was significantly reduced to 200-400µg/ml. This demonstrates the synergistic effect between ajwain extract and ampicillin

GC-MS analysis

The GC-MS analysis identified 7 major compounds in ethanolic ajwain extract; they are listed in Table 4. It was observed that carvacrol (iso-thymol) was present in the highest concentration in ajwain extract followed by emersol. Other components like Phenol 4methoxy 2, 3- 6 trimethyl, Ethyl ester, Terpineol, Terpinene and Cymene were present in small concentrations. The retention times of the identified components are indicated in Table 4.

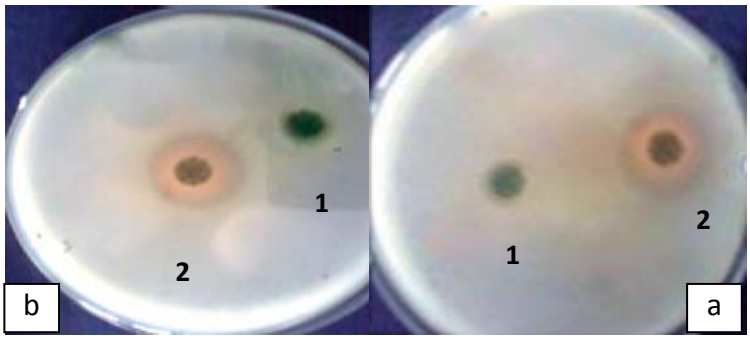


Fig. 1: Well diffusion test showing zones of inhibition observed for ESBL producing *E.coli* (a) and MBL producing *Citrobacter amalonaticus* (b). In the figure, 1 and 2 denotes control (50µl ethanol) and Test (50µl ethanolic ajwain extract) respectively.

Table 2: Well diffusion test showing zones of inhibition observed against test pathogens by ajwain extracts

Test pathogens	No. of isolates	Mean zones of inhibition (mm) ± SD (n=3)
ESBL Producers		
E.coli	10	17.67 ± 0.40
Klebsiella pneumoniae	10	15.72 ± 0.58
Citrobacter amalonaticus	3	18.67 ± 0.57
Citrobacter diversus	7	17.33 ± 0.39
Pseudomonas aeruginosa	10	15.33 ± 0.67
Proteus mirabilis	5	18.67 ± 0.63
Proteus vulgaris	5	19.84 ± 0.52
MBL producers		
E.coli strain 1	1	15.33 ± 0.57
E.coli strain 2	1	19.67 ± 0.57
E.coli strain 3	1	15.33 ± 0.57
Klebsiella pneumoniae strain 1	1	18.33 ± 0.57
Klebsiella pneumoniae strain 2	1	18.67 ± 0.57
Citrobacter amalonaticus	1	15.33 ± 0.57
Pseudomonas aeruginosa	1	16.33 ± 0.57

Table 3: Minimum Bactericidal Concentration of ampicillin and ajwain extracts individually and synergistically against the test pathogens

