Study on synergistic action of Coriandrum sativum seed extracts on antibiotics against multidrug resistant P. aeruginosa

Bezalwar Pratik M.¹ and Charde Vijay N.²

Received: 01.07.2019   Revised: 28.08.2019   Accepted: 19.10.2019

Abstract

Increase in resistance of antibiotic against P. aeruginosa, the antibiotic treatment therapy fail to exterminate the infection. The approach of new strategy is must to deal with multidrug resistant P. aeruginosa. In the present study total 300 isolates from different clinical samples (Urine, Blood, Sputum, and Pus) were studied for isolation, identification and analysis of resistance pattern against 22 antibiotics. Total 5 most resistant isolates (U004, P017, P078, U105, and U237) were screened to search for synergistic approach of Coriandrum sativum extract prepared by both Soxhlet method and Maceration process in different solvents; petroleum ether, chloroform, acetone, methanol and water. Encouraging results were obtained where HDME extract could synergise the activity of total 8 antibiotics that are; MZ, CPZ, CTX, PB, LE, DOR, AZ and CAZ. Thus the present study provides and landmark approach to deal with multidrug resistant P. aeruginosa by synergistic action of antibiotic and Coriandrum sativum extract.

Key Words: Coriandrum sativum, P. aeruginosa, Multidrug resistant, Synergistic action, Antibiotics, Solvents

Introduction

Mortality rate is still significantly high worldwide despite of development of synthetic antibiotics. Emergence of antimicrobial resistance is a main problem in the treatment of infectious diseases (Russell, 2003). Therefore, a quest for new and sustainable antibiotics or other treatment therapy is a necessity. Many researches have proved substantial information on antibacterial activity of natural products on resistant microbial strains (Bakkali et al., 2008; Miladinović et al., 2012). Tremendous increase in antibiotic resistance due to ESBL production in gram negative bacteria created a problem (Duman, et al., 2010; Duman, et al., 2014; Toner, et al., 2016). Development of Resistance in P. aeruginosa is by spontaneous mutations process or by plasmids acquisition (extrachromosomal DNA) having resistance genes (Livermore, 2002). Efflux pumps and beta-lactamases are common mechanisms of resistance detected in P. aeruginosa (Singh et al., 2000). Nosocomial outbreaks and failure of treatment by cephalosporins is due to plasmid-mediated AmpC enzymes in enteric bacteria (Pai et al., 2004). Enzymatic degradation of antibiotics causes resistance to extended-spectrum β-lactam antibiotics in Enterobacteriaceae (Mylvaganam et al., 2017). The distinctive attribute of P. aeruginosa is that it has low permeability of the cell wall, produce cephalosporinases, have active efflux pumps and have low affinity for DNAgyrase (Al-Tawfiq, 2007). P. aeruginosa is resistant for β lactams antibiotics, Fluoroquinolones, and carbapenem (Marilee, 2005). Coriandrum sativum (Coriander) is an annual herb that belongs to Apiaceae family. The medicinal properties of Fructus Coriandri are practice for the treatment of carminative, dyspeptic action loss of appetite and digestive problems (Baytop, 1999). Losing of antibiotic potential as antimicrobial agents due to drug resistance shifted the interest of researchers use plant derived products that increases in the use of plant extracts to practice it as conventional medicine for antibacterial effects. Coriander shown to have antibacterial effects against various organism studied (Laribi et al., 2015; Jiang et al., 2015). Plantaricin CS is a novel antimicrobial peptide in Coriander leaf extract and reported to have significant antibacterial activity against gram negative bacteria. The phytochemical such as the essential oils (EOs) of plants displayed

Author’s Address

¹Department of Microbiology, Chintamani College of Arts and Science, Gondpipri. Dist- Chandrapur (MS), India-442702
²Department of Microbiology, Taywade College, Mahadula-Koradi, Dist- Nagpur (MS), India- 441 111.
E-mail.: pratikmbezalwar@gmail.com
synergistic antibacterial action with antibiotics (Zare-Zardini et al., 20012; Zare-Shehneh et al., 2014). The use of plant extracts with conventional antibiotics in combination provides affordable treatment therapy even against drug-resistant bacterial infections (Majee et al., 2018). Plants combinations with antimicrobial agents have confirmed its efficiency as synergistic potential during the last decade (Balouiri et al., 2016, Hemaiswarya et al., 2008). The Petroleum Ether Coriander leaf extract potentiate cefoxitin synergism but it expresses less synergism than Methanol Coriander leaf extract on cefoxitin combination against gram negative organism (Nilay et al., 2018). Strategy of synergism could frame potential therapeutic choices for the treatment of resistant pathogens (Musumeci et al., 2003; Duarte et al., 2012). The mechanisms adopted by synergism of drug and extract combinations includes, common biochemical pathway inhibition, degradation of enzymes, cell wall-active agents (Zhang et al., 2011, Eliopoulos and Moellering, 1991).

