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In Vitro Management of MDR Pseudomonas Sp. By Combined Activity of Antibiotics and Foeniculum Vulgare Miller Extract

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Abstract: *Pseudomonasis widespread in nature, inhabiting soil, water, plants, and animals (including humans). Pseudomonas aeruginosa has become an important cause of infection, especially in patients with compromised host defense mechanisms. In the present study, total 106 Clinical samples were studied and 33 suspected isolates of Pseudomonas aeruginosa were identified on the basis of morphological, cultural & biochemical characteristics and all of them were found to be* of *Pseudomonas sp. Out of 33 Pseudomonas, 20(60%) isolates were associated with pus samples, 7 (21 %) with urinary tract infection (UTI), 5 (15 %) were associated with blood and 1 (3 %) with stool sample. Antibiogram study of these isolates revealed that all these isolates are resistant to many antibiotics out of 15 antibiotics tested. Total 5 MDR isolates of Pseudomonassp. Namely U004, P017, P039, P076 and U0105 were selected for further studies on the basis of their resistance to more than 60% antibiotics. Hot and cold extracts of solvents with increasing order of polarity from petroleum ether, chloroform, acetone, methanol and water were used for study. Combined effect of herbal extracts of Foeniculum vulgare Millerand antibiotics on susceptibility of Multidrug resistant (MDR) isolates showed promising effect although individual extracts were not effective against any isolates. Concept of this synergism provides a new thought of antibiotics and bioactive plant extracts in development combined antimicrobial therapy for effective management of MDR Pseudomonas isolates.*

Keywords: *Pseudomonas aeruginosa, Foeniculum vulgare Miller, Multidrug resistant.*

1. INTRODUCTION

In the past decade, study over phytochemicals witnessed the diverse application of herbs pharmacologically. Discoveries in herbal medicines have turned the interest of pharmaceutical industries to view it as reemerging health aid in a number of countries [1] as plants and their parts offer antimicrobial properties against microbial infections. This can be traced to the increasing cost of prescription drugs, for personal health care and developed antibiotic-resistant strains in the case of infectious diseases. Herbal spices being a source of phenolics, flavonoids, anthocyanins, carotenoids etc., imparts flavour and enhance the shelf-life of cuisine and processed food products. Essential oils, extracts and bioactive constituents of several spices and herbs are reported to exert antioxidant and anti- microbial activities [2].

Different parts of the plant have long being used as a potent phytomedicine in traditional as well as in different sectors of scientific medicinal practices like, Ayurved, Unani, Homoeopathy and Sidhha/Tamil types of alternative medicinal systems. A major problem encountered with antibiotics in clinical use is drug resistance, which mostly leads to treatment failure. Different classes of antibiotics have been introduced the wide use of antibiotics in the treatment of bacterial

infections and has led to the emergence and spread of resistant strains. Often used first-line antibiotics has lost clinical efficacy against previously susceptible pathogens and treatment of such pathogens shifted to second-line and third-line antibiotics that are often more expensive with numerous side effects and these pathogens has been nicknamed 'superbug' by clinicians because of its rapid adaptive resistance through several mechanisms. Past records over study of antibiotic resistance shows that resistant strains often appear a few years after the first clinical use of any antibiotics [2].

Natural medicine are known to cure cardiovascular disease, diabetes, dental conditions, erectile dysfunction, antibiotic resistant bacterial infections, skin diseases, wound, earache, leprosy, leucorrhea, hepatitis, snakebite, infant brain ischemia, male infertility, Alzheimer's disease, arthritis, asthma, epilepsy, fever, malaria, diarrhea, dysentery and ultraviolet radiation-induced skin damage. These medicines also provide many special health benefits which includes hepatoprotective, bronchovasodilatory, antiparasitic, antidiarrhoeal, larvicidal, an antioxidant, anticarcinogenic, pesticidal, antiplasmodial, antomalarial, antileishmanial, schizonticidal and anti-inflammatory properties, free radical scavenging, immunostimulating, molluscicidal. Other potential applications include analgesic, antipsychotic, wound healing, anti-asthmatic, expectorant, antiparasitic and phytotoxic properties and obesity [3, 4, 5, 6, 7].

Search of chemical constituents of the plants and advancement of pharmacological test have endowed the basis for progress in the development of new agents. The importance of conventional medicines in solving the best of health problem solutions is invaluable on a global pharmaceutical market [8]. Therefore it is necessary to limits the depletion of biogenetic resources in forests [9] and promote the farming of medicinally important plants. In industrialized countries, the extraction and development of many drugs, and chemotherapeutics from medicinal plants have been increasing [10]. In the survey of WHO, IHO, almost 70-80% populations living almost solely on conventional medicine for their basic health care needs and nearly 61% of drugs marketed worldwide can be outlined to natural products. Natural product always considered a first choice in the global market.

