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Antimicrobial Efficacy of *Cinnamonum verum* Essential Oil Alone and in Combination with Antibiotics and Other Essential Oils

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A B S T R A C T

Microbial resistance to antibiotics is a global health problem. Infectious diseases are increasing day by day for various untackable reasons. Thus, there is an urgent need to develop novel antimicrobial agents from natural substances such as plants which are easily available with no toxic effects which are generally associated with synthetic drugs. Besides there is every chance of getting a new drug or lead to drug molecule because of the vast diversity which they possess. The present study was designed to examine synergistic antimicrobial activity of Cinnamon (*Cinnamonum verum*) oil with some commercial antibiotics and some other essential oils against a panel of multidrug resistant microorganisms. The composition of Cinnamon oil was characterized by GC-MS analysis, the principal compound identified was cinnamaldehyde (62.28%) and benzyl alcohol (24.60%). Combination of Cinnamon oil with antibiotics and other essential oils showed synergistic antimicrobial activity by disc diffusion method. Microbes which showed significant sensitivity were further assayed with various concentrations of the potent combination of Cinnamon oil in a broth dilution method and determined by fractional inhibitory concentration (∑FIC) index. Among the different combinations, Cinnamon oil with ampicillin and Cinnamon oil with Clove oil was found to be most effective combination against pathogenic microorganisms. Synergistic potential of Cinnamon oil can be a new and important way for the treatment of infectious diseases.

Introduction

The emergence of drug resistant pathogens has becomes one of the most severe threats against the treatment of infectious diseases and significant global economic and healthcare crisis (Huttner *et al.*, 2013). Life-threatening infections caused by bacteria and fungi are the serious problem in agricultural, food, water and medical tradition. Furthermore, the treatments of infectious disease by conventional antimicrobial agents are not acceptable because they cause resistance over a time and have an undesirables side effect (Ibrahim *et al.*, 2012).

This has led to an increased thrust towards identification of novel antimicrobial agents from the natural sources. The use of plant based herbal medicine for health care is known since ancient times. Plants are rich source of active phytoconstituents which can be used for curing various diseases (Nakhuru *et al.*, 2013; Padalia and Chanda, 2015).

One of the most promising active constituent of plants for development of novel antimicrobial agents is essential oil. Essential oils are yield of secondary metabolism of aromatic plants and mixture of diverse volatile compounds (Pinto *et al.,* 2013). Essential oils are generally extracted from plant by steam or hydro distillation methods and they are mixture of wide range of compound mainly terpenoids particularly monoterpenes and sesquiterpenes and a variety of low molecular weight aliphatic hydrocarbons (Dorman and Deans, 2000). Essential oils and their components have many applications in food flavouring and preservation, traditional medicine as well as in cosmetic and pharmaceutical industries (Gendy *et al.,* 2015).

Essential oils have broad spectrum of activity including antibacterial, antifungal, antiviral, antimycotic and insecticidal against wide range of microorganisms like bacteria, fungi, virus, yeast etc. (Akthar *et al.,* 2014; Ghabraie *et al.,* 2016; Luis *et al.,* 2016). To improve the effectiveness of antimicrobial agents, their combined use with the essential oils is one of the promising approach (Giordani *et al.,* 2004). Essential oil is mixture of number of complex phytoconstituents which have individual biological effects. The effects of compounds depend on their concentration when they are used alone or in combination. Interactions between antimicrobial agents may lead to additive, synergistic or antagonistic effects (Bakkali *et al.,* 2008).

Some essential oils such as cinnamon, eucalyptus, lavender, lemon, lime, mint, rosemary, basil, clove and neem are traditionally used by people in different parts of the world. Cinnamon oil is derived from *Cinnamonum verum* (Lauraceae family) and is generally used as spices. It is therapeutically used in neuralgia, headache, diarrhea and to treat problems of the digestive system. It is also used in the treatment of cancer and many diseases associated with microorganisms (Prabuseenivasan *et al.,* 2006; Clemente *et al.,* 2016). The aim of the present investigation was to evaluate synergistic potential of cinnamon essential oil. The synergistic potential was evaluated with some commercial antibiotics and some other essential oils against multidrug resistant microorganisms.

Materials and Methods

Plant material

Different essential oils viz. cinnamon (*Cinnamomum verum*) (CN), clove (*Syzygium aromaticum)* (CL), orange (*Citrus reticulata*) (OR), lemon (*Citrus lemon*) (LE), karanja (*Pongamia pinnata*) (KJ) and neem (*Azadirachta indica*) (NE) were purchased from the Yucca Enterprise, Mumbai, India.

Synergistic antimicrobial activity

Synergistic antimicrobial activity of cinnamon oil was done by disk diffusion method against Gram positive bacteria, Gram negative bacteria and fungal strains.

Microorganisms tested

The microorganisms were obtained from National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4 °C. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Bacillus subtilis* (BS) ATCC6633, *Staphylococcus aureus* (SA) ATCC29737, *Corynebacterium rubrum* (CR) ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa* (PA) ATCC9027, *Klebsiella pneumoniae* (KP) NCIM2719 and *Salmonella typhimurium* (ST) ATCC23564. The fungi studied were *Candida glabrata* NCIM3448, *Candida*

albicans ATCC2091, *Cryptococcus neoformans* ATCC34664, *Candida epicola* (CE) NCIM3367.

Antibiotics used in this study

Antibiotic used in the study Ampicillin (AP), Gentamicin (GEN), Chloramphenicol (CH), Penicillin-G (P), Tetracycline (TE), Amphotericin B (AMP), Clotrimazole (CC), Ketoconazole (KT), Fluconazole (FLC), Nystatin (NYS), Itraconazole (IT). All antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., Mumbai, India.

Disk diffusion assay

Synergistic antimicrobial activity of cinnamon oil with antibiotics (AP, CH, GEN, P, TE, AMP, CC, FLC, KT, IT and NYS) and other essential oils (CL, OR, LE, KJ and NE) was assessed against Gram positive bacteria, Gram negative bacteria and fungi by using agar disc diffusion method (Rakholiya and Chanda, 2012). The petri plates were prepared by pouring 20 ml of sterilized molten Mueller Hinton Agar (MHA) for bacteria and 20 ml Sabouraud dextrose agar for fungi, seeded with 200 µl test culture containing 1×10^8cfu/ml as McFarland 0.5 turbidity standard. Plates were allowed to solidify.

Standard antibiotics paper discs (6 mm) were impregnated with 20 µl of cinnamon oil (20% in ethanol) separately. The sterile paper discs were impregnated with 20 µl combination of cinnamon oil (20% in ethanol) with other essential oil (20% in ethanol) and allowed to saturate for 30 min and were placed on the surface of the agar plates which had previously been inoculated with tested microorganisms.