The present study deals with evaluation of synergistic activity of Coriandrum sativum extract indifferent solvents with resistant antibiotics against P. aeruginosa. Thus, this study will provide promising approach to deal with multidrug resistant P. aeruginosa.

Materials and Method

Collection of clinical Samples: Clinical samples of urine (U), pus (P), blood (B) and sputum (S) were collected aseptically from different pathology laboratories of Nagpur (MS), India.

Isolation of P. aeruginosa:
The sterile cotton swab was dipped in samples and that was then released in sterile Tryptone Soy Broth. The inoculated Tryptone Soy Broth is incubated at 37°C for 48 hrs. Loopful of culture from enriched Tryptone Soy Broth was streaked on Pseudomonas isolation agar and incubated at 37°C for 48 hrs. After incubation P. aeruginosa shows luxurious growth with green pigmentation. These typical colonies on selective media were maintained and used for further study.

Microscopic examination of P. aeruginosa:
The isolated colonies were subjected to standard Gram staining procedure was used for finding Gram character. After gram staining dry slides were focused on microscope.

Biochemical identification of P. aeruginosa:
Isolates were identified by biochemical characteristics for Indole test, Methyl red test, Catalase test, Citrate utilization test, Urease test, Oxidase test, Triple sugar iron test, and pigment production and the results were compared with Bergey’s Manual of Determinative Bacteriology 9th edition as well as confirmed by biochemical identification using Vitek 2 System.

Preparation of Inoculums:
The inoculum was prepared according to 0.5 McFarland standards which correspond to size of 1.5×10^8 CFU/ml.

Antimicrobial susceptibility testing:
Antimicrobial susceptibility testing was performed by the disc diffusion method with commercially available discs (HiMedia, Mumbai, India) of; Amikacin (AK) 30 mcg, Gatifloxacine (GAT) 5 mcg, Doripenem (DOR) 10 mcg, Tobramycin (TOB) 10 mcg, Aztreonam (AT) 30 mcg, Polymyxin-B (PB) 300 units, Cefoperazone (CPZ) 75 mcg, Levofloxacin (LE) 5 mcg, Cefotaxime (CTX) 30 mcg, Gentamycin (GEN) 10 mcg, Piperacillin/Tozobactam (PIT) 10 mcg, Ticarcillin (TI) 75 mcg, Carbencillin (CB), Norfloxacin (NX) 10 mcg, Cefotaxime (CTR) 30 mcg, Imepenem (IPM) 10 mcg, Netilamin (NET) 30 mcg, Azlocillin (AZ) 75 mcg, Meropenem (MRP) 10 mcg, and Ceftizoxime (CZX) 30 mcg, Ceftazidine (CAZ) 30 mcg, Mezlocillin (MZ) 75 mcg

100 µl of broth culture was seeded over previously sterilised and solidified Hi sensitivity test agar plates and antibiotic discs were placed over it. The plates are then shifted to incubator at 37ºC for 18-24hrs. After completion of incubation period, zones were matched with standard CLSI recommendation (CLSI 2007).

Collection of Plant material:
Coriandrum sativum seeds were collected from spice shop of Nagpur (MS), India and authenticated by Dr. Mrs. Suvarna. P. Patil, Assistant Professor and Head, department of Botany, Taywade College, Koradi, Nagpur. The plant material was cleaned by traditional method, pulverised with mortar and pestle and filled in amber coloured bottle and stored in refrigerator.
Preparation of Herb Extracts:
For preparation of extracts solvent were used in a sequence with increasing order of polarity; petroleum ether (60 °C- 80°C), chloroform, acetone, methanol and then water.

Hot Extract (Soxhlet Extract):
25 g of powdered herb was filled in Soxhlet thimble and refluxed with 250 ml of solvents for 24 hrs.

Cold Extract (Maceration Process):
25 g of powdered herb loosely tied in nylon mesh and immersed in 100 ml of solvent in conical flasks. The conical flasks is plugged with cotton and wrapped with aluminium foil and placed in orbital shaking incubator for 24 hrs adjusted at 25°C under 150 rpm (Charde et. al., 2014).