Synergistic interaction of phytomedicine with antibiotics can explain efficacy in dealing resistant pathogens. Abundance and cheaper value of herbal drug complements its use at large scale. Despite of increasing interest in medicinal plant research very few formulations are available in market worldwide as compared to chemical based chemotherapeutics. Development of drug resistance in *Pseudomonas* is worldwide and is real challenge to medical practitioners to deal with it [11].*Foeniculum vulgare* Miller,(common name 'Sauf' in Hindi, an Indian language) a plant belonging to the family *Apiaceae*, has a long history of herbal uses. Fennel seeds for its properties of anti-inflammatory, analgesic, carminative, diuretic and antispasmodic were used traditionally. Recently fennel seed extracts and essential oil is explored for its antioxidant potential and antimicrobial activities.

The current study involves the isolation of *Pseudomonas* from the patients suffering from the ailments like respiratory tract infection, UTI, from the patients who are hospitalized for long period, etc. and studying the antibiotic resistant pattern of these isolates and to investigate synergistic effect of *Foeniculum vulgare* Miller extracts on antibacterial efficacy of antibiotics often used to treat MDR pathogens.

2. MATERIAL AND METHODS

2.1. Collection of Clinical Samples

Clinical samples of urine, pus, blood and sputum sample were collected from different pathology laboratories of Nagpur (MS), India.

2.2. Isolation of Pseudomonassp

Collected sample was then immediately transferred to sterile nutrient broth for enrichment under aseptic condition and incubated at 37^0C for 48 hrs. Loopful of culture from enriched nutrient broth was plated on selective media, Pseudomonas isolation agar after 48 hours, so as to get well isolated colonies. Suspected colonies of *Pseudomonas* showing typical cultural characteristics on selective media were picked up and were maintained on nutrient agar slant for further identification.

2.3. Identification of Isolates

Isolates were identified on the basis of morphological, cultural & biochemical characteristics 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.

2.4. Preparation of Inoculums

A loopful of culture from slants was inoculated into the screw cap tube containing 5ml sterile nutrient broth and incubated at 37^0C for 24hrs. Again loopful of culture from same broth was transferred to fresh 5ml of sterile nutrient broth and incubated at 37^0C for 6-8 hrs. Turbidity was adjusted according to 0.5 McFarland standards which were then used as an inoculum which corresponds to size of 1.5×10^8 CFU/ml. This suspension was used as inoculums.

2.5. Antibiotic Susceptibility Test

Antimicrobial susceptibility testing was performed by the disc diffusion method with commercially available discs (HiMedia, Mumbai, India) of Tobramycin (TOB) 10 mcg, Gatifloxacine (GAT) 5 mcg, Gentamycin (GEN) 10 mcg, Doripenem (DOR) 10 mcg, Polymyxin-B (PB) 300 units, Cefoperazone (CPZ) 75 mcg, Amikacin (AK) 30 mcg, Levofloxacin (LE) 5 mcg, Ticarcillin (TI) 75 mcg, Cefotaxime (CTX) 30 mcg, Piperacillin/Tozobactam (PIT) 10 mcg, Ciprofloxacin (CIP) 5 mcg, Cefepime (CPM) 30 mcg, Mezlocillin (MZ) 75 mcg and Ceftizoxime (CZX) 30 mcg. Selected antibiotic discs placed over plates seeded with 100 □l broth culture (0.5 McFarland standards) over surface of Hi sensitivity test agar and plates were kept undisturbed in a refrigerator for 1 hour. Then plates were removed from refrigerator and shifted to incubator maintained at 37^0C for 18-24hrs. After incubation all plates were examined for zone of inhibition. Isolates were considered susceptible, intermediate, or resistant to a particular antimicrobial agent on the basis of the diameters of the inhibitory zones that matched the criteria of the manufacturer's interpretive table, which followed the recommendations of the performance standard for antimicrobial disk susceptibility test, CLSI (CLSI 2007) (formerly NCCLS) [12].

2.6. Antimicrobial Activity of Extracts

Antimicrobial activity of extract was tested by agar well diffusion method. 25 ml of Hi-sensitivity agar medium poured into sterilized petri plates and allowed it to solidify. 100 µl of *Pseudomonas* inoculum was inoculated over solidified agar medium and spread with the help of sterilized L-spreader. Wells were then cut with the help of sterilized cork borer (10 mm diameter) equidistantly. 100 µl of herb extract is then filled in a wells including control for each solvent. Plates were kept undisturbed in a refrigerator for 1 hour and then shifted to incubator maintained at 37^0C for 24hrs. Zone of inhibitions were measured after incubation.

2.7. Collection and Maintenance of Plant Material

The spice, Seeds of *Foeniculum vulgare* Miller (Sauf) were purchased from local market of Nagpur (MS). The seeds were dried under shade, pulverized with hand mortar and pestle, filled in air tight bottle and stored in refrigerator till its use.