All the plates were incubated for 24 h at 37 °C for bacteria and 48 h 28 °C for fungi. Results were recorded by measuring the zone of inhibition appearing around the discs. All the tests were performed in triplicate and the mean values are presented.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To determine MIC of six essential oils and nine antibiotics, the broth micro-dilution method was performed (Edziri *et al.,* 2012) with some modifications against bacteria and fungi. The inoculums of the test bacteria were prepared using the colony suspension method (EUCAST, 2003). Ninety-six-well culture plates (Tarsons Products Pvt. Ltd.) were used, and serial two-fold dilutions of essential oil (200-6.25 µl/ml) were dispensed into the plate wells. Two-fold dilutions of all antibiotics (32-1 µg/ml) were used. The volume of dispensed drug was 20 µl per well along with 150µl of Mueller Hinton Broth. 30 µl of bacterial culture at a density of 6×10^5 CFU/ml was added to the wells. Three control wells were maintained for each test batch; the positive control (antibiotic, Mueller-Hinton broth and test organism) and sterility control (Mueller-Hinton broth and ethanol) and negative control (Mueller-Hinton broth, test organism and ethanol).The plates were incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. The bacterial activity in the test wells were detected by adding 40 µl of 0.2 mg/ml of 2-(4-Iodo phenyl)-3-(4-nitro phenyl)5-phenyltetrazolium chloride (I.N.T.) (Himedia, India) solution dissolved in sterile distilled water to each well (Frey and Meyers, 2011). The plates were incubated for further 30 min. After addition of INT, inhibition of bacterial growth was visible as a clear well and presence of growth was detected by presence of pink red color (Singh *et al.*, 2010). The lowest concentration (highest dilution) of the essential oil and antibiotics required to inhibit visible growth of the tested microorganism was designated as its MIC.

For determination of minimum bactericidal concentration (MBC), wells showing no growth as well as from the lowest concentration showing growth in the MIC assay for all the samples were chosen. Bacterial cells from the MIC test plates were sub-cultured on freshly prepared solid nutrient agar by making streaks on the surface of the agar. The plates were incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Plates that did not show growth was considered to be the MBC for the drug used (Akinyemi *et al.*, 2005).

Determination of combinations of cinnamon oil with antibiotics/other essential oil

Synergistic antimicrobial activity of the cinnamon oil with antibiotics and other essential oils was assessed against Gram positive bacteria, Gram negative bacteria and fungi. All combinations were mixed at a ratio of 1:1 and were tested for MICs which were determined by micro well dilution method as described above.

The fractional inhibitory concentration (∑FIC) index was calculated by adding the FIC values of antimicrobial compounds (FIC_A+FIC_B). The FIC_A and FIC_B values represent the lowest concentration of oil and antibiotics/extracts respectively that caused the inhibition of bacterial growth in the combination tests. Each of the combination was calculated according to the following formula: $\sum FICI = FIC_A + FIC_B$

For interpretation of the results, ≤ 0.5 was assigned as a synergistic effect, 0.5> ∑FIC ≤ 0.75 represented as a partial synergy, 0.76 to 1.0 represented as an additive effect, >1.0 to 4.0 represented as an indifferent effect and ∑FIC > 4.0 antagonistic effect between two tested antimicrobial agents (Sabate *et al.*, 2012).

Results and Discussion

Synergistic antimicrobial activity

The synergistic antimicrobial activity of cinnamon oil alone, other essential oil alone, commercial antibiotics alone and in combination i.e. their synergistic effect was assessed by measuring their zones of inhibition against various microorganisms.

Antimicrobial activity of cinnamon oil with antibiotics

The antibacterial activity of cinnamon oil alone, five antibiotics alone (AP, CH, GEN, P, and TE) and their synergistic effect against four Gram positive bacteria is given in Fig.1. The essential oil CN alone showed antibacterial activity against all the four Gram positive bacteria though the level of activity varied. The highest zone of inhibition was against *B. cereus* (39.5 mm). All the four Gram positive bacteria were susceptible to all the five antibiotics alone except *B. cereus* which was resistant to AP and P; and *C. rubrum* which was resistant to GEN. The synergistic activity was envisaged almost with all the 5 antibiotics against all the four bacterial strains with different level of inhibitory activity (Fig.1). Cinnamon oil with antibiotic AP showed synergistic antibacterial activity against *S. aureus* and *C. rubrum* (Fig. 1A); while antibiotic CH showed synergistic activity against *B. subtilis* and *C. rubrum* (Fig. 1B); antibiotic GEN showed synergistic antibacterial activity against *B. cereus* (Fig. 1C); antibiotic P showed synergistic antibacterial activity against *S. aureus* and *C. rubrum* (Fig. 1D) and antibiotic TE showed synergistic antibacterial activity against *B. cereus* and *C. rubrum* (Fig. 1E).

The antibacterial activity of cinnamon oil alone, five antibiotics alone (AP, CH, GEN, P, and TE) and their synergistic effect against

four Gram negative bacteria is given in Fig. 2. The cinnamon oil alone showed antibacterial activity against three Gram negative bacteria (*E. coil*, *P. aeruginosa* and *S. typhimurium*) except *K. pneumoniae*. The highest zone of inhibition was against *E. coil* (15 mm). *E. coil* was susceptible to four antibiotics (AP, CH, GEN and TE) but resistant to antibiotic P. *K. pneumoniae* was resistant but *S. typhimurium* was susceptible to all the five antibiotics. *P. aeruginosa* was susceptible to GEN and TE but resistant to the other three antibiotics. The antibiotics AP, GEN and P did not show any synergistic activity against any of the four Gram negative bacteria. Antibiotic CH and TE showed synergistic activity against *E. coil* and *S. typhimurium* (Fig. 2B and 2E).

The cinnamon oil alone showed inhibitory antifungal activity against all the four fungi, though the level of activity varied (Fig. 3). The highest zone of inhibition was against *C. albicans* (38.5 mm).

All the four fungi were susceptible to all the six antibiotics except *C. albicans* which was resistant to AP and *C. neoformans* which was resistant to FLC. A very slight synergistic activity was envisaged against fungi. Cinnamon oil plus antibiotic AMP and KT showed synergistic activity against only *C. glabrata* (Fig. 3A and 3E).

Antimicrobial activity of cinnamon oil with other essential oils

The antibacterial activity of cinnamon oil alone, other essential oils alone (CL, KJ, LE, OR and NE) and their synergistic effect against four Gram positive bacteria is given in Fig.4. CL oil alone showed very slight antibacterial activity against *B. cereus* and *C. rubrum*. The other 4 essential oils (KJ, LE, OR and NE) alone did not show any activity. Cinnamon oil with KJ oil showed synergistic antibacterial activity against *C. rubrum and B. subtilis*. None of the oils showed antagonistic effects except OR oil against *S. aureus* (Fig. 4). CL oil alone showed activity against *K. pneumoniae* and *P. aeruginosa* while KJ, LE and NE oils alone showed activity against only KP while OR oil alone did not show any activity. Cinnamon oil with CL, KJ, LE, OR and NE oil showed synergistic antibacterial activity against KP. However, no antagonistic activity was envisaged (Fig. 5). CL oil alone showed slight antifungal activity against all the four fungi. The other KJ, LE, OR and NE essential oils alone did not show any antifungal activity. Five essential oils in combination with cinnamon oil showed antifungal activity against fungi but no synergistic activity was envisaged. However, no antagonistic activity was envisaged (Fig. 6).