The extracts were concentrated up to 50 ml in volumetric flask by recovering extra solvent by Rotary Vacuum Evaporator under reduced pressure. The *Coriandrum sativum* (Regional name, Hindi-Dhania) extract were coded as, Hot Dhania Acetone extract (HDAT), Cold Dhania Acetone extract (CDAT), Hot Dhania Methanol extract (HDME), Cold Dhania Methanol extract (CDME), Hot Dhania Chloroform extract (HDCF), Cold Dhania Chloroform extract (CDCF), Hot Dhania Water extract (HDW), Cold Dhania Water extract (CDW), Hot Dhania Petroleum ether extract (HDPE) and Cold Dhania Petroleum ether extract (CDPE).

Testing of Synergistic Activity:
Hi-sensitivity test agar medium and empty petri plates were sterilised separately. 100 μl of test extract was added in empty petri plates and then 15ml of sterilized molten Hi-sensitivity test agar is added. The plates were rotated for few seconds for even mixing of medium with extract. After solidification of medium, 100 μl of inoculum was spread with L-spreader. Then, antibiotic discs were placed. The plates were incubated at 37°C for 18-24hrs. After incubation results were noted (Charde et al., 2014).

Results and Discussion
Total 300 isolates were screened from different clinical samples. Each isolates were subjected to identification by cultural, biochemical and morphological analysis. On Pseudomonas Isolation Agar the isolates observed to have luxurious growth with green pigmentation. The results of biochemical test are shown in Table 1.

Table 1: Biochemical test for *P. aeruginosa* isolates.

| Indole test | Methyl red test | Citrate utilization test | Catalase test | Oxidase test | Urease test | Triple sugar iron test | Pigment production |
|-------------|----------------|-------------------------|---------------|------------|-----------|-----------------------|------------------|
| Negative    | Negative       | Positive                | Positive      | Positive   | Negative  | K/K or K/- No H2S     | Yes (Green)      |

The suspected isolates on the basis of cultural and biochemical identification was subjected to Gram staining for morphological identification. The Gram staining results are shown in Fig.1.

The isolates; U004, P017, P078, U105, and U237 were selected on the basis of resistance to maximum and diverse antibiotics. Results of antibiotic sensitivity show the isolates were resistant to CTX, DOR, PB, AZ, GEN, LE, MZ, CPZ, and CAZ. The Soxhlet Extract of *Coriandrum sativum* in methanol (HDME) shows potential synergistic activity on MZ, CPZ, CTX, PB, LE, DOR, AZ, CAZ but not on GEN. The overall results of shift of antibiotic sensitivity pattern are given in Table 2.

The extract HDME shows the prominent synergistic activity. This extract was then preceded for the GC-MS analysis. In result it shows various phytochemicals. Chromatogram of HDME is shown in Fig. 2.
Table 2: Combined action of Antibiotics and Extracts

| Antibiotics | MZ | CPZ | CTX | PB | LE | DOR | AZ | CAZ | GEN |
|-------------|----|----|----|----|----|-----|----|-----|-----|
| Isolate AS | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| HDAT        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| CDAT        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| HDME        | 18s | 23s | 24s | 15s | 19s | 21s | 20s| 22s | 11r |
| CDME        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| HDCF        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| CDCF        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| HDW         | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| CDW         | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| HDPE        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |
| CDPE        | 12r | 14r | 12r | 11r | 13r | 15r | 11r| 13r | 11r |

Where: Isolate AS = Isolate Antibiotic Sensitivity.

Fig.2: Chromatogram Generated of HDME Extract In GCMS Analysis.

Table 3: Peak Report of GCMS Analysis of HDME Extract.

