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Synergistic Effect of the Combination of Commercial Essential Oils with Standard Antibiotics: *In vitro* Evaluation

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Abstract. The aim of this investigation was to determine the antibacterial activity of essential oils and to assess the outcomes produced by the combinations of antibiotics and essential oils. To execute this research, gold standard and conventional methods were used. Antibacterial potency of five essential oils namely *Citrus limon*, *Elettaria cardamomum*, *Lavandula angustifolia*, *Nigella sativa* and *Prunus dulcis* were tested against *Escherichia coli*, *Serratia fonticola*, *Serratia liquefaciens*, *Citrobacter freundii* and *Staphylococcus aureus* recouped from street foods of Karachi. Among five of them, *Citrus limon* and *Lavandula angustifolia* were the most potent essential oils showing highest antibacterial activity in their undiluted form with the exception of *Staphylococcus aureus* but their mix at different concentrations successfully inhibited the growth of *Staphylococcus aureus* and *Serratia fonticola*. Synergistic outcomes were achieved against all the tested bacterial strains from the mix of essential oils and antibiotics, however antagonistic results were also obtained. This exploration underpins the application of essential oils alone and in combinations with antimicrobial agents to improve the affectability of ineffective drugs and aides in the advancement of new antimicrobial drugs to treat bacterial infections utilizing therapeutic plants.

Keywords: *Citrus limon*, *Elettaria cardamomum*, *Lavandula angustifolia*, *Nigella sativa*, *Prunus dulcis*, synergy, foodborne bacteria

Introduction

Expanding resistance against antimicrobial agents will swiftly increase the odds of morbidity and mortality rate which could seriously influence the health of public. Antimicrobial resistance is turning into a noteworthy issue and the ultimate challenge for the researchers these days. Ready-to-eat foods contaminated with micro-organisms are liable for serious life threatening illnesses and an important source of extending resistance from one bacterium to another. From a previous couple of decades, it has been seen that there is an expansion in the cases of foodborne sicknesses and with the progression of time, food isolates are becoming more resistant to antibacterial agents subsequently, their treatment has become more trouble some (Angulo *et al*., 2009; Yücel *et al*., 2005; Threlfall *et al*., 2000).

Traditionally, essential oils have been utilized for down the ages to treat several infectious diseases around the world. Essential oils can be used in different fields such as food, beverages, fragrance and cosmetic industry. The application of essential oils is broad spectrum and also possess varieties of biological activities that can perform an extraordinary part in battling micro-organisms (Silva *et al*., 2010). Several researches have been proven the inborne antimicrobial attributes of essential oils and currently natural therapeutic remedies are the choice of interest over synthetic ones because essential oils are not only used in monotherapy but have been used in combination for many years to reduce the harm of antibiotics (de Rapper *et al*., 2013; Liu and Nakano, 1996). It was stated in several studies that constituents of essential oils are partitioned into two broad range groups of terpenes, terpenoids, flavonoids, aliphatic compounds and some hydrocarbon that supposed to harm and disturb the cell wall and membrane of micro-organisms by their mechanism of action (Faleiro, 2011; Pichersky *et al*., 2006). Compounds that are actually present in essential oils obtained from plants, spices and herbs are the primary constituents that can combat against micro-organisms (Kim *et al*., 1995; Deans and Ritchie, 1987; Janssen *et al*., 1985). Therefore, essential oils are comprehensively utilized as a part of the medicinal world as natural therapeutic agent. Mode of action of essential oils could be bacteriostatic or bactericidal relying on the concentrations

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used (Guynot *et al*., 2003). Essential oils of *Citrus limon*, *Lavandula angustifolia*, *Nigella sativa*, *Elettaria cardamomum*, and *Prunus dulcis* are well known for their antibacterial, antifungal, antioxidant, and insecticidal properties and many researches have been done on these essential oils (Prabuseenivasan *et al*., 2006; Cavanagh and Wilkinson, 2002; Randhawa and Al-Ghamdi, 2002) however, very limited literature is available on essential oil of *Prunus dulcis* and its antimicrobial competences.