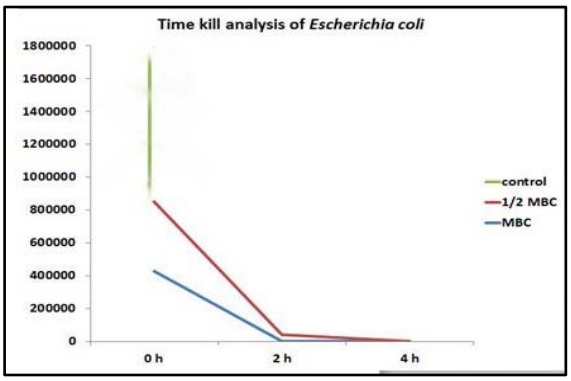
Test pathogens	No. of isolates	MBC of ampicillin	MBC of Ajwain extracts	Synergism:- MBC of ampicillin in presence of sub-lethal concentration of Ajwain extract.
ESBL Producers				
E.coli	10	> 10	1.5-5mg/ml	200µg/ml
Klebsiella pneumoniae	10	mg/ml	1.5-2.5mg/ml	300-400µg/ml
Citrobacter amalonaticus	3		1-2.5mg/ml	300-500µg/ml
Citrobacter diversus	7		1-2.5mg/ml	300-500µg/ml
Pseudomonas aeruginosa	10		1-10mg/ml	300-400µg/ml
Proteus mirabilis	5		1mg/ml	300-400µg/ml
Proteus vulgaris	5		1mg/ml	300-400µg/ml
MBL Producers				
E.coli strain 1	1	> 10	10mg/ml	200µg/ml
E.coli strain 2	1	mg/ml	10mg/ml	200µg/ml
E.coli strain 3	1		5mg/ml	200µg/ml
Klebsiella pneumoniae strain 1	1		1.5mg/ml	200µg/ml
Klebsiella pneumoniae strain 2	1		1.5mg/ml	200µg/ml
Citrobacter amalonaticus	1		1.5mg/ml	200µg/ml
Pseudomonas aeruginosa	1		2.5mg/ml	200µg/ml

Table 4: Components identified from ethanolic ajwain extract by GC-MS analysis

S. No.	Retention time	Compound
1	3.9	Cymene
2	4.7	Terpinene
3	5.5	Terpineol
4	10.1	Iso-thymol (carvacrol)
5	22.7	Emersol
6	22.8	Ethyl ester
7	27.9	Phenol 4methoxy 2, 3- 6 trimethyl

Time kill analysis

The time kill curves of 4 ESBL and 4 MBL producers are shown in figures 2a, 2b, 2c, 2d and 3a, 3b, 3c, 3d respectively. All isolates showed complete loss of viability within 2 h of growth at MBC concentration for both ESBL and MBL producers. A significant reduction in viability was observed at ½ MBC concentrations gradually over 4h time period analyzed.



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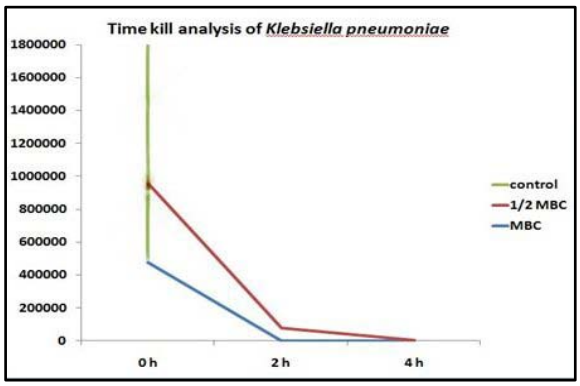

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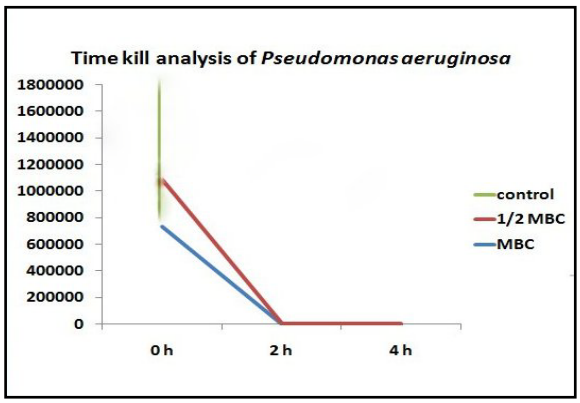