2.8. Preparation of Herb Extracts

2.8.1. Hot Extract (Soxhlet Extract)

25 g of powdered herb were filled in Soxhlet thimble and refluxed in Soxhlet apparatus with 250 ml of solvent for 24 hrs. Solvent were used in a sequence with increasing order of polarity of the solvent from petroleum ether (60^0C- 80^0C), chloroform, acetone, methanol and then water. Before every extraction, material inside the thimble was dried in open air to evaporate previous solvent. Solvent is recovered after 24 hrs. Still approximately 55 -60ml of extract remained in round bottom flask. The solvent was then evaporated at appropriate temperature until a very concentrated extract (less than 50 ml) was obtained and final volume was made up to 50 ml in volumetric flask.

2.8.2. Cold Extract

25 g of powdered herb were tide loosely in nylon cloth (400 mesh) and immersed in 100 ml of solvent in 250 ml stoppered conical flask and kept at 25^0C under 150 rpm in orbital shaking

incubator for 24 hrs. Successive extraction of same powdered material was carried out in selected series of solvents as used in hot extraction. Before every extraction, material was exposed to air to evaporate preceding solvent absorbed in material. After 24 hrs.solvent is evaporated at room temperature to obtain final volume of 50 ml [13].

Total 10 extracts were prepared and they were abbreviated and hereafter referred by their abbreviation. They are Hot *Foeniculum vulgare* Acetone extract (HFAT), Cold *Foeniculum vulgare* Acetone extract (CFAT), Hot *Foeniculum vulgare* Methanol extract (HFME), Cold *Foeniculum vulgare* Methanol extract (CFME), Hot *Foeniculum vulgare* Chloroform extract (HFCF), Cold *Foeniculum vulgare* Chloroform extract (CFCF), Hot *Foeniculum vulgare* Water extract (HFW), Cold *Foeniculum vulgare* Water extract (CFW), Hot *Foeniculum vulgare* Petroleum ether 60^0C- 80^0C extract (HSPE) and Cold *Foeniculum vulgare* Petroleum ether 40^0C-60^0C extract (CFPE).

Cold and hot concentrated extracts were prepared in sufficient volume (50 ml) and preserved at 4^0C in sealed vials for further use to avoid batch to batch variations.

2.9. Sterility Testing of Plant Extracts

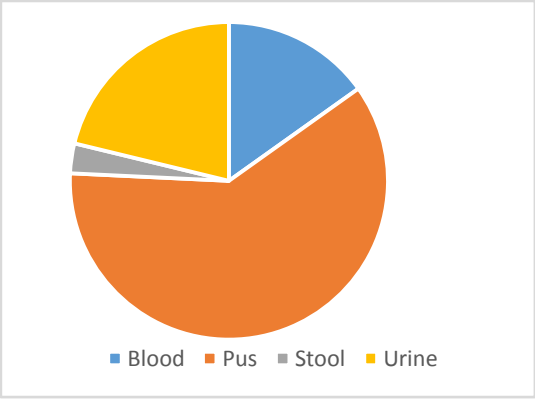
100 µl of hot and cold extract were inoculated on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates for checking for bacterial and fungal contaminants respectively [14].

2.10. Testing of Synergistic Activity

100 µl of extract was transferred to sterile petri plates and 15ml of sterilized molten Hi-sensitivity test agar maintained 55^0C in constant temperature water bath was then poured in a plates, then plate is rotated for about 30 to 35 seconds to ensure even mixing of extract with the agar medium. Agar medium was then allowed to solidify. 100 µl of inoculum was added on solidified Hi-sensitivity test agar and spread over agar medium with the help of sterile disposable L-spreader. Four or five antibiotic discs were placed over it equidistantly. Plates were kept undisturbed in a refrigerator for 1 hour. Then plates were removed from refrigerator and shifted to incubator maintained at 37^0C for 18-24hrs. After incubation all plates were examined for zone of inhibition. Zone of inhibition is then measured and classified as susceptible, intermediate, or on the basis of manufacturer's interpretive table, CLSI standard.

3. Results and Discussion

In the present study, total 106 Clinical samples of urine, pus, blood and sputum were collected from different pathology laboratories of Nagpur (MS),India for isolation of *Pseudomonas sp*.Total 33 suspected isolates of *Pseudomonas sp*.were identified on the basis of morphological, cultural & biochemical characteristics and all of them were found to be of *Pseudomonas sp*. 20 isolates were associated with pus samples, 7 with urinary tract infection (UTI), 5 was associated with blood and 1 from stool sample. Percentage prevalence of *Pseudomonas sp*.in clinical samples is graphically illustrated in Graph 1.