The antimicrobial activity of CN oil alone and its synergistic activity i.e. combination with antibiotics/ other essential oils were investigated which showed varied levels of antimicrobial activity by disc diffusion method. CN oil with all the five antibiotics (AP, CH, GEN, P, TE) and CN with KJ oil showed maximum synergistic antibacterial activity against Gram positive bacteria *C. rubrum*.

CN oil with antibiotics CH and TE also showed synergistic antibacterial activity against *E. coli*; CN oil with OR oil showed synergistic antibacterial activity against *K. pneumoniae.* CN oil plus antibiotic AMP and KT showed synergistic activity against only *C. glabrata***.**

So, combination of CN oil with antibiotics/other essential oils were taken up for further study against two Gram positive (*C. rubrum* and *B. subtilis*), Gram negative (*E. coli* and *P. aeruginosa*) bacteria and Fungi (*C. albicans* and *C. neoformans*) by minimum inhibitory concentration.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC values of essential oil (CN, CL, OR, LE, NE and KJ) and antibiotic (AP, CH, GEN, P, TE, AMP, FLC, KT and NS) were tested for its antimicrobial activity at various concentration against two Gram positive, two Gram negative and two fungi strain. The values are presented in Table 1 and 2.

For Gram positive bacteria strains, MIC and MBC values of all five antibiotics ranged from 1 to 32 µg/ml and 4 to >32 µg/ml respectively. *B. subtilis* was most susceptible bacterial pathogen to GEN and TE (MIC-1µg/ml). *C. rubrum* was also susceptible bacterial pathogen to GEN and P (MIC-1µg/ml). The MIC and MBC value of all six essential oil ranged from 12.5 to 200 µl/ml and 50 to >200 µl/ml respectively. *B. subtilis* was most susceptible bacterial pathogen to CL and LE oil (MIC – 12.5µl/ml).

Fig.1 Synergistic antibacterial activity of cinnamon oil with antibiotics against Gram positive bacteria (A = Ampicillin, B = Chloramphenicol, C = Gentamicin, D = Penicillin, E =Tetracycline)

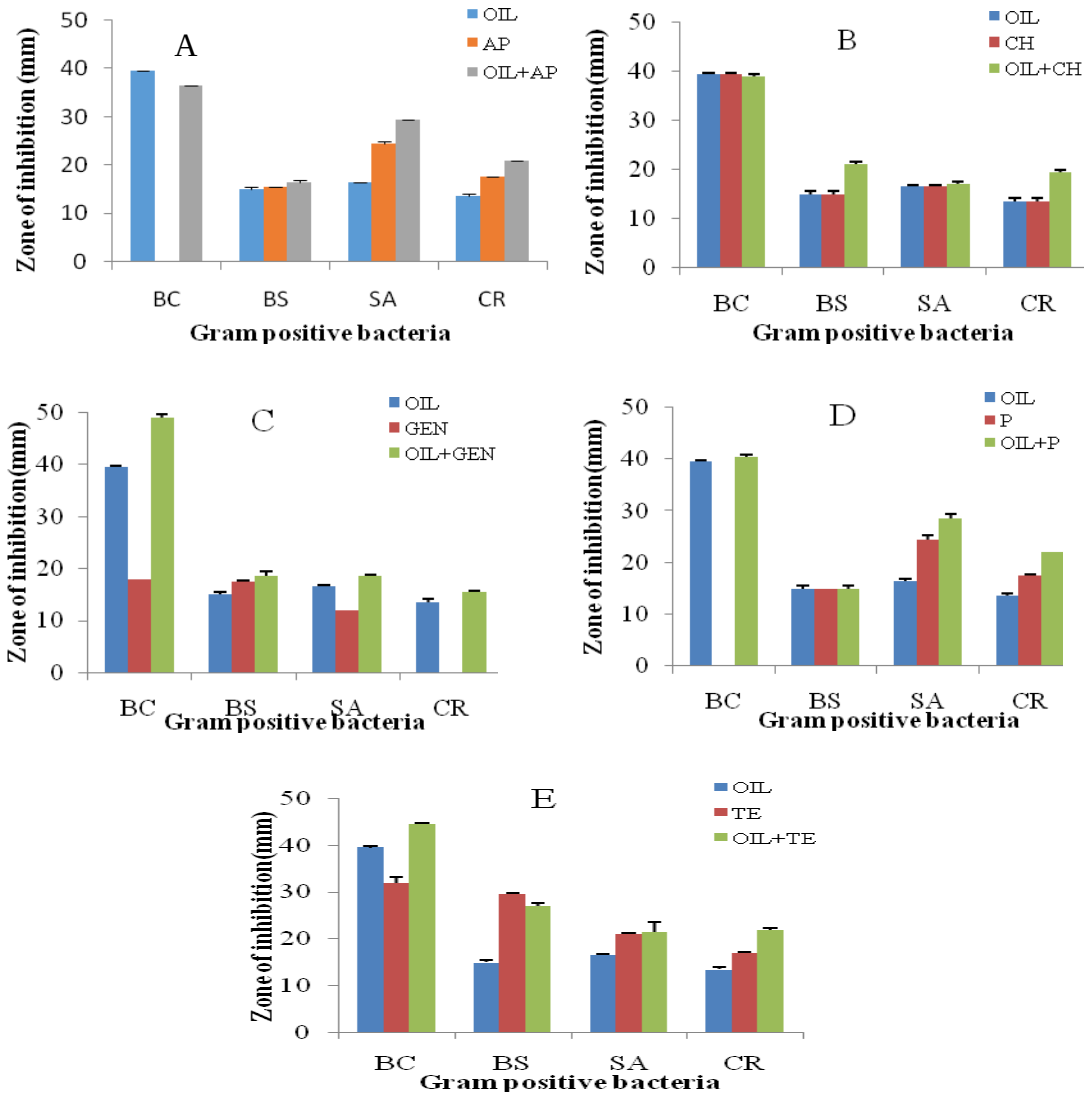

Fig.2 Synergistic antibacterial activity of cinnamon oil with antibiotics against Gram negative bacteria (A = Ampicillin, B = Chloramphenicol, C = Gentamicin, D = Penicillin, E =Tetracycline)