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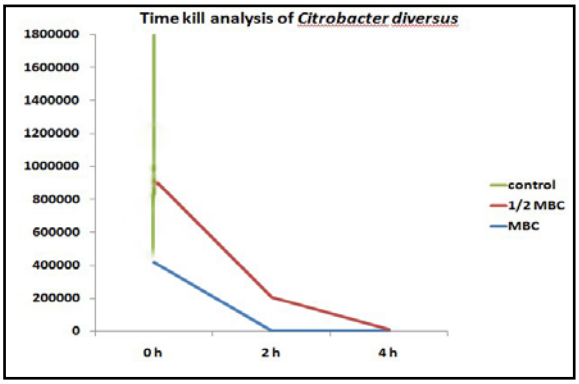
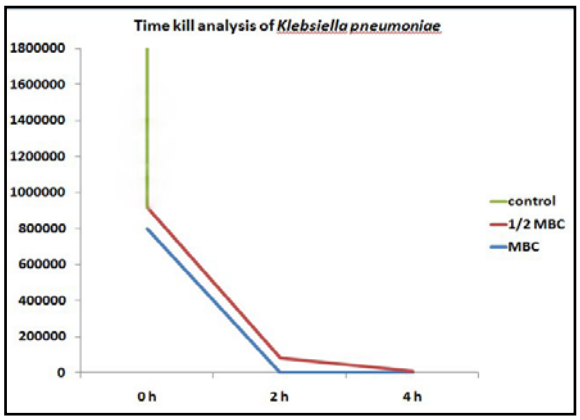


D

Fig. 2: Time kill curves of ESBL producing (a) *E.coli* (b) *K.pneumoniae* (c) *P.aeruginosa* and (d) *Citrobacter diversus* over 4h at MBC and ½ MBC.



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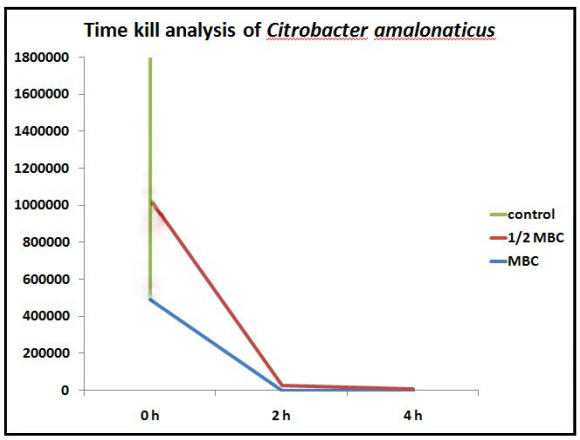

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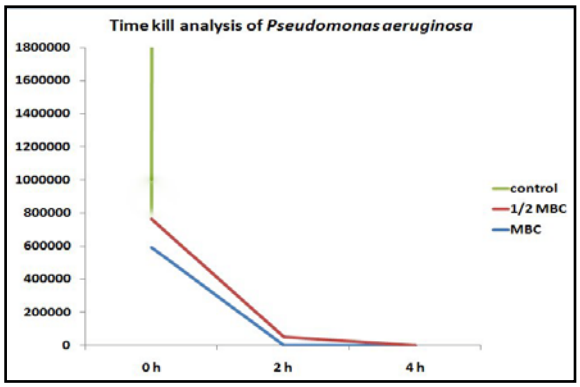

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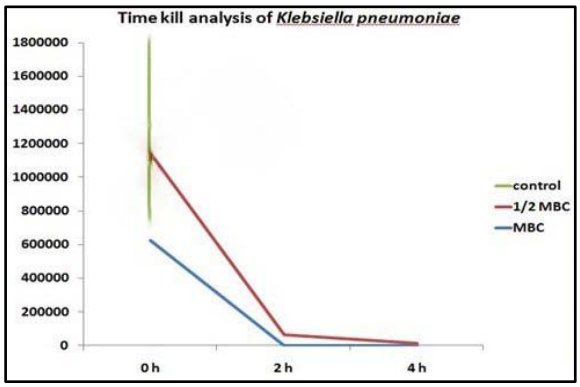

D

Fig. 3: Time kill curves of MBL producing (a) *K.pneumoniae* strain 1 (b) *Citrobacter* amalonaticus (c) *P.aeruginosa* and (d) *K.pneumoniae* strain 2 over 4h at MBC and ½ MBC.