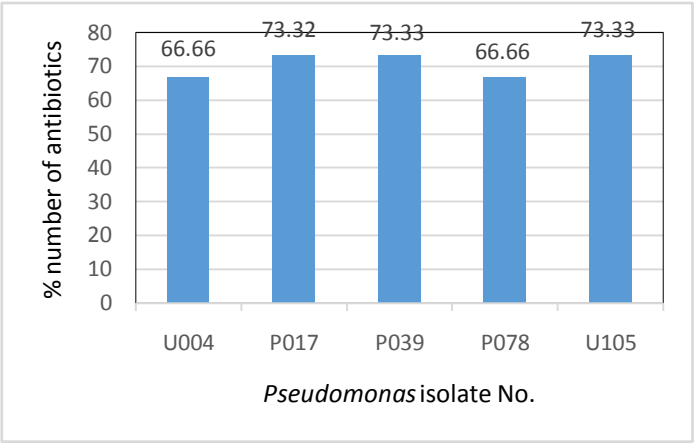


Graph1. *Graph showing % prevalence of Pseudomonas in clinical samples.*

More than 60% *Pseudomonas sp*. were found to be associated with pus samples, 21 %, 15 % and 3 % with Urine samples, blood samples and stool sample respectively.

These 33 isolates were preliminary screened for the selection of MDR using 15 different antibiotics. Antibiogram study of these isolates revealed that all these isolates are resistant to

several antibiotics out of 15 antibiotics tested.Total 5 MDR isolates of *Pseudomonassp*. namely U004, P017, P039, P076 and U0105 were selected for further studies out of 33 isolates on the basis of their resistance to more than 60% antibiotics. Graph2 illustrates the % resistance of each isolate to number of antibiotics.



Graph2. *Graph showing resistance of isolate to % number of antibiotics.*

These isolates were resistant to GEN, DOR, PB, CPZ, AK, CIP, CPM, CZX, MZ and intermediate to LE & CTX. Each individual extract was then tested for antimicrobial action against selected MDR isolate and no extract found to have antimicrobial property against MDR isolate. Total 10 extracts of *Foeniculum vulgare* were studied; HFAT, CFAT, HFME, CFME, HFCF, CFCF, HFW, CFW, HFPE and CFPE. The results of antibiogram and synergistic effect of herbal extracts on susceptibility of MDR isolates are given in Table 1.

Table1. *Antibiogram and synergistic effect of herbal extracts and antibiotics on MDR Pseudomonasisolates*

	TOB	GAT	GEN	DOR	PB	CPZ	AK	LE	TI	CTX	PIT	CIP	CPM	MZ	CZX
U004															
Antibiotics	17 (S)	22 (S)	15 (R)	(R)	(R)	17 (I)	11 (R)	16 (I)	22 (S)	25 (I)	25 (S)	13 (R)	18 (S)	14 (R)	20 (R)
HFAT	24 (S)	27 (S)	17 (S)	29 (S)	(R)	(R)	(R)	26 (S)	28 (S)	34 (S)	27 (S)	18 (I)	(R)	27 (S)	(R)
CFAT	26 (S)	29 (S)	16 (S)	28 (S)	(R)	(R)	(R)	25 (S)	30 (S)	36 (S)	26 (S)	17 (I)	(R)	26 (S)	(R)
HFME	24 (S)	29 (S)	18 (S)	27 (S)	(R)	10 (R)	(R)	24 (S)	31 (S)	37 (S)	28 (S)	16 (I)	(R)	28 (S)	11 (R
CFME	24 (S)	31 (S)	17 (S)	26 (S)	(R)	11 (R)	(R)	26 (S)	28 (S)	32 (S)	27 (S)	18 (I)	(R)	27 (S)	(R)
HFCF	26 (S)	30 (S)	18 (S)	28 (S)	(R)	(R)	(R)	22 (S)	26 (S)	32 (S)	28 (S)	17 (I)	(R)	27 (S)	(R)
CFCF	28 (S)	29 (S)	18 (S)	27 (S)	(R)	(R)	(R)	26 (S)	24 (S)	33 (S)	26 (S)	16 (I)	(R)	26 (S)	(R)
HFW	26 (S)	28 (S)	17 (S)	28 (S)	(R)	(R)	(R)	26 (S)	29 (S)	32 (S)	27 (S)	18 (I)	10 (R)	28 (S)	(R)
CFW	26 (S)	27 (S)	16 (S)	26 (S)	(R)	10 (R)	(R)	23 (S)	30 (S)	34 (S)	31 (S)	17 (I)	(R)	28 (S)	(R)
HFPE	26 (S)	28 (S)	18 (S)	26 (S)	(R)	(R)	(R)	22 (S)	31 (S)	32 (S)	32 (S)	16 (I)	(R)	29 (S)	(R)
CFPE	24 (S)	27 (S)	17 (S)	28 (S)	(R)	(R)	(R)	24 (S)	29 (S)	32 (S)	26 (S)	18 (I)	(R)	30 (S)	(R)
P017															
Antibiotics	18 (S)	18 (S)	15 (R)	(R)	(R)	18 (I)	11 (R)	17 (S)	18 (I)	27 (R)	25 (S)	12 (R)	17 (I)	16 (R)	22 (I)
HFAT	24 (S)	28 (S)	17 (S)	29 (S)	(R)	(R)	(R)	22 (S)	29 (S)	31 (S)	27 (S)	18 (I)	(R)	30 (S)	(R)
CFAT	26 (S)	27 (S)	18 (S)	30 (S)	(R)	10 (R)	(R)	23 (S)	28 (S)	29 (S)	28 (S)	16 (I)	(R)	31 (S)	(R)
HFME	26 (S)	26 (S)	16 (S)	31 (S)	(R)	(R)	(R)	24 (S)	29 (S)	28 (S)	27 (S)	18 (I)	(R)	28 (S)	(R)
CFME	28 (S)	27 (S)	18 (S)	28 (S)	(R)	(R)	(R)	25 (S)	28 (S)	30 (S)	26 (S)	17 (I)	(R)	27 (S)	(R)
HFCF	27 (S)	26 (S)	17 (S)	27 (S)	(R)	(R)	(R)	22 (S)	28 (S)	32 (S)	25 (S)	16 (I)	(R)	26 (S)	(R)
CFCF	26 (S)	26 (S)	18 (S)	28 (S)	(R)	10 R	(R)	23 (S)	29 (S)	32 (S)	26 (S)	17 (I)	(R)	28 (S)	(R)

