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Research Article

Synergistic Antimicrobial Effects of Volatile Oils used in a Polyherbal Formulation

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ABSTRACT

Synergistic antimicrobial activity is performed using a polyherbal formulation. The study involved evaluation of minimum inhibitory concentration activity against a variety of pathogens. The results portray that different types of microbes have different sensitivity against the formulation. The study was done separately with essential oil and as a combined formulation for evaluation of synergistic effect. Extracted essential oil showed synergistic activity with ciprofloxacin and streptomycin against test strains.

Keywords: antimicrobial effects, volatile oils, polyherbal formulation

INTRODUCTION

Essential oils are natural oils which are mostly obtained by distillation and possesses characteristics odour of the plant or the other source from where they were extracted[5]. Cardamom, Fennel, Coriander, Caraway and Ajowan is produced from cultivated or wild plants in the mountainous regions of southern India[6-8]. Essential oils are long used for ailing medical conditions and are present in ancient text of Chinese and Indian civilizations. The present study is an attempt to investigate the synergistic effect of few essential oils namely Cardamom, Fennel, Coriander, Caraway and Ajowan for evaluation of their antimicrobial activity against common pathogens. This study is the second study by authors and provides more detailed reports for synergistic antimicrobial effect[1].

MATERIALS AND METHODS

Plant Material

The seeds of Cardamom, Fennel, Coriander, Caraway and Ajown were obtained from trade market, humidity 8%.

Extraction of Essential Oils

A number of extraction procedures like Ethanol extraction, solvent extraction, supercritical fluid extraction and steam distillation techniques[2-4] were used in the experiment design to extract of essential oil from crude material. Out of these techniques it was found that, steam distillation technique is relatively a cheaper process to operate at basic level whereas in supercritical extraction technique the CO_2 & 1,1,1,2- tetrafluoroethane gas came out as the main constraint. After optimization of all techniques, steam distillation technique was used for further extraction of essential oil based on the yield, reproducibility and easy methodology. 250 g of each seed boiled with 500 mL of distilled water in a Clevenger apparatus up to 6 hours. The volume of essential oils was determined from a calibrated trap. The obtained oils were dried over anhydrous Na_2SO_4 and kept in the dried vial at -4°C. A Headspace-Gas chromatographic method was developed to identify and characterize the individual oil against the Marker compound procured from Ultra international, Uttarpradesh. Each of the Marker compound was characterized by GC/MS method.

Preparation of blend of sample formulation

A blend of oil was prepared by transferring 1g of individual oil in 20 mL volumetric flask. The volume was made using sunflower oil as a vehicle. The sample was stored in a close tight, amber colored bottle.

Determination of antibacterial activity

Antimicrobial activity of the essential oils extracted was determined against reference microbial strains. The strains are deposited in the Microbial Culture Collection. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of essential oils extract were determined by serial broth dilution method in accordance with CLSI reference method (CLSI Standards, 1990). A stock solution to be tested was prepared by diluting the respective extracted oils sample in DMSO (Sigma-Aldrich Co.). Antimicrobial activity of the extract was determined in concentration of 10 % (w/v). The antibacterial activity of the seed extracts was carried out by disc diffusion assay (Kumar et al., 2001; Gulluce et al., 2003). Muller Hinton agar (MHA) plates were swabbed with the respective broth culture of the organisms (diluted to 0.5 McFarland Standard with saline) and kept for absorption to take place. Sterile 6 mm diameter filter paper discs were impregnated with 10% w/v of seed extract that was dissolved in sterile Dimethylsulfoxide (DMSO). Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ciprofloxacin (5µg/mL) and streptomycin (5µg/mL) were used as positive reference standards to

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Table 1: The antibacterial activity of essential oils by Agar Disc method.

Name	Concentration	Zone of inhibition(mm)			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Cardamom oil	10%W/V	24.0	20.0	22.0	24.0
Coriander oil	10%W/V	18.0	20.0	18.0	22.0
Ajawan oil	10%W/V	18.0	16.0	18.0	16.0
Fennel oil	10%W/V	16.0	14.0	16.0	18.0
Caraway oil	10%W/V	14.0	16.0	14.0	14.0
Ciprofloxacin	5µg/mL	30.0	30.0	28.00	30.0
Streptomycin	5µg/mL	32.0	32.0	30.0	32.0
Control	DMSO	-	-	-	-

Table 2: The synergistic antibacterial activity of essential oils with ciprofloxacin and streptomycin by Agar Disc method.