DISCUSSION

Qualitative as well as quantitative assays of bacterial inhibition using several plant extracts have been carried out by various scientists [43, 44, 45, 46, 47]. However, most of the studies deal with the effect of the extract on general, non-pathogenic bacteria. Very little data is available on its benefits in treating pathogenic, drug-resistant bacteria [48, 49, 50]. Although the antibacterial and antifungal activity of ajwain is reported [51, 52, 53, 54, 55], the amount of data published about its effectiveness against drug resistant pathogens is very scanty [56]. To that extent, the present study was focused on the bactericidal as well as synergistic activity of ethanolic ajwain extracts with ampicillin. Qualitative antibacterial analysis of ajwain extracts was carried out by the well diffusion method.

The ajwain extracts showed prominent zones of inhibition of about 15-19mm in diameter against ESBL and MBL producing uropathogens. Several other antimicrobial studies of ajwain done on sensitive isolates have shown similar activities [57, 58]. It is known that herbal extracts provide similar activity against drug resistant as well as drug sensitive organisms [59, 60, 61, 62, 63, 64, 65]. This may be due to the difference in modes of action of various compounds present in the extracts, to which the organism was never exposed before and hence never had a chance to develop resistance. Another possibility is that the presence of multiple bioactive components in the crude extracts may exhibit synergistic activity collectively against the pathogens. Whereas the development of resistance against one single compound is comparatively easier, the development of resistance against combined action of multiple compounds is difficult.

Quantitative antibacterial analysis of ethanolic ajwain extracts, evaluated by determining MBC showed complete inhibition of the growth of ESBL and MBL producing pathogens at concentrations ranging from 1-10mg/ml. Since the extract used in the current study was prepared by simple crude extraction using a soxhlet apparatus, the potency of the extract can be increased by introducing modifications in the extraction system and choice of appropriate solvents.

More than 99% reduction in the MBC of ampicillin was observed in the presence of sub-lethal (1/2 MBC) concentration of ajwain extracts, hence indicating promising synergistic activity between ajwain extracts and ampicillin. Herbal medicines have always been a rich source of drug discovery programs. Many plant derived compounds have shown promising activity against MDR bacteria and have caused a reversal of antibiotic resistance due to its synergistic interaction with the drug (10, 12, 13, 48, 49]. Plant antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties individually, but when they are taken concurrently with the standard drug, they enhance the activity of the drug [48].

Several published data show synergistic activities between plant extracts and antibiotics [66, 67, 68]. To our knowledge, there is no published data that deals specifically with the synergistic activity of ajwain and ampicillin. Ampicillin remains the most important antibiotic to be used for common infections, the reason being its broad spectrum activity, low production cost and comparatively negligible side effects [69]. However, the development of resistance against beta-lactam antibiotics including ampicillin limits its use as a therapeutic agent. With the reversal of antibiotic resistance by ajwain extracts, ampicillin can be safely used even against drug-resistant pathogens. The use of antimicrobial agents displaying synergy is one of the well-established indications for combination antimicrobial therapy. Combinations of antimicrobials that demonstrate an *in vitro* synergistic effect against infecting strains are more likely to result in successful therapeutic result. In addition, combinations of agents that exhibit synergy or partial synergy could potentially improve the outcome for patients with difficult to treat infections [49]. Thus, evidence of in vitro synergism could be useful in selecting more favourable combinations of antimicrobials for the practical therapy of serious bacterial infections.

Our results revealed that the combined use of plant extracts and antibiotics could be useful in the treatment of infectious diseases, and in fighting the problem of emerging drug resistance. In-vivo experiments, however, are needed to confirm the phenomenon of synergy between drugs and plant extracts.