HFW	26 (S)	27 (S)	17 (S)	27 (S)	(R)	(R)	(R)	24 (S)	30 (S)	30 (S)	27 (S)	18 (I)	(R)	29 (S)	(R)
CFW	24 (S)	28 (S)	16 (S)	26 (S)	(R)	(R)	(R)	25 (S)	28 (S)	29 (S)	27 (S)	19 (I)	(R)	30 (S)	(R)
HFPE	26 (S)	27 (S)	18 (S)	28 (S)	(R)	(R)	(R)	24 (S)	29 (S)	30 (S)	26 (S)	18 (I)	(R)	30 (S)	(R)
CFPE	24 (S)	26 (S)	19 (S)	27 (S)	(R)	11 (R)	(R)	24 (S)	29 (S)	31 (S)	29 (S)	16 (I)	(R)	29 (S)	(R)
P039															
Antibiotics	17 (S)	19 (S)	16 R	(R)	(R)	17 (I)	12 (R)	14 (I)	19 (I)	25 (R)	25 (S)	14 (R)	17 (I)	20 (I)	23 (S)
HFAT	24 (S)	26 (S)	19 (S)	28 (S)	(R)	(R)	(R)	23 (S)	28 (S)	32 (S)	31 (S)	16 (I)	(R)	28 (S)	(R)
CFAT	26 (S)	26 (S)	18 (S)	26 (S)	(R)	(R)	(R)	24 (S)	27 (S)	33 (S)	32 (S)	18 (I)	(R)	27 (S)	(R)
HFME	28 (S)	27 (S)	17 (S)	28 (S)	(R)	(R)	(R)	26 (S)	26 (S)	30 (S)	28 (S)	18 (I)	(R)	26 (S)	11 (R)
CFME	26 (S)	26 (S)	16 (S)	27 (S)	(R)	(R)	(R)	26 (S)	28 (S)	29 (S)	26 (S)	18 (I)	(R)	28 (S)	(R)
HFCF	27 (S)	27 (S)	18 (S)	26 (S)	(R)	(R)	(R)	25 (S)	29 (S)	28 (S)	27 (S)	17 (I)	(R)	27 (S)	(R)
CFCF	30 (S)	26 (S)	17 (S)	27 (S)	(R)	10 (R)	(R)	24 (S)	29 (S)	32 (S)	27 (S)	18 (I)	(R)	27 (S)	(R)
HFW	26 (S)	27 (S)	16 (S)	28 (S)	(R)	(R)	(R)	26 (S)	30 (S)	31 (S)	26 (S)	16 (I)	(R)	26 (S)	(R)
CFW	26 (S)	26 (S)	18 (S)	27 (S)	(R)	(R)	(R)	25 (S)	31 (S)	28 (S)	28 (S)	17 (I)	(R)	28 (S)	(R)
HFPE	25 (S)	26 (S)	17 (S)	29 (S)	(R)	(R)	(R)	26 (S)	29 (S)	30 (S)	29 (S)	19 (I)	(R)	28 (S)	(R)
CFPE	24 (S)	27 (S)	18 (S)	30 (S)	(R)	(R)	(R)	27 (S)	31 (S)	31 (S)	28 (S)	17 (I)	(R)	27 (S)	(R)
P078															
Antibiotics	14 (I)	20 (S)	15 (R)	(R)	(R)	17 (I)	14 (R)	14 (I)	20 (S)	25 (R)	27 (S)	15 (R)	17 (I)	16 (R)	27 (R)
HFAT	24 (S)	27 (S)	18 (S)	29 (S)	(R)	(R)	(R)	26 (S)	28 (S)	32 (S)	28 (S)	19 (I)	(R)	27 (S)	(R)
CFAT	26 (S)	28 (S)	17 (S)	30 (S)	(R)	10 (R)	(R)	23 (S)	26 (S)	33 (S)	27 (S)	19 (I)	(R)	28 (S)	11 (R)