The bacterial strains selected in this investigation were recovered from street foods, a research was conducted on the microbial quality of street foods vending in Karachi city (Mehboob and Abbas, 2019). Out of five foodborne bacteria, four were commensal bacteria and one was foodborne pathogen. Food-related diseases are becoming one of the most hazardous worldwide problems. It is estimated that around 30% of food poisoning cases are reported each year along with 70% of potential outbreaks risks are associated with street foods consumption (Canini *et al*., 2013). On the contrary, commensal bacteria are generally harmless for an immuno competent person but they can transfer the resistant gene from one bacterium to another (Paterson, 2006). Researchers mainly focus on the foodborne pathogens like *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus* and *Shigella* spp but these commensal bacteria cannot be neglected as the commensal *Escherichia coli* have the higher prevalence rate of drug resistance than same herd of MDR *Salmonella* species was depicted by (DeFrancesco *et al*., 2004).

Because of massive resistance produced by microbes through various mutational process against antimicrobial agents creating pressure on researchers to discover new approaches or remake old ones by using essential oils and plant extracts alone or in combinations for the treatment of diseases (Fisher and Phillips, 2009; Rios and Recio, 2005). Therefore, the aim of this study was the assessment of the antimicrobial significance of essential oils and their *in vitro* possible interactions with antibiotics against bacterial strains recovered from the street foods of Karachi.

Materials and Methods

Essential oils. Five essential oil samples of almond (*Prunus dulcis*), black seed (*Nigella sativa*), green cardamom (*Elettaria cardamomum*), lavender (*Lavandula angustifolia*), and lemon (*Citrus Limon*) were used in this study to evaluate their antimicrobial potentials. Essential oils of *Prunus dulcis* and *Nigella sativa* were commercially purchased from local market, while essential oils of *Elettaria cardamomum*, *Lavandula angustifolia,* and *Citrus limon* were kindly supplied by Noor Oil depot, Karachi, Pakistan.

Tested microorganisms. The antibacterial activity of these commercially purchased EO was assessed against five foodborne bacteria that recuperated from the famous street food shops of Karachi. All these five bacterial strains including gram-negative *Escherichia coli, Citrobacter freundii, Serratia fonticola, Serratia liquefaciens,* and gram-positive *Staphylococcus aureus* were isolated and identified according to Bergey's manual of determinative biology (Holt *et al*., 1994) and ABIS online software (Costin and Ionut, 2017).

Antimicrobial screening of essential oils. *Drop agar diffusion method.* Drop agar diffusion technique was executed to evaluate the antibacterial potentials of undiluted EO proposed by (Lopes-Lutz *et al*., 2008; Cruz *et al*., 2007; Hili, 2001; Hammer *et al*., 1999). The Mueller Hinton agar plates were inoculated with the test organisms standardize to 0.5 McFarland concentration. 10 µL drop of essential oil was placed on Mueller Hinton agar plates and then left untouched for proper dispersion of oils at ambient temperature. After a while, plates were incubated at 37 ± 1 °C for 24 h. After incubation, zone of inhibition around each drop was measured in millimeters.

Agar well diffusion method. To analyze the alone and combined effects of EO, the agar well diffusion method was performed according to the method proposed by (Pasha *et al*., 2018; Martins *et al*., 2013).

Minimal inhibitory concentration (MIC). Minimum inhibitory concentration is generally considered as a measure of antimicrobial performance of EO. Determination of MIC assay was accomplished as described by (Weerakkody *et al*., 2010). Different concentrations of EO (1000, 500, 250, 125, 62.5, 15.625 µg/mL) were prepared in 40% DMSO. 1000 µL aliquots of sterile Mueller Hinton broth was dispensed in Eppendorf tubes containing various concentrations of EO and 10 µL bacterial suspension (0.5 McFarland concentration). Positive control also run concurrently. Then tubes were

incubated for overnight at 37 ± 1 °C. After incubation, bacterial growth was observed by the presence and absence of turbidity (Han *et al*., 2008).

Antibiotics. The standard antibiotics used were tetracycline (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), amoxicillin (10 µg), levofloxacin (5 µg), streptomycin (10 µg), and oxacillin (1 µg). These antibiotics were purchased from Thermo Fischer Scientific Oxoid ltd.

Determination of the synergistic effect of antibiotics and essential oils on tested organisms. To evaluate the combined impact of both EO and standard antibiotics on tested organisms, disk diffusion method was performed as described by (Mahmoud *et al*., 2016; Moussaoui and Alaoui, 2016; Toroglu, 2007). 10 µL drop of undiluted EO were soaked on antibiotics disc, then plates were allowed to dry for proper diffusion of oils and then plates were incubated at 37 ± 1 °C for overnight. Next day the zone of inhibition around each disc was observed and results were compared between alone and combined interaction of EO and antibiotics.