A time kill analysis of ethanolic ajwain extract against ESBL and MBL producing uropathogens showed a significant reduction in their viability over a 4h time period at ½ MBC concentrations. A complete loss of viability was observed within 2h of incubation of the pathogen at MBC concentrations. The growth curves of pathogens, grown in the presence of ajwain extracts, indicate that the activity of ajwain is not just bacteriostatic but also bactericidal. Thymol and carvacrol are considered to be the active antimicrobial components of ajwain. They act as either bactericidal or bacteriostatic depending on the concentration used [44].

Phytochemical analysis of ajwain extract by GC-MS showed the presence of carvacrol as its major constituent. The major component of ajwain is thymol followed by carvacrol [70, 71, 72]. However thymol was not detected in the extract as shown in Table 4. This may be due its fragmentation in the harsh program conditions of GC-MS. Another reason could be the possible obscuring of the thymol peak in comparison with carvacrol in the chromatogram. In any case, the bactericidal activity of ajwain and synergistic activity between ajwain and ampicillin can be attributed to carvacrol and other phytochemicals present in minor concentrations in ajwain.

The ethanolic extracts of ajwain showed significant activity against ESBL and MBL producing uropathogens. Further, its potency can be evaluated against other pathogenic drug resistant bacteria causing other infections. Moreover, the ability of crude extracts to give such promising antibacterial results only enhance the chances of it becoming an important alternate remedy towards the treatment of serious infections.

CONCLUSION

In the present study, the effectiveness of ethanolic ajwain extracts was investigated against ESBL and MBL producing pathogens. These results confirm the potential of ajwain to be used alone and in synergy with ampicillin against ESBL and MBL producing pathogens.