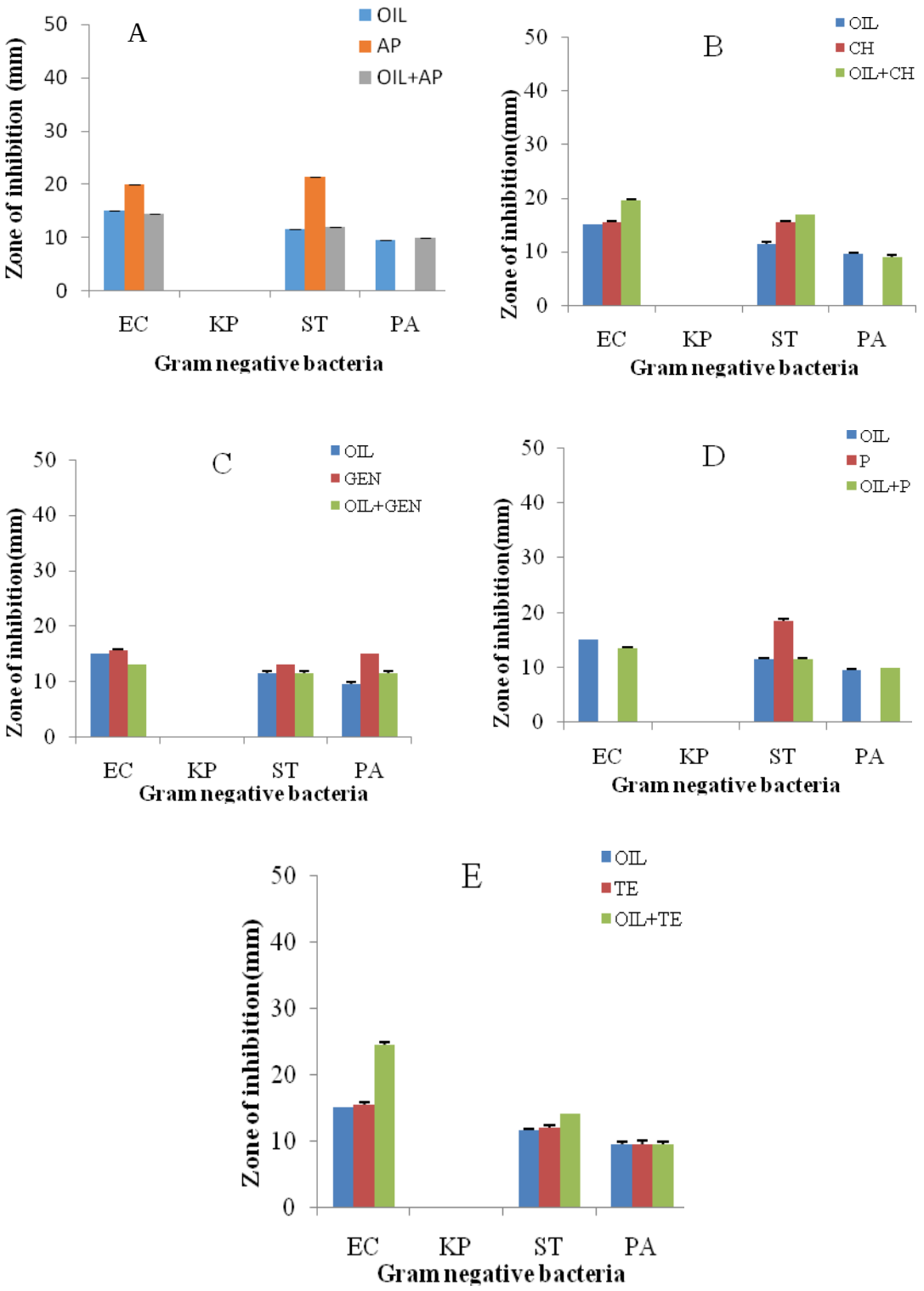

Fig.3 Synergistic antifungal activity of cinnamon oil with antibiotics against Fungi (A= Amphotericin, B= Clotrimazole, C= Fluconazole, D= Itraconazole, E=Ketoconazole, F= Nystatin)

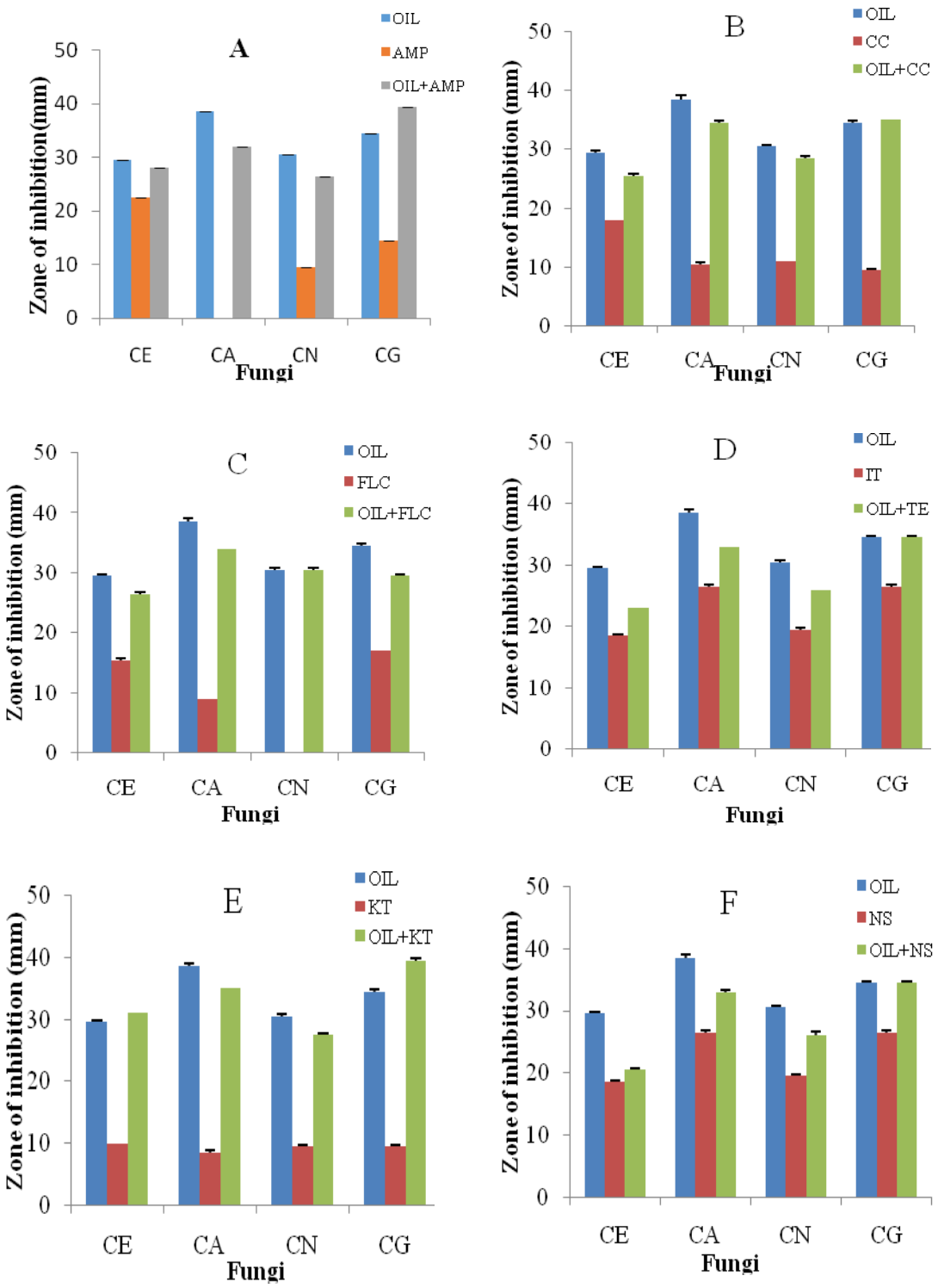

Fig.4 Synergistic antibacterial activity of cinnamon oil with other oil against Gram positive bacteria (A= Clove oil, B= Karanja oil, C= Lemon oil, D= Orange oil, E= Neem oil)