| Peak Report TIC |
|-----------------|
| Peak# | R.Time | L.Time | F.Time | Area% | Height% | A/H | Mark | Name |
|-------|--------|--------|--------|-------|---------|-----|------|------|
| 1     | 5.194  | 5.150  | 5.250  | 1.59  | 3.04    | 2.20| 2-PENTANOINE, 4-METHOXY-4-METHYL BUTANE, 1,1-DIETHOXY-3-METHYL | |
| 2     | 5.904  | 5.767  | 5.892  | 1.48  | 2.98    | 2.09| Guanosine | |
| 3     | 5.987  | 5.950  | 6.050  | 0.91  | 1.82    | 2.10| 1-BUTANOL, 3-METHYL | |
| 4     | 8.284  | 8.200  | 8.308  | 0.35  | 0.40    | 3.70| DECANOIC ACID, 8-METHYL- | |
| 5     | 19.192 | 19.150 | 19.267 | 1.41  | 2.15    | 2.75| PENTENITRILE, 3-HYDOXYMETHYL | |
| 6     | 19.592 | 19.575 | 19.842 | 1.44  | 0.24    | 25.62| NONACETIC ACID, ETHYL ESTER | |
| 7     | 19.886 | 19.842 | 19.967 | 1.76  | 2.40    | 3.08| 9-DECAN-3-ONE, 2,2,6,6-TETRAMETHYL | |
| 8     | 20.858 | 20.817 | 20.950 | 0.86  | 0.74    | 4.89| 7-Hexdecenoic acid, methyl ester, (Z)- | |
| 9     | 21.042 | 20.950 | 21.383 | 41.01 | 46.73   | 3.69| NITROSO DIMETHYLAMINE | |
| 10    | 21.276 | 21.533 | 21.308 | 0.40  | 0.61    | 2.73| 2,2'-BIPYRIDINE, 3,5-DICHLORACET | |
| 11    | 21.569 | 21.517 | 21.583 | 0.51  | 1.08    | 1.99| 1H,1,2,4-Triazole-3-carboxylic acid | |
| 12    | 21.617 | 21.583 | 21.642 | 0.82  | 1.19    | 2.88| 2-DEOXYURIDINE, 3',5'-DICHLORACET | |
| 13    | 21.766 | 21.642 | 22.733 | 44.19 | 33.94   | 5.47| Ethyl 9-hexadecenoate S9-9-Hexadecenoic acid | |
| 14    | 22.058 | 21.950 | 22.083 | 0.63  | 0.57    | 4.67| TV, 2.2'-BIPYRIDINE, 3,5-DICHLORACET | |
| 15    | 22.183 | 22.092 | 22.292 | 0.38  | 0.10    | 15.42| FURU(3,4-BIFURAN-2,6-DIHYDROFURAN | |
| 16    | 22.311 | 22.392 | 22.492 | 0.42  | 0.31    | 5.65| 4-[4-(6-HYDROXYCARBONYL)-TRANS-5-HYDROXYCARBONYL]-TRANS-5-HYDROXYCARBONYL | |
| 17    | 22.713 | 22.550 | 22.733 | 0.35  | 0.38    | 3.78| 2-BETA-VIPIROLEACTONE | |
| 18    | 25.335 | 25.250 | 25.358 | 0.34  | 0.40    | 3.51| (S)-1-PHENYL-1-NONANOL | |
| 19    | 28.761 | 28.692 | 28.792 | 0.50  | 0.56    | 3.79| Phthalic acid, 4-cyanophenyl ester | |
| 20    | 29.872 | 29.783 | 29.983 | 0.64  | 0.36    | 7.39| 2,2'-BIS(N-TERT-BUTYLPHENYL)-1,1-DIPHENYL | |

The chromatogram generated by GCMS analysis is used to identify the compounds present in the extract subjected for presence of phytochemicals by analysing Peak time, Area% and Height% to...
From the overall results obtained and report generated it can be undertake that the specific phytochemicals extracted in methanol can act on component to develop synergistic action of antibiotics and phytochemicals. Very few studies are conducted on synergistic action of extracts of Coriandrum sativum and antibiotics against P. aeruginosa. P. aeruginosa detected to have highest sensitivity to piperacillin/tazobactam followed by meropenem, ciprofloxacin, cefepime, amikacin, and gentamicin (Abdul et al., 2017). P. aeruginosa is prevalent pathogen in immune compromised patient. Experimental evidences proved its prevalence up to 60% and 12.7 % in hospital settings (Masomeh, et al., 2013; Lutfusavas et al., 2005) The combination of chloramphenicol and thyme oil exhibited more pronounced action on P. aeruginosa than their individual activity. Coriander essential oil and linalool has shown have synergistic interactions on oxacillin, amoxicillin, gentamicin, ciprofloxacin, tetracycline against Gram-negative bacteria (Petruta et al., 2018). Silva et al., appraised coriander essential oil can damage membrane against Gram positive and Gram negative bacteria (Silva et al., 2011). The activity of norfloxacin is potentiated by essential oil of P. graveolens against B. cereus, and S. aureus (Rosato et al., 2007). The Methanolic extract of Coriandrum sativum showed better activity against the most tested organisms Pseudomonas aeruginosa. The combinations of chloramphenicol with coriander oil have synergistic effect against A. baumannii less potentiation activity is observed with tetracycline, gentamicin or ciprofloxacin (Duarte et al., 2012). The active component, Geraniol of essential oil of H. italicum can synergise with chloramphenicol by targeting efflux mechanisms (Lorenzi et al., 2009). In another combination study on ketoconazole and P. graveolens essential oil also supposed to have synergism against T. schoenleinii and T. soudanense (Shin and Lim, 2004). The overall researches in study give evidence of synergistic action of Methanolic extract of Coriandrum sativum on antibiotics.

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