REFERENCES

1. Naber KG, Schito G, Botto H, Palou J, Mazzei T. Surveillance study in Europe and Brazil on clinical aspects and Antimicrobial Resistance Epidemiology in Females with Cystitis (ARESC): implications for empiric therapy. European urology 2008;54(5):1164-75.
2. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. The American journal of medicine 2002;113 Suppl 1A:14S-9S.
3. Saint S, Kowalski CP, Kaufman SR, Hofer TP, Kauffman CA, Olmsted RN, et al. Preventing hospital-acquired urinary tract infection in the United States: a national study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2008;46(2):243-50.
4. Singhal A, Sharma R, Jain M, Vyas L. Hospital and Community Isolates of Uropathogens and their Antibiotic Sensitivity Pattern from a Tertiary Care Hospital in North West India. Annals of medical and health sciences research 2014;4(1):51-6.
5. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrobial agents and chemotherapy 1995;39(6):1211-33.
6. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrobial agents and chemotherapy 2009;53(12):5046-54.
7. Laurent P, Jose M, Nashwan N, Yvette J, Debets O. Rodríguez M, Characterization of DIM-1, an Integron Encoded Metallo-β-Lactamase from Pseudomonas stutzeri clinical Isolate. Antimicrob Agents Chemother 2010;54:2420-4.
8. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clinical microbiology reviews 2007;20(3):440-58, table of contents.
9. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Archives of medical research 2005;36(6):697-705.
10. Shriram V, Jahagirdar S, Latha C, Kumar V, Dhakephalkar P, Rojatkar S, et al. Antibacterial & antiplasmid activities of Helicteres isora L. The Indian journal of medical research 2010;132:94-9.
11. Suchita S, Kamat SD, Kamat DV, J. Effect of aqueous extract of Terminalia chebula on Metallo beta-lactamase. Int Pharm Sci 2010;2:172-5.
12. Belofsky G, Percivill D, Lewis K, Tegos GP, Ekart J. Phenolic metabolites of Dalea versicolor that enhance antibiotic activity against model pathogenic bacteria. Journal of natural products 2004;67(3):481-4.
13. Beg AZ, Ahmad I, J. Effect of Plumbago zeylanica extract and certain curing agents on multidrug resistance bacteria of clinical origin. World Biot 2004;16:841-4.
14. Elias A, Ziad D, Abdei MR, Int J. Leaf and branch extracts of Eriobotrya japonica exert antibacterial activity against ESBL producing Escherichia coli and Klebsiella pneumonia. phytomed 2011;3:120-8.
15. Hashemi A, Shams S, Barati M, Samedani A. Antibacterial effects of methanolic extracts of Zataria multiflora, Myrtus communis and Peganum harmala on Pseudomonas aeruginosa producing ESBL. Arak University of Medical Sciences Journal 2011;14:104-12.
16. Kumar V, Shriram V, Mulla J, J. Antibiotic resistance reversal of multiple drug resistant bacteria using Piper longum fruit extract. Pharm Sci 2013;3:112-6.
17. Shriram V, Kumar V, Mulla J, Latha C. Curing of Plasmid-Mediated Antibiotic Resistance in Multi-Drug Resistant Pathogens using Alpinia galanga Rhizome Extract. Adv Bio Tech 2013;13:01-5.
18. Gibbons S, Oluwatuyi M, Veitch NC, Gray AI. Bacterial resistance modifying agents from Lycopus europaeus. Phytochemistry 2003;62(1):83-7.