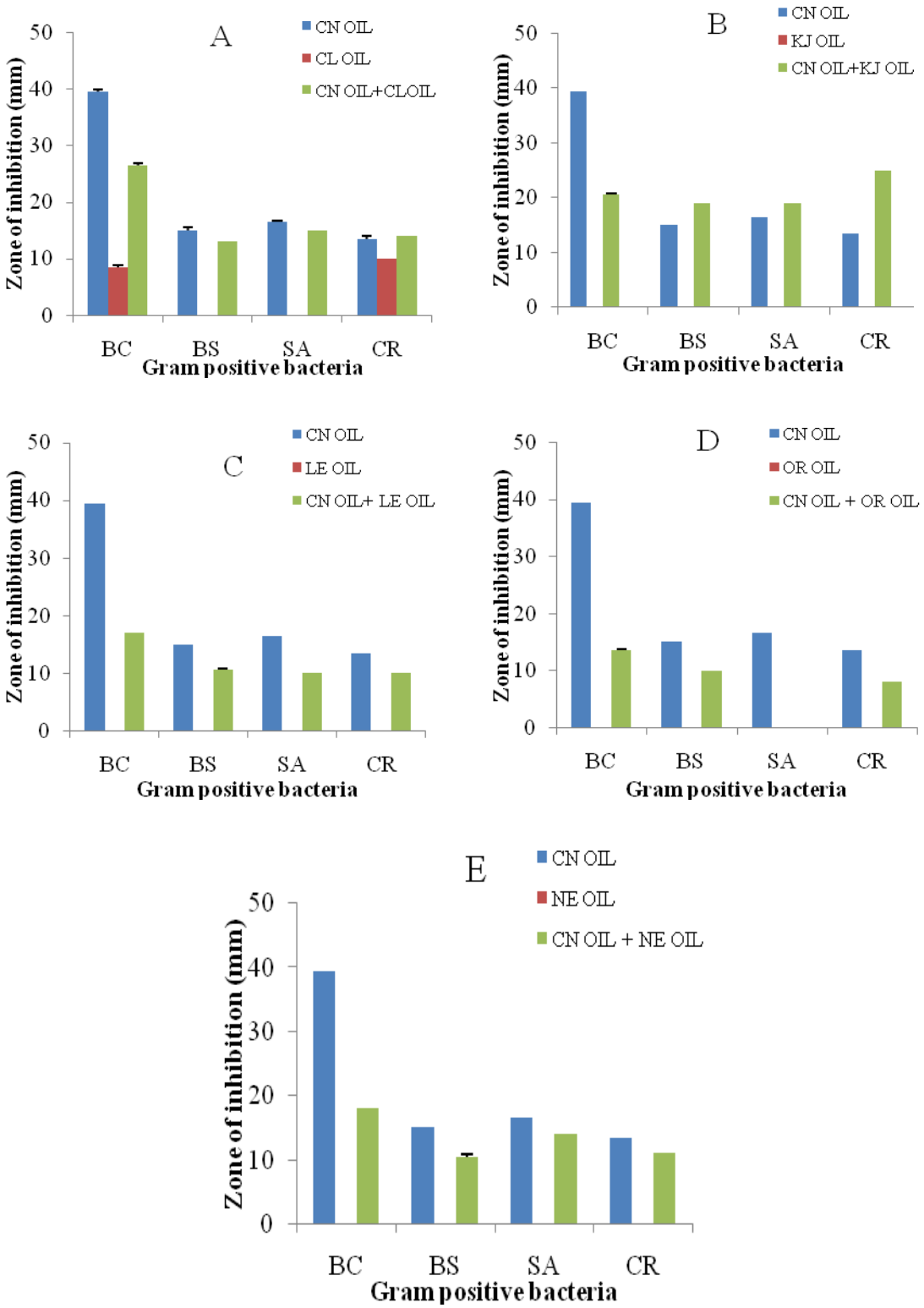

Fig.5 Synergistic antibacterial activity of cinnamon oil with other oil against Gram negative bacteria (A= Clove oil, B= Karanja oil, C= Lemon oil, D= Orange oil, E= Neem oil)