Scanning electron microscopy. Scanning electron microscopy was performed with JEOL from Japan (model # JSM-6380) located at Centralized Science Laboratories, University of Karachi. Fresh culture of selected strains of *Escherichia coli* and *Staphylococcus aureus* (18 h old) were standardized with 0.5 McFarl and index. 10 µL of each selected bacterial suspension was inoculated into the micro centrifuge tube containing Mueller Hinton broth and MIC of EO and incubated at 37 °C for 18 h. Control tubes having bacterial suspension were also run alongside. After overnight incubation, 10 µL crystal violet was added in micro-centrifuge tubes and let it stand for 1 min. The tubes were centrifuged at 11,000 rpm for 10 mins. After 3 washes with ethanol at 70, 80 and 90% , the specimens (bacterial cells) were coated up to 300 A° with gold using smart coater with an ion sputtering device (model JFC-1500) and energy dispersive X-ray spectroscopy detector (model EX-54175JMU; JEOL, Tokyo, Japan). Finally, morphological alterations in the bacterial cell before and after treatment were observed under scanning electron microscope.

Statistical analysis. All experimental analyses were done in triplicates and interpretations were presented as Mean ± Standard deviation.

Results and Discussion

Citrus limon and *Lavandula angustifolia* were showed as the most potent EO among five of them as clearly observed in (Table 1). The least MIC of *Lavandula angustifolia* was 250 µg/mL for *Staphylococcus aureus*, *Serratia liquefaciens* and *Citrobacter freundii* but for *Escherichia coli* and *Serratia fonticola*, the MIC were 1000 µg/mL and 500 µg/mL respectively. While, the lowest MIC of *Citrus limon* against all the selected strains was 500 µg/mL except for *Escherichia coli* with the MIC was 1000 µg/mL. Although, rest of the EO were not able to demonstrate their antibacterial proficiency in agar well diffusion method. Macro broth dilution method was performed to determine the MIC of EO that inhibit the visible growth of bacteria (Delaquis *et al*., 2002).

All the EO in broth medium produced competent results against all the tested organisms as presented in (Table 2). EO of *Elettaria cardamomum* was the most effective EO, repressed the growth of all food-borne bacteria at 62.5 µg/mL aside from *Serratia liquefaciens* with the MIC was 250 µg/mL followed by *Nigella sativa* and *Prunus dulcis,* both the EO showed variable levels of inhibition. For *Nigella sativa* EO, *Escherichia coli, Staphylococcus aureus* and *Serratia liquefaciens* and in case of *Prunus dulcis* EO, *Citrobacter freundii*, *Serratia fonticola* and *Staphylococcus aureus* were the most susceptible bacteria because of their low MIC (62.5 µg/mL). Similarly the highest MIC values of the EO of *Nigella sativa* (125-1000 µg/mL) were determined against *Serratia fonticola* and *Citrobacter freundii*. Whereas, the highest MIC values (250 – 1000 µg/mL) of *Prunus dulcis* EO were found against *Serratia liquefaciens* and *Escherichia coli*. The obtained MIC of *Citrus limon* and *Lavandula angustifolia* EO ranges in between (125, 250 and 1000 µg/mL) against all the tested strains. The variations were noticed in the MIC values of agar well diffusion and macro broth dilution method when compared are due to the nature of antimicrobial compounds. Some of the antimicrobial compounds were not properly diffused into the solid medium because of their low polarities hence unable to show their antibacterial traits. Several studies have been done on EO possessed biological activities such as *Elettaria cardamomum* proved itself as a competent candidate to battle against different diseases such as

Table 1. Antibacterial activity of essential oils of *Citrus limon*, *Lavandula angustifolia*, *Nigella sativa*, *Elettaria cardamomum*, and *Prunus dulcis* by agar well diffusion method[a]

Bacterial strains	*Citrus limon*			*Lavandula angustifolia*				*Nigella sativa, Elettaria cardamomum, & Prunus dulcis*
	Essential Oils concentration in (µg/mL) Zone of inhibition in mm							
	1000	500	250, 125,	1000 62.5 & 15.6	500	250	125, 62.5 & 15.6	1000 to 15.6
Staphylococcus aureus	20±0.0	20±0	N/D	19.6±0.34	18.9±0.17	29.9 ±0.17	N/D	N/D
Escherichia coli	15.5±0.5	N/D	N/D	29.43±0.51	N/D	N/D	N/D	N/D
Serratia liquefaciens	19.8±0.34	20±0	N/D	19.93±0.11	19.66±0.28	12.67±0.57	N/D	N/D
Citrobacter freundii	20 ± 0	19.8±0.34	N/D	N/D	N/D	14.67±0.5	N/D	N/D
Serratia fonticola	20 ± 0	20±0	N/D	20±0	20±0	10±0	N/D	N/D

[a]N/D = No detection of antibacterial activity; values are means of triplicates ± SD.