19. Hebshi AN, Haroni AM, Skaug N. In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. Arch Oral Biol 2006;51:183-8.
20. Dey D, Debnath S, Hazra S, Ghosh S, Ray R, Hazra B. Pomegranate pericarp extract enhances the antibacterial activity of ciprofloxacin against extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing Gram-negative bacilli. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 2012;50(12):4302-9.
21. Jeet K, Devi N, Thakur N, Tomar S, Shalta L, Thakur R. *Trachyspermum ammi*: A comprehensive review. Int Res J Pharm 2012;3:133-138.
22. Katasani D, Srinu B, Bala R. Phytochemical screening, quantitative estimation of total Phenolic, flavanoids and antimicrobial evaluation of Trachyspermum ammi. Journal of Atoms and Molecules 2011;1:1-8.
23. Singh DB, Singh SP, Gupta RC, T. Anti-fungal effect of volatiles from seeds of some Umbelliferae. Mycol Soc 1979;73:349-50.
24. Choudhury S, Riyazuddin A, Kanjilal PA, B. P, J. Composition of the seed oil of Trachyspermum ammi (L.) Sprague from Northeast India. Oil Res 1998;10:588-90.
25. Iqbal Z, Lateef M, Khan MN, Jabbar A, Akhtar MS. Anthelmintic activity of Swertia chirata against gastrointestinal nematodes of sheep. Fitoterapia 2006;77(6):463-5.
26. Javed I, Iqbal Z, Rahman ZU, Khan FH, Muhammad F, Aslam B, et al. Comparative anti-hyperlipidaemic efficacy of Trachyspermum ammi-extracts in albino rabbits. Pak 2006;26:23-9.
27. Thangam C, Dhananjayan R, J. Anti-inflammatory potential of the seeds of Carum copticum Linn. Indian 2003;35:388-91.
28. Chialva F, Monguzzi F, Manitto P, J. Akgül A. Essential oil constituents of Trachyspermum copticum L Link fruits Oil Res 1993;5:105-6.
29. Chialva F, Monguzzi F, Manitto P, Akgül A. Essential oil constituents of *Trachyspermum copticum* (L.) Link fruits. J Essent Oil Res 1993;5:105-106.
30. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. Journal of ethnopharmacology 2000;71(1-2):23-43.
31. Kaur M, Kaur J, Ojha S, Mahmood A. Ethanol effects on lipid peroxidation and glutathione-mediated defense in rat small intestine: role of dietary fats. Alcohol (Fayetteville, N.Y.) 1998;15(1):65-9.
32. Boskabady MH, Jandaghi P, Kiani S, Hasanzadeh L. Antitussive effect of Carum copticum in guinea pigs. Journal of ethnopharmacology 2005;97(1):79-82.
33. Ahsan SK, Shah AH, Tanira MM, Ahmad MS, Tariq M, Ageel AM. Studies on some herbal drugs used against kidney stones in Saudi folk medicine. Fitoterapia 1990;61:435-58.
34. Aeschbach R, Löliger J, Scott BC, Murcia A, Butler J, Halliwell B, et al. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 1994;32(1):31-6.
35. Bairwa R, Sodha RS, Rajawat BS. Trachyspermum ammi. Pharmacognosy reviews 2012;6(11):56-60.
36. Indian Spices-Ajwain2013.
37. Aruna K, Tariq M. Prevalance of extended spectrum beta-lactamase production among uropathogens in south Mumbai and its antibiogram pattern. EXCLI 2012;11:363-72.
38. Sule LO, Agbabiaka TO. Antibacterial effect of some plant extracts on selected Enterobacteriaceae. Ethno botanical leaflets 2008;12:1035-42.
39. Toda M, Okubo S, Hiyoshi R, Shimamura T. The bactericidal activity of tea and coffee. Lett Appl Microbiol 1989;8:123-125.
40. Clinical and Laboratory Standards Institute (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow