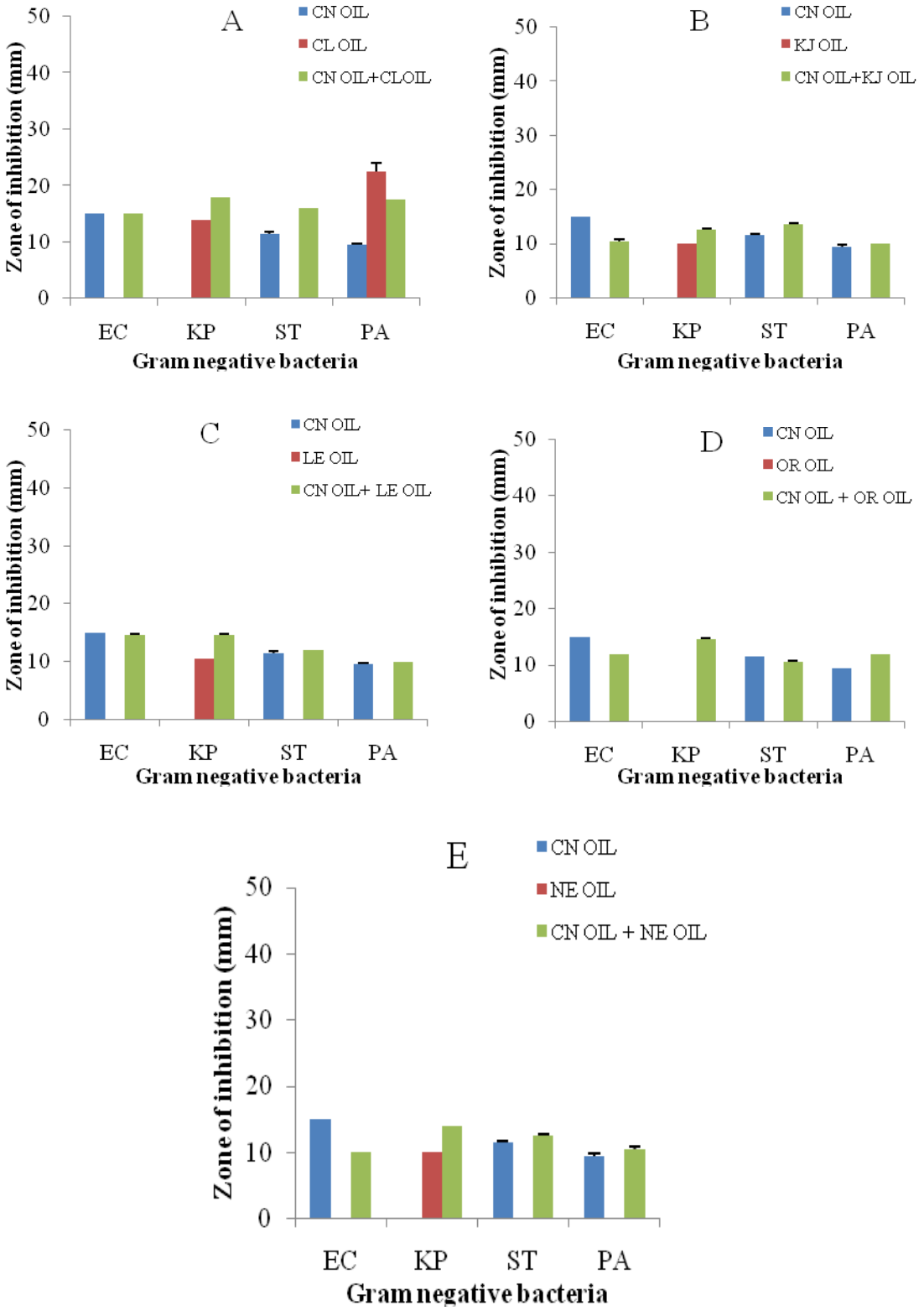

Fig.6 Synergistic antifungal activity of cinnamon oil with other oil against Fungi (A= Clove oil, B= Karanja oil, C= Lemon oil, D= Orange oil, E= Neem oil)

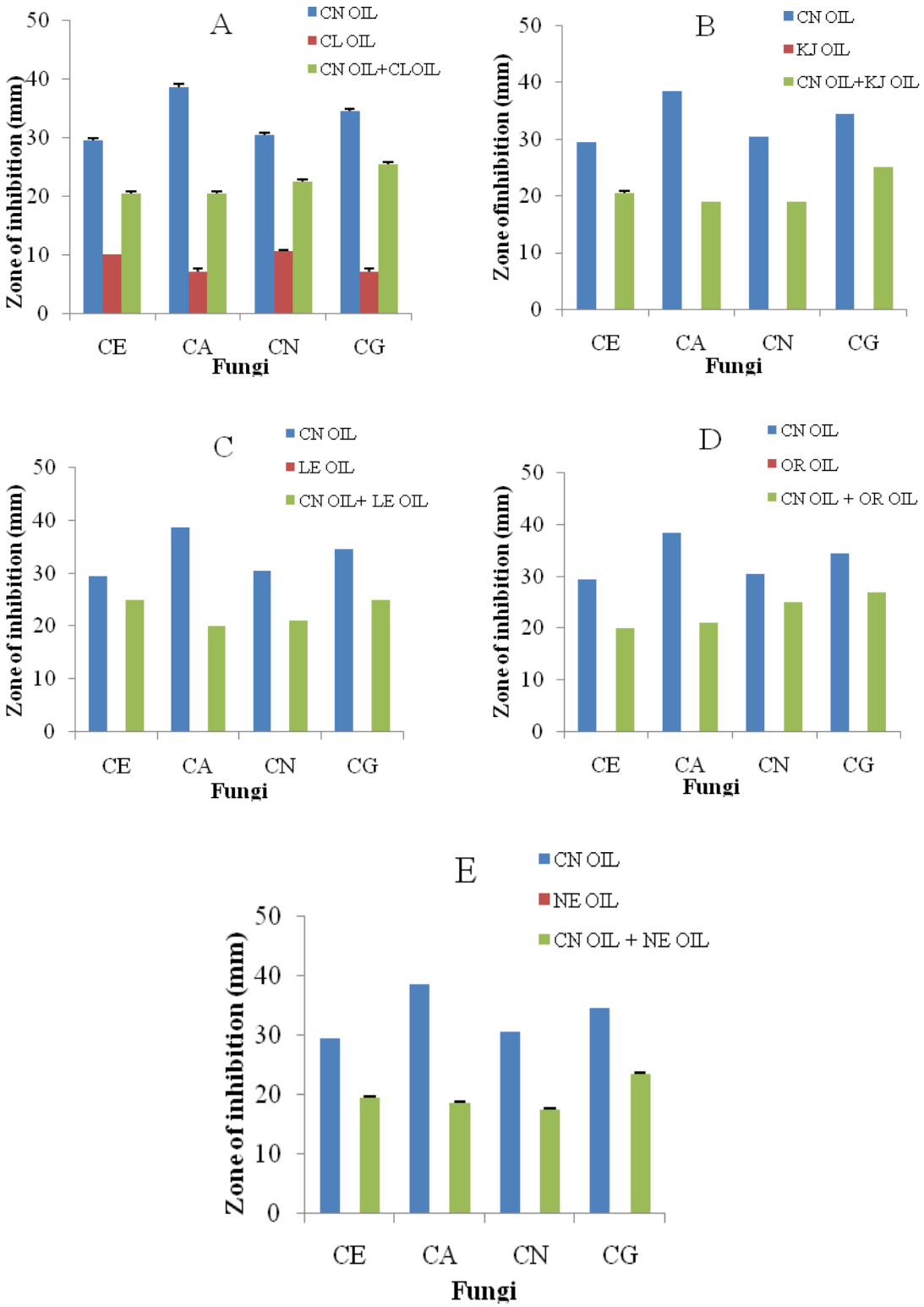

Table.1 MIC and MBC values of antibiotics/essential oil against Gram positive and negative bacteria

Antibiotics/Oils	Organisms							
	CR		BS		EC		PA	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ampicillin	4	16	32	>32	32	>32	>32	>32
Chloramphenicol	32	>32	16	32	16	32	8	16
Gentamicin	1	32	1	16	2	8	1	8
Penicillin	1	16	32	>32	>32	>32	>32	>32
Tetracycline	2	4	1	8	2	4	1	4
Cinnamon oil	100	100	200	>200	25	>200	12.5	>200
Clove oil	50	>200	12.5	50	12.5	>200	25	>200
Orange oil	25	>200	25	100	12.5	>100	12.5	100
Lemon oil	25	50	12.5	100	25	>200	6.25	>200
Karanj oil	12.5	100	50	>200	100	>200	25	>200
Neem oil	100	>200	50	>200	200	>200	100	100

Table.2 MIC and MBC values of antibiotics/essential oil against fungi

Antibiotics/Oils	Fungi			
	CA		CN	
	MIC	MBC	MIC	MBC
Amphotericin	>32	>32	>32	>32
Fluconazole	8	>32	2	>32
Ketoconazole	>32	>32	>32	>32
Nystatin	32	>32	>32	>32
Cinnamon oil	50	>200	100	>200
Clove oil	6.25	>200	12.5	>200
Orange oil	100	>200	100	>200
Lemon oil	6.25	>200	6.25	>200
Karanj oil	25	>200	25	>200
Neem oil	100	>200	>200	>200