Sample	Concentration	Zone of inhibition(mm)			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Cardamom + Ciprofloxacin	10%w/v	36.0	34.0	38.0	36.0
Cardamom + Streptomycin	10%w/v	36.0	32.0	32.0	34.0
Coriander + Ciprofloxacin	10%w/v	30.0	32.0	34.0	34.0
Coriander + Streptomycin	10%w/v	32.0	34.0	38.0	38.0
Fennel + Ciprofloxacin	10%w/v	30.0	30.0	30.0	30.0
Fennel + Streptomycin	10%w/v	36.0	34.0	36.0	38.0
Caraway + Ciprofloxacin	10%w/v	34.0	30.0	30.0	30.0
Caraway + Streptomycin	10%w/v	30.0	36.0	34.0	34.0
Ajawan + Ciprofloxacin	10%w/v	34.0	32.0	32.0	30.0
Ajawan + Streptomycin	10%w/v	38.0	36.0	38.0	36.0
Cardamom + Coriander	10%w/v	26.0	24.0	20.0	22.0
Cardamom + Fennel	10%w/v	24.0	22.0	18.0	20.0
Cardamom + Caraway	10%w/v	20.0	22.0	20.0	24.0
Cardamom + Ajawan	10%w/v	22.0	20.0	20.0	22.0
Coriander + Fennel	10%w/v	22.0	24.0	22.0	20.0
Coriander + Caraway	10%w/v	20.0	22.0	24.0	20.0
Coriander + Ajawan	10%w/v	20.0	20.0	20.0	22.0
Fennel + Caraway	10%w/v	22.0	20.0	22.0	24.0
Caraway + Ajawan	10%w/v	24.0	20.0	20.0	22.0
Blend of essential oil	10%W/V	28.0	24.0	26.0	26.0
Ciprofloxacin	5µg/mL	30.0	32.0	30.0	32.0
Streptomycin	5µg/mL	30.0	30.0	28.00	30.0
Control	Distilled water	-	-	-	-

determine the sensitivity of one strain in each bacterial species tested. The plates were incubated overnight at 37°C. The antimicrobial activity was evaluated by measuring the zone expressed as mm of inhibition against test organism. Six discs per plate were used for each separate stain was run.

Preparation of bacteria strain

Four different bacteria were used. Two species of Gram positive bacteria, S. aureus, B. Subtilis and two Gram negative bacteria, E. *coli*, P. *aeruginosa* were obtained. Bacteria were sub-cultured on nutrient agar at 37°C prior to being grown in nutrient broth overnight. All overnight (ON) cultures were standardized by matching to the McFarland 0.5 turbidity standard using sterile saline to produce approximately 1.5x108 colony forming units (cfu) per mL.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) values were also studied for the bacteria which was determined as sensitive to the extracts in disc diffusion assay[9]. The inoculated bacteria as prepared from 24 h nutrient broth cultures and suspensions were adjusted to 0.5 McFarland turbidity standards. Extracts dissolved in DMSO were first diluted to the highest concentration (50 µg/mL) to be tested and then serial two fold dilutions were made in a concentration range from 2µg/mL to 50 µg/mL, which are 2.5µg/mL, 3µg/mL, 4µg/mL, 5µg/mL, 6.25µg/mL, 10µg/mL, 12.5µg/mL 25µg/mL and 50 µg/mL. With a standardized micropipette a drop of the diluted broth culture of the test organism (approximately 0.01 mL) was added to all the tubes, including the control. The contents of the test tube were mixed gently and incubated at 25°C for 24 hrs for *Staphylococcus aureus* NCIM 2079, *Pseudomonas aeruginosa* NCIM 2036, *Escherichia coli* NCIM 2118 and *Bacillus subtilis* NCIM 2063. The least concentration where each extract showed clear inhibition was taken as the MIC level. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was taken as minimum inhibitory concentration (MIC). The highest dilution streaked on nutrient agar plates which did