HFME	26 (S)	28 (S)	16 (S)	28 (S)	(R)	(R)	(R)	24 (S)	28 (S)	30 (S)	26 (S)	20 (I)	(R)	27 (S)	(R)
CFME	28 (S)	27 (S)	18 (S)	27 (S)	(R)	(R)	(R)	23 (S)	29 (S)	29 (S)	28 (S)	21 (I)	10 (R)	30 (S)	(R)
HFCF	24 (S)	26 (S)	17 (S)	26 (S)	(R)	(R)	(R)	24 (S)	29 (S)	28 (S)	27 (S)	19 (I)	(R)	28 (S)	(R)
CFCF	25 (S)	25 (S)	16 (S)	27 (S)	(R)	(R)	(R)	23 (S)	31 (S)	32 (S)	29 (S)	18 (I)	(R)	27 (S)	(R)
HFW	26 (S)	28 (S)	18 (S)	28 (S)	(R)	(R)	(R)	22 (S)	28 (S)	31 (S)	31 (S)	17 (I)	(R)	26 (S)	(R)
CFW	26 (S)	27 (S)	17 (S)	27 (S)	(R)	(R)	(R)	23 (S)	27 (S)	28 (S)	28 (S)	16 (I)	(R)	28 (S)	(R)
HFPE	27 (S)	28 (S)	17 (S)	28 (S)	(R)	(R)	(R)	24 (S)	26 (S)	30 (S)	27 (S)	18 (I)	(R)	30 (S)	10 (R)
CFPE	26 (S)	27 (S)	18 (S)	27 (S)	(R)	(R)	(R)	26 (S)	28 (S)	31 (S)	26 (S)	17 (I)	(R)	29 (S)	(R)
U105															
Antibiotics	17 (S)	21 (S)	14 (R)	(R)	(R)	17 (I)	11 R	16 (I)	18 (I)	26 (R)	24 (S)	14 (R)	(R)	18 (I)	25 (S)
HFAT	27 (S)	28 (S)	18 (S)	28 (S)	(R)	(R)	(R)	24 (S)	28 (S)	33 (S)	28 (S)	18 (I)	(R)	28 (S)	(R)
CFAT	26 (S)	27 (S)	16 (S)	27 (S)	(R)	(R)	(R)	25 (S)	26 (S)	32 (S)	27 (S)	17 (I)	(R)	30 (S)	(R)
HFME	26 (S)	26 (S)	18 (S)	29 (S)	(R)	(R)	(R)	26 (S)	28 (S)	34 (S)	26 (S)	16 (I)	(R)	29 (S)	(R)
CFME	27 (S)	28 (S)	17 (S)	29 (S)	(R)	10 (R)	(R)	28 (S)	29 (S)	33 (S)	26 (S)	18 (I)	(R)	27 (S)	10 (R)
HFCF	27 (S)	27 (S)	16 (S)	28 (S)	(R)	(R)	11 (R)	26 (S)	31 (S)	32 (S)	30 (S)	17 (I)	(R)	26 (S)	(R)
CFCF	26 (S)	26 (S)	18 (S)	27 (S)	(R)	(R)	(R)	26 (S)	31 (S)	32 (S)	31 (S)	16 (I)	(R)	28 (S)	(R)
HFW	26 (S)	27 (S)	17 (S)	28 (S)	(R)	(R)	(R)	25 (S)	29 (S)	33 (S)	28 (S)	18 (I)	(R)	27 (S)	(R)
CFW	27 (S)	28 (S)	16 (S)	29 (S)	(R)	(R)	(R)	24 (S)	30 (S)	33 (S)	30 (S)	18 (I)	(R)	26 (S)	(R)
HFPE	27 (S)	27 (S)	18 (S)	30 (S)	(R)	(R)	(R)	26 (S)	31 (S)	32 (S)	31 (S)	18 (I)	(R)	28 (S)	10 (R)
CFPE	26 (S)	26(S)	18 (S)	28 (S)	(R)	(R)	(R)	27 (S)	29 (S)	31 (S)	31 (S)	18 (I)	(R)	27 (S)	(R)