aerobically-seventh edition: approved standard M07-A7. CLSI, Wayne, PA.
41. Carson CF, Mee BJ, Riley TV. Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob Agents Chem 2000;46:1914-20.
42. Indian Pharmacopoeia 5th ed2007.
43. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). Journal of applied microbiology 2000;88(1):170-5.
44. Caccioni DLR, Guizzardi M, Biondi DM, J. Relationships between volatile components of citrus fruit essential oil and antimicrobial action on Penicillium digitatum and Penicillium italicum. Int Microbiol 2000;88:170-5.
45. Tassou CC, Koutsoumanis K, Nychas GJ. Inhibition of Salmonella enteridis and Staphylococcus aureus on nutrient broth by mint essential oil. Food Res Int 2000;48:273-80.
46. Toda M, Okubo S, Hiyoshi R, Shimamura T. The bactericidal activity of tea and coffee. Lett Appl Microbiol 1989;8:123-5.
47. Pednekar PP, Vakil B V, Sane RT, Datar AG. Antimicrobial activity of essential oil of *Blumea eriantha DC* against skin pathogens. Int J Pharm Pharm Sci 2012;4:296-299.
48. Marquez B, Neuville L, Moreau NJ, Genet J-P, dos Santos AF, Caño de Andrade MC, et al. Multidrug resistance reversal agent from Jatropha elliptica. Phytochemistry 2005;66(15):1804-11.
49. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, et al. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules (Basel, Switzerland) 2009;14(2):586-97.
50. Shriram V, Jahagirdar S, Latha C, Kumar V, Puranik V, Rojatkar S, et al. A potential plasmid-curing agent, 8-epidiosbulbin E acetate, from Dioscorea bulbifera L. against multidrug-resistant bacteria. International journal of antimicrobial agents 2008;32(5):405-10.
51. Dwivedi SK, Singh KP, Frag J. Fungi toxicity of some higher plant products against Macrophmina phascolina (Tassi) Gold. Flavour. American journal of therapeutics 1998;13:397-9.
52. Singh G, Maurya S, Catalan C, De Lampasona MP. Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. Journal of agricultural and food chemistry 2004;52(11):3292-6.
53. Syed M, Sabir AW, Chaudhary FM, Bhatty MK, J. Antimicrobial activity of essential oils of umbelliferae part II-Trachyspermum ammi, Daucus carota, Anethum graveolens and Apium graveolens. Pak Ind Res 1986;28:189-92.
54. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC complementary and alternative medicine 2009;9:30.
55. Masih U, Shrimali R, Naqvi S. Antibacterial Activity of Acetone and Ethanol Extracts of Cinnamon (Cinnamomum zeylanicum) and Ajowan (Trachyspermum ammi) on four Food Spoilage Bacteria. International Research Journal of Biological Sciences 2012;1:7-11.
56. Rosina K, Mohammad Z, Sadul HA, Abdul L, J. AsadK. Activity of solvent extracts of Prosopis spicigera, Zingiber officinale and Trachyspermum ammi against multidrug resistant bacterial and fungal strains. Dev Ctries 2010;4:292-300.
57. Vivek KS, Deepali S, Deepti P, Archana S, J. Dermatophytes and related keratinophilic fungi isolated from the soil in Gwalior region of India and in vitro evaluation of antifungal activity of the selected plant extracts against these fungi. Plant Res 2013;7:2136-9.
58. Savita A, Sanjay G, J. In Vitro Antimicrobial Studies of Trychyspermum ammi. Int Bio Sci 2012;3:64-8.
59. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant Salmonella typhi. Phytotherapy research : PTR 2004;18(8):670-3.
60. Parekh J, Chanda S, J. In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr Res 2007;1:92-9.
61. Tambekar DH, Khante BS, Dahikar SB, Banginwar YS, J. Antibacterial properties of contents of Triphala: A traditional Indian herbal preparation. Continental 2007;1:8-12.
62. Shafiqur M, Anwar MN, J. Rahman, Antimicrobial activity of crude extract obtained from the root of Plumbago zeylanica Bangladesh. American journal of therapeutics 2007;24:73-5.
63. Mazumder R, Bhattacharya S, Mazumder A, Pattnaik AK, Tiwary PM, Chaudhary S. Antidiarrhoeal evaluation of Aegle Marmelos (Correa) Linn. root extract. Phytotherapy research : PTR 2006;20(1):82-4.
64. Rahman MU, Gul S, Ejaz AO, J. Antimicrobial activities of Ferula assafoetida oil against Gram positive and Gram negative bacteria. Env Sci 2008;4:203-6.
65. Bhatnagar SS, Santapau H, Desa JD, Mamar AC, Ghadielly NC, Solomon MJ, et al. Biological Activity of Indian Medicinal Plants: Anti-bacterial, anti-tubercular, and anti-fungal action. Indian Res 1961;49:799-813.
66. Yang Z-C, Wang B-C, Yang X-S, Wang Q, Ran L. The synergistic activity of antibiotics combined with eight traditional Chinese medicines against two different strains of Staphylococcus aureus. Colloids and surfaces. B, Biointerfaces 2005;41(2-3):79-81.
67. Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes Junior A. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memorias do Instituto Oswaldo Cruz 2006;101(4):387-90.
68. Freitas E, Aires A, de Santos Rosa EA, Saavedra MJ. Antibacterial activity and synergistic effect between watercress extracts, 2-phenylethyl isothiocyanate and antibiotics against 11 isolates of Escherichia coli from clinical and animal source. Letters in applied microbiology 2013;57(4):266-73.
69. Timothy J, Vicky D, J. Management of uncomplicated urinary tract infections. West 2002;176:51-5.
70. Seo S-M, Park H-M, Park I-K. Larvicidal activity of ajowan (Trachyspermum ammi) and Peru balsam (Myroxylon pereira) oils and blends of their constituents against mosquito, Aedes aegypti , acute toxicity on water flea, Daphnia magna , and aqueous residue. Journal of agricultural and food chemistry 2012;60(23):5909-14.
71. Zarshenas MM, Samani SM, Petramfar P, Moein M. Analysis of the essential oil components from different Carum copticum L. samples from Iran. Pharmacognosy research 2014;6(1):62-6.
72. Rafiul H, Ansari S, Abul KN, Kamran JN, Hptlc, J. Validated Method For Quantification of Thymol Content In Trachyspermum ammi and Poly herbal Unani Formulation Arq Zeera. Int Pharm Sci 2012;4:478-82.