Table 2. Minimum Inhibitory concentration determined by broth dilution method

Bacterial strains	MIC of Essential oils (µg/mL)				
	Prunus dulcis	*Elettaria cardamomum*	*Nigella sativa*	*Lavandula angustifolia*	*Citrus limon*
Escherichia coli	1000	62.5	62.5	250	250
Staphylococcus aureus	62.5	62.5	62.5	125	250
Serratia liquefaciens	250	250	62.5	1000	125
Citrobacter freundii	62.5	62.5	1000	1000	1000
Serratia fonticola	62.5	62.5	125	1000	1000

gastrointestinal disturbances and known to be utilized in cooking to augment the deliciousness of food (Evans, 2002). Similarly, *Citrus limon* and *Nigella sativa* are heavily loaded with bioactive compounds to combat against a wide range of bacteria (Prabuseenivasan *et al*., 2006). While the EO of *Lavandula angustifolia* is well known to refresh mind and have healing properties. EO of *Lavandula angustifolia* reportedly used in combination with multiple oils and produced synergistic effects against microorganisms (Buckle, 2014; de Rapper *et al*., 2013; Shealy, 1998; Lawless, 1995).

EO of *Citrus limon* and *Lavandula angustifolia* exhibited significant antibacterial activity against all the tested foodborne isolates with an exception of *Staphylococcus aureus* while no antibacterial activity was distinguished against rest of the EO in their neat (undiluted) form as summarized in Table 3-7. As demonstrated by the obtained results, the combinations of five EO with seven tested antibiotics produced synergistic impact against all the selected foodborne bacteria. EO of *Citrus limon* with all the tested antibiotics showed synergistic effect against *Staphylococcus aureus* and antagonistic effect against *Serratia liquefaciens* and *Citrobacter freundii*. Synergistic effect was seen against *Escherichia coli* and *Serratia liquefaciens*, while the combination of EO of *Nigella sativa* and chloramphenicol and gentamicin were applied, respectively. The mix of *Nigella sativa* with levofloxacin, gentamicin, tetracycline, and streptomycin produced synergistic effect against *Citrobacter*

Table 3. Antibacterial activity of essential oil of *Citrus limon* and its combined effects with standard antibiotics[a]

Bacterial strains	Zone of inhibition in millimeters (mm)														
	A				B									C	
	CL	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	14.86 ±0.2	29.5 ±0.7	R	R	24.3 ±0.5	17±0	26±0	11.93 ±0.11	25±0a	6±0a	19.9± 0.11s	25±0a	20±0a	24.93± 0.11a	15±0a
SF	10±0	26.5 ±0.7	R	R	24.2 ±0.4	16.3 ±0.5	28.2 ±0.40	10.5 ±0.70	9.93 ±0.1a	6±0a	28.96 ±0.05s	23±0a	14.83 ±0.28a	22.56 ± 0.49a	6±0a
SL	9.93± 0.1	25.6± 0.5	R	R	21.9± 0.05	14.1± 0.28	25.8± 0.28	13.1± 0.17	22±0a	6.93± 0.11a	8.93± 0.11a	20±0a	18±0a	18±0a	15±0a
SA	N/D	26±0	R	R	22.1± 0.1	22± 0.2	26.1± 0.17	17.2±0 .34	29.8± 0.34s	14.8± 0.26s	30±0s	34.5± 0.5s	35.73± 0.25s	40±0s	24.96± 0.05s
CF	19±0	22±0	R	R	22.3± 0.5	16±0	23.7± 0.57	12±0	25±0a	10±0a	8.93± 0.11a	25±0a	16.8± 0.34a	22±0a	12±0a

[a]A = Inhibition zones that occurred with essential oil (essential oil of *Citrus limon*: 10 µL); B = Inhibition zones that occurred with standard antibiotic disc, levofloxacin (L), oxacillin (O), amoxicillin; (M), chloramphenicol (C), gentamicin (CN), tetracycline (T), and streptomycin (P); C = Inhibition zones that occurred when essential oil and standard antibiotic were used together, (essential oil of *Citrus limon* 10 µL/disc); (CL = *Citrus limon*; EC = *Escherichia coli