(S)- Sensitive, (I) - Intermediate, (R)- Resistant

Extract found to be potentiate the activity of antibiotics; GEN, DOR, CIP, MZ, LE and CTX. These extracts found to potentiate the susceptibility of even sensitive antibiotic. *Foeniculum vulgare* extract found to have pronounced effect on activity of DOR and CIP as compared to other antibiotics. Synergistic study of extracts of *Foeniculum vulgare* seeds and antibiotics on susceptibility of resistant *Pseudomonas* isolates showed that most of the extracts exhibited potentiating effect on antibiotics.

Combined antibiotic therapy of antibiotics and herbal extract has been shown to have a synergistic effect. Concept of synergism provides new thought of antibiotics and bioactive plant extracts in development of combined antimicrobial therapy [15].Charde VN *et. al*(2014) studied synergy of polar and nonpolar solvent extracts of *Trachyspermumammi* and antibiotics against MDR *Staphyllococcusaureus* and demonstrated that such synergy is very effective in *invitro* management of MDR organisms [13].

Gulfraz*et al*. (2008) reported strong inhibitory potential of fennel oil against *Bacillus cereus, B. magaterium, B. pumilus, B. subtilis, E. coli, K. pneumonia, M. lutus, Pseudomonas pupida, P. syringae,* and *Candida albicans* than the seed extracts prepared inmethanol and ethanol [16] which is in accord with the present study were no antimicrobial activity was found in any of solvent extracts including methanol against *Pseudomonas* isolates. Phytochemicals have various protective and therapeutic effects which confers prevention of diseases due to its bactericidal or bacteriostatic effect. It is difficult to find the major content forantibacterial and anti-fungal activity of natural essential oils which are present in complex mixtures but it is valid to interpret the activity may be due to the presence of its key component i.e., eugenol in case of clove oilin high concentration. Phytochemicals have different modes of action to confer its antimicrobial activity. Essential oils are good lipophilic and can travel through the cell wall and cytoplasmic membrane. Hydrophobicity of component makes them permeable to partition into the lipid bilayer of the cell membrane successive leakage of cell contents [17].Phytoconstituents of *Foeniculum vulgare, Anethumgraveolens, Pimpinellaanisum, Carumcarvi* and *Coriandrumsativum* in its methanol extract proved to be rich in total phenols and total flavonoids, inGC–MS study and they found to contain trans-anethol as major component in addition to fenchone, estragoleand limonene[18,19] and also contains aldehydes, ketones, and alcohols [20,21,22].

F. vulgare seed oil is rich in trans- anethole and other compounds which are effective against microbes. Total phenolic and flavonoids of *Foeniculum vulgare* are very much higher than the content of *Coriandrumsativum.* Essential oil of fennel plant roots, stem, leaves and seeds have anticandidal activity against *C. albicans*and *C. tropicalis*[23]whereas in study of Asgary S. *et. al.* (2005), Kaviarasan S. *et. al.* (2004),Valente MJ.*et. al.* (2011), and Biswas S. *et. Al* have demonstrated that polyphenolic compounds in *Foeniculum vulgare* protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants with various ways[24, 25, 26, 27] such as, metal chelating agents, reducing agents, singlet oxygen quenchers, and hydrogen donors [28,29].

4. CONCLUSION

The extracts of *Foeniculum vulgare* were found to be effective in combination with antibiotics against *Pseudomonassp*. This study paves the path for further attention of researchers to identify the active responsible compounds of plants for their biological activity. The different solvent extracts of *Foeniculum vulgare* showed significant activity against MDR pathogens in combination with antibiotics which were previously least effective or ineffective. To conclude, this clears the view of validity of synergistic activity of plant extract with antibiotic which is a promising approach in the field of medical microbiology and provides a new hope for development of drug therapy. Further studies are needed to confirm the phenomenon of synergy between drugs and plant extracts and to avoid allergy and other side effects.

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REFERENCES

[1] UNESCO., Culture and Health, Orientaion texts, World Decade for Cultural Dvelopment 1988-1997 (1996) Document CLT/DEC/PRO- 1996, UNESCO, Paris, France p 129.

[2] Mohammed I., Rubina L, MD Nasruddin A., MohdS.,andEbenezer JK.,Synergistic effects of *ocimum sanctum* extract and antibiotics on methicillin resistant *staphylococcus aureus*isolated from clinical specimens, Journal of recent advances in applied sciences. 27:99-107 (2012)

[3] R´ıosJ.L.,Recio M.C., Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 100:80–84 (2005)

[4] Sunday OO., Uguru MO., and Ogbonna A., Antimicrobial and hypoglycemic effects of *Momordicabalsamina.*Linn.Journal of Natural Products. 1:03-09(2008)

[5] Julie J., Therapeutic MT., Applications of Pomegranate (*Punicagranatum*L.): A Review. Alternative Medicine Review.13(2):128-1442008

[6] KrishnamoorthyV.,andMadalageri M.B., Bishop weeds (*Trachyspermumammi*): an essential crop for north Karnataka. J Med and Aromat Plants Sci. 21(4): 996- 998 (1999).