Table.3 (A) MIC and MBC values for the combination of cinnamon oil with antibiotic and other essential oils against Gram positive bacteria

Combinations	Organisms											
	CR						BS					
	Alone		Combination		∑FIC		Alone		Combination		∑FIC	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
CN+AP	100/4	100/16	6.25/1	>200/>32	0.312	ND	200/32	>200/>32	12.5/2	>200/>32	0.125	ND
CN+ CH	100/32	100/>32	25/4	>200/>32	0.375	ND	200/16	>200/32	12.5/2	>200/>32	0.187	ND
CN +GEN	100/1	100/32	12.5/2	>200/>32	2.125	ND	200/1	>200/16	50/8	>200/>32	8.25	ND
CN+ P	100/1	100/16	50/8	100/16	8.5	2	200/32	>200/>32	12.5/2	100/16	0.125	ND
CN+ TE	100/2	100/4	12.5/2	>200/>32	1.125	ND	200/1	>200/8	25/4	>200/>32	4.125	ND
CN +CL	100/50	100/>200	50/50	100/100	1.5	ND	200/12.5	>200/50	12.5/12.5	>200/>200	1.062	ND
CN +OR	100/25	100/>200	50/50	>200/>200	2.5	ND	200/25	>200/100	50/50	>200/>200	2.25	ND
CN+ LE	100/25	100/50	100/100	>200/>200	5	ND	200/12.5	>200/100	6.25/6.25	100/100	0.527	ND
CN +KJ	100/12.5	100/100	100/100	>200/>200	9	ND	200/50	>200/>200	100/100	>200/>200	2.5	ND
CN+ NE	100/100	100/>200	50/50	200/200	1	ND	200/50	>200/>200	100/100	>200/>200	2.5	ND

MIC and MBC values for essential oil were expressed in µl/ml (v/v). MIC and MBC values for antibiotics were expressed in µg/ml (w/v). ΣFIC (Fractional Inhibitory Concentration Index) = $FIC_A + FIC_B$; FIC_A = (MIC_A combination /MIC_A alone);FIC_B = (MIC_B combination /MIC_B alone); Results interpreted as follows : ≤ 0.5 was assigned as a synergistic effect, 0.5> ∑FIC ≤ 0.75 represented as a partial synergy, 0.76 to 1.0 represented as an additive effect, >1.0 to 4.0 represented as an indifferent effect and ∑FIC > 4.0 antagonistic effect ND = Not determined because of high MIC value

Table.3 (B) MIC and MBC values for the combination of cinnamon oil with antibiotic and other essential oils against Gram negative bacteria

Combinations	Organisms											
	EC						PA					
	Alone		Combination		∑FIC		Alone		Combination		∑FIC	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
CN+AP	25/32	>200/>32	12.5/2	100/16	0.562	ND	12.5/>32	>200/>32	25/4	200/32	ND	ND
CN+ CH	25/16	>200/32	12.5/2	200/32	0.625	ND	12.5/8	>200/16	50/8	200/32	5	ND
CN +GEN	25/2	>200/8	25/4	100/16	3	ND	12.5/1	>200/8	100/16	>200/32	24	ND
CN+ P	25/>32	>200/>32	100/16	>200/>32	ND	ND	12.5/>32	>200/>32	12.5/2	50/8	ND	ND
CN+ TE	25/2	>200/4	6.25/1	50/8	0.75	ND	12.5/1	>200/4	12.5/2	100/16	3	ND
CN +CL	25/12.5	>200/>200	6.25/6.25	50/50	0.75	ND	12.5/25	>200/>200	100/100	100/100	12	ND
CN +OR	25/12.5	>200/>100	6.25/6.25	100/100	0.75	ND	12.5/12.5	>200/100	50/50	200/200	8	ND
CN+ LE	25/25	>200/>200	25/25	200/200	2	ND	12.5/6.25	>200/>200	200/200	>200/>200	48	ND
CN +KJ	25/100	>200/>200	100/100	>200/>200	5	ND	12.5/25	>200/>200	100/100	200/200	12	ND
CN+ NE	25/200	>200/>200	12.5/12.5	100/100	0.50	ND	12.5/100	>200/100	100/100	>200/>200	9	ND

MIC and MBC values for essential oil were expressed in µl/ml (v/v). MIC and MBC values for antibiotics were expressed in µg/ml (w/v). ΣFIC (Fractional Inhibitory Concentration Index) = FIC_A + FIC_B; FIC_A = (MIC_A combination /MIC_A alone);FIC_B = (MIC_B combination /MIC_B alone); Results interpreted as follows : ≤ 0.5 was assigned as a synergistic effect, 0.5> ∑FIC ≤ 0.75 represented as a partial synergy, 0.76 to 1.0 represented as an additive effect, >1.0 to 4.0 represented as an indifferent effect and ∑FIC > 4.0 antagonistic effect ND = Not determined because of high MIC value

Table.3 (C) MIC and MBC values for the combination of cinnamon oil with antibiotic and other essential oils against Fungi.

Organisms												
Combinations	**CA**						**CN**					
	Alone		**Combination**		**∑FIC**		**Alone**		**Combination**		**∑FIC**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CN+ AMP	50/>32	>200/>32	50/8	100/16	ND	ND	100/>32	>200/>32	200/32	>200/>32	ND	ND
CN+FLC	50/8	>200/>32	50/8	>200/>32	2	ND	100/2	>200/>32	50/8	>200/>32	4.5	ND
CN+ KT	50/>32	>200/>32	50/8	200/32	ND	ND	100/>32	>200/>32	6.25/1	100/16	ND	ND
CN +NS	50/32	>200/>32	100/16	>200/>32	2.5	ND	100/>32	>200/>32	200/32	>200/>32	ND	ND
CN +CL	50/6.25	>200/>200	6.25/6.25	50/50	1.125	ND	100/12.5	>200/>200	6.25/6.25	100/100	0.562	ND
CN +OR	50/100	>200/>200	200/200	>200/>200	6	ND	100/100	>200/>200	25/25	>200/>200	0.5	ND
CN+ LE	50/6.25	>200/>200	100/100	>200/>200	18	ND	100/6.25	>200/>200	>200/>200	>200/>200	ND	ND
CN +KJ	50/25	>200/>200	200/200	>200/>200	12	ND	100/25	>200/>200	12.5/12.5	>200/>200	0.625	ND
CN+ NE	50/100	>200/>200	100/100	>200/>200	3	ND	100/>200	>200/>200	200/200	>200/>200	ND	ND

MIC and MBC values for essential oil were expressed in µl/ml (v/v). MIC and MBC values for antibiotics were expressed in µg/ml (w/v). ΣFIC (Fractional Inhibitory Concentration Index) = FIC_A + FIC_B; FIC_A = (MIC_A combination /MIC_A alone); FIC_B = (MIC_B combination /MIC_B alone); Results interpreted as follows : ≤ 0.5 was assigned as a synergistic effect, 0.5> ∑FIC ≤ 0.75 represented as a partial synergy, 0.76 to 1.0 represented as an additive effect, >1.0 to 4.0 represented as an indifferent effect and ∑FIC > 4.0 antagonistic effect ND = Not determined because of high MIC value

For Gram negative bacteria strains, MIC and MBC values of all five antibiotic ranged from 1 to >32 µg/ml and 4 to >32 µg/ml respectively. *P. aeruginosa* was most susceptible bacterial pathogen to GEN and TE (MIC- 1µg/ml). The MIC and MBC value of all six essential oil ranged from 6.25 to 200 µl/ml and 100 to >200 µl/ml respectively. *P. aeruginosa* was most susceptible bacterial pathogen to LE oil (MIC – 6.25 µl/ml).