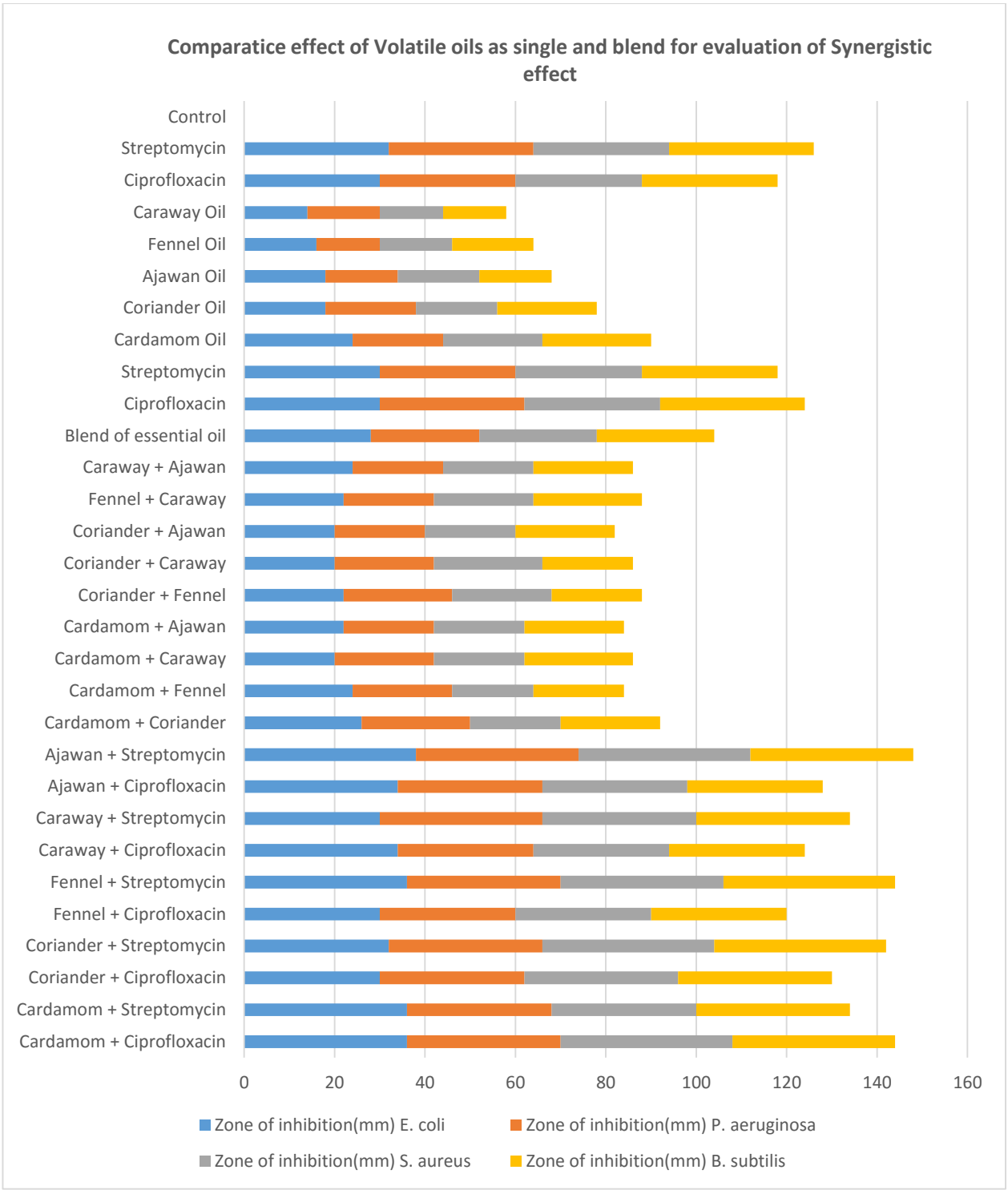

Figure 1: Graphical Representation of The synergistic antibacterial activity of essential oils with ciprofloxacin and streptomycin by Agar Disc method.

not show any bacterial growth after overnight incubation was taken as minimum bactericidal concentration (MBC).

RESULTS AND DISCUSSION

The results for the antibacterial activity were shown in the Table 1 and synergistic activities were shown in Table 2. The antimicrobial activity of essential oil may be influenced by some factors such as quality of the extraction of essential oil. The activity of the different essential oil collected from different seeds varied significantly among the various bacterial species studied. The cardamom extract demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria. The extract was less active against strains of P. aeruginosa, which belong to the group of the most resistible bacterial strains. The ability of P. aeruginosa to produce extracellular polysaccharides

increased antimicrobial resistance. The synergistic antimicrobial activity for the different combination of extracted essential oils shown that when essential oils are combined (50:50) and tested, it having synergistic effect. Essential oil extracted from cardamom alone at 10%W/V tested against E. coli, P. aeruginosa, S. aureus and B. subtilis produced zone of inhibition (in mm) 24, 20, 22 and 24 respectively. When, Essential oil extracted from cardamom combined with essential oil extracted from coriander at 1:1 ratio, produced zone of inhibition in mm as 26, 24, 20 and 22 respectively. The results proved the synergistic activity when they were combined and tested. The synergistic antimicrobial activity of blend of essential oils extracted from cardamom, coriander, fennel, caraway and Ajowan (1:1:1:1:1) were found to be remarkable when compared with standard. The individual Essential oils also showed synergistic activity with combination with ciprofloxacin. It has observed that individual essential oil having antimicrobial activity but when combination of all, the rate of growth of inhibition increased significantly. In high concentration Cardamom and Coriander oil having more bactericidal properties than other oil where in small concentration of all the oil having the same properties that was confirmed from MIC study. The MIC of blend of essential oil sample, positive response was found at lower concentration as 2.5µg/mL in all bacterial stain rather than individual oil.

CONCLUSION

The results showed that the gram positive and gram negative strains of bacteria had different sensitivities to individual essential oils. In this present study, individual essential oils demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria however the combination of these essential oils shown greater biological activity as compared when they were used separately. The results shown significant antibacterial properties with combination of synthetic drug. This claim is good indications for future aspect to formulate an herbal formulation which can be recommend as combination with synthetic antibiotics to the patients who are having drug resistance disorders. Due to its similar kind of antibacterial properties, this can be reduced the use of synthetic medicine in preliminary health disease.

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