[7] Supayang PV., and Hazel M., Inhibitory and killing activity of medicinal plant against multiple drug resistant *helicobactor pylori*. Journal of health science. 54(1):81-88 (2008)

[8] Javed A., Annu K., Mohd. NK., and Siva KM., Evaluation of the Combinational Antimicrobial Effect of *AnnonaSquamosa*and*Phoenix Dactylifera*SeedsMethanolic Extract on Standard Microbial Strains. Int. Res. J. Biological Sci.2(5):68-73 (2013)

[9] Bhone MK., Shuchi A., Win Mar Soe, JayaramanPremkumar, Chu Sing Lim. Dynamic Drug Combination Response on Pathogenic Mutations of *Staphylococcus aureus.* International Conference on Biomedical Engineering and Technology. 11 (2011)

[10] UNESCO. *FIT/504-RAF-48* Terminal Report: Promotion of ethnobotany and the sustainable use of plant resources in Africa.UNESCO, Paris, France p. 60. (1998)

[11] Blanc, DS., Petignat C., Janin B., Bille J., and Fancioli P., Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: a prospective epidemiologic study. Clin. Microbiol. Infect. 4:242-247 (1998)

[12] CLSI. Performance standards for antimicrobial susceptibility testing: 17th Informational supplement. Approved standard M100-S17, Wayne, USA: Clinical and Laboratory Standards Institute. (2007)

[13] ChardeVN., BezalwarPM., GomasheAV., Synergistic Activity of Antibiotics and*Trachyspermumammi*(Ajwain) Extract on MDR *Staphylococcus aureus.* Indian Journal of Applied Research. 4(11):450-454 (2014)

[14] Sule, LO.,Agbabiaka TO., Antibacterial effect of some plant extracts on selected Enterobacteriaceae. Ethno botanical leaflets.12:1035-42 (2008)

[15] Gibbons, S., Anti-staphylococcal plant natural and P.A.K. Duke, 2003. CRC Handbook of Medical products. Nat. Prod. Rep. 21: 263-277 (2004)

[16] Gulfraz M., Mehmood S., Minhas N., Jabeen N., Kausar R., Jabeen K., and ArshadG.,Composition and antimicrobial properties of essential oil of Foeniculum vulgare,African Journal of Biotechnology.7(24): 4364-4368 (2008)

[17] Burt S., Essential oils: their antibacterial properties and potential applications in foods – a review.Int J Food Microbiol. 94:223–253 (2004)

[18] Ozcan MM., Chalchat JC., Arslan D., Ates A., Unver A., Comparative essential oil composition and antifungal effect of bitter fennel (Foeniculum vulgare ssp. piperitum) fruit oils obtained during different vegetation. J. Med Food. 9(4):552-561 (2006)

[19] Anwar F., Alia M., Hussain AI., Shahid M., Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan, FlavourFragr. J. 24: 170–176 (2009).

[20] Azzouz MA., Bullerman LB., Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J Food Protect .45:1298–1301 (1982)

[21] Shelef LA., Antimicrobial effects of spices, J. Food Safety. 6:29– 44 (1983)

[22] Akgul A., Antimicrobial activity of black cumin (Nigella sativa L.) essential oil. J GaziPharmacol Faculty. 6: 63–68 (1989)

[23] Kawther FA., Antimicrobial Activity of Essential Oils of some Medicinal Plants from Saudi Arabia, Saudi J. Biological Sciences. 14 (1): 53-60 (2007)

[24] Asgary S, Naderi G, Askari. Protective effect of flavonoids against red blood cell hemolysis by free radicals. Exp. Clin. Cardiol. 10:88–90 (2005)

[25] Kaviarasan S., Vijayalakshmi K., Anuradha C., Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. Plant Foods Hum. Nutr. 59:143– 147 (2004)

[26] Valente MJ., Baltazar AF., Henrique R., Estevinho L., Carvalho M., Biological activities of Portuguese propolis: Protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth in vitro, Food.Chem. Toxicol. 49:86–92 (2011)

[27] Biswas S., Bhattacharyya J., Dutta AG., Oxidant induced injury of erythrocyte-role of green tea leaf and ascorbic acid. Mol. Cell. Biochem. 276:205–210 (2005)

[28] Rice-Evans C., Miller N., Paganga G., Structure- antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med. 20:933–956 (1996)

[29] Cao G., Sofic E., Prior RL.. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic. Biol. Med. 22:749–760 (1997)