For fungal strains, MIC and MBC values of all six antibiotic ranged from 2 to >32 µg/ml and >32 µg/ml respectively. *C. neoformans* was most susceptible fungal pathogen to FLC (MIC- 2 µg/ml). The MIC and MBC values of all six essential oil ranged from 6.25 to >200 µl/ml and >200 µl/ml respectively. CL and KR oil effectively inhibit both the fungi (MIC – 6.25µl/ml).

Amongst the tested microbial strains *C. neoformans*, *C. albicans*, *P. aeruginosa* was inhibited by LE oil with list MIC values 6.25 µl/ml. Its MBC values were >200 µl/ml. While amongst six essential oils CL and LE oil showed good antimicrobial activity followed by OR oil against bacteria and fungi.

Synergistic antimicrobial activity of cinnamon oil

Synergistic antimicrobial activity of cinnamon oil with antibiotic and other essential oil were evaluated against two Gram positive, two Gram negative and two fungi. Synergistic effect was determined by calculation FIC index. Results are interpreted as follows: ≤ 0.5 = synergistic; >0.5 to 0.75 = partially synergistic; 0.76 to 1.0 = additive; >1.0 to 4.0 = indifferent and > 4.0 = antagonistic; ND = Note determined because of high MIC value >32µg/ml and >200 µl/ml.

Synergistic antibacterial activity of cinnamon oil against Gram positive bacteria is summarized in the Table 3(A). The FIC indices of combination of CN oil ranged from 0.312 to 2.125 against *C. rubrum* and 0.125 to 8.25 against *B. subtilis.* The combination of CN+AP and CN+CH showed synergistic antibacterial activity against *C. rubrum* and *B. subtilis*. Combination of CN+P and CN+LE showed synergistic and partial synergies antibacterial activity against *B. subtilis* with FIC indices 0.125 and 0.527 respectively. The remaining combination showed additive / indifferent/antagonistic effect against *C. rubrum* and *B. subtilis*.

Synergistic antibacterial activity of cinnamon oil against Gram negative bacteria is summarized in Table 3(B). The FIC indices of combination of CN oil ranged from 0.50 to 5 against *E. coli* and 3 to 48 against *P. aeruginosa*. The combinations of CN +NE showed synergistic antibacterial activity against *E. coli* with FIC indices was 0.50. The combinations of CN+AP, CN+TE, CN+CH, CN+CL and CN+OR showed partial synergistic antibacterial activity against *E. coli*. Remaining combination showed additive / indifferent /antagonistic effect against *E. coli*. None of the combination showed synergistic antibacterial activity against *P. aeruginosa*.

Synergistic antifungal activity of cinnamon oil against fungi is summarized in Table 3(C). The FIC indices of combination of CN oil ranged from 1.124 to 18 against *C. albicans* and 0.562 to 4.5 against *C. neoformans*.

The combination of CN+CL and CN+KJ showed partial synergistic antifungal activity against fungi *C. neoformans* with FIC indices 0.562 to 0.625 respectively.

The remaining combinations showed additive/indifferent/antagonistic effect against *C. neoformans*. None of the combinations showed synergistic antifungal activity.

Comparison of CN oil combinations with antibiotic/other essential oils, combination of CN oil with antibiotics showed better synergistic antimicrobial activity as compared to CN oil with other essential oils. Combination of CN oil with AP and CH antibiotic showed best synergistic antibacterial activity against *C. rubrum, B. subtilis* and partial synergistic activity against *E. coli*. Luis *et al.,* (2016) also reported synergistic antibacterial activity of two essential oils *Eucalyptus globulus* and *Eucalyptus radiate* with conventional antibiotic against *Acinetobacter baumannii*. Silva *et al.,* (2011) also found synergistic antifungal activity of *Coriandrum sativum* essential oil with amphotericin B against *Candida tropicalis and Candida albicans.*

Combination of CN oil with NE oil showed synergistic activity against *E. coli* while Combination of CN oil with CL and KJ oil showed partial synergistic activity against *C. neoformans*. Matam *et al.,* (2006) analyzed that mixture of cinnamon and clove essential oils showed synergistic antimicrobial activity against food spoilage microorganisms. Similar results are reported by Pekmezovic *et al.,* (2015). According to them *Thymus vulgaris* and *Cinnamomum cassia* essential oil combination showed synergistic antifungal activity. *C. rubrum* and *E. coil* were the most susceptible bacterial pathogens.

Synergistic effect of natural product and conventional antimicrobial agents against infectious pathogen is growing field of herbal medicine (Wagner and Ulrich-Merzenich, 2009).

The increase in synergistic antimicrobial effect may be caused by the reaction between essential oil and antibiotics or other essential oil. The cinnamon oil contains major bioactive components Cinnamaldehyde which can synchronize easily with antibiotic and other essential oil functional group and exert synergistic antimicrobial activity. It has been previously reported that cinnamaldehyde was able to inhibit the synthesis of essential enzyme of microorganisms and resulted in the damage and cell death of the microorganisms (Helander *et al.,* 1998; Di Pasqua *et al.,* 2007). There for it the observed synergistic antimicrobial activity of cinnamon oil may be due to the various compounds presentin the oil especially cinnamaldehyde.

The present findings showed that most of the tested essential oil combinations inhibited *in vitro* microbial growth with different degree depending on the type of oil or antibiotic, microorganism, concentration etc. Among the different combination of CN oil, CN oil with antibiotic AP and CN oil with CL oil was found to be the most effective. This essential oil combination i.e. CN oil plus AP and CN oil plus CL oil can be used as new treatment modalities to treat the bacterial infections. This combination may reduce antibiotic minimum effective dose and thus can minimize potential antibiotic side effects and prevent the emergence of antibiotic resistance.

Combination of cinnamon oil with antibiotic and other essential oil exhibited stronger antimicrobial properties against pathogens by disc diffusion method and broth dilution method (MIC).

In addition, combination also showed partial synergistic and additive effect can also be beneficial for development of novel antimicrobial agents. Among the different combinations, Cinnamon oil with ampicillin and Cinnamon oil with Clove oil showed good antimicrobial activity against pathogenic microorganisms even at low MIC value. However more studies are needed to understand the mechanism of action responsible for antimicrobial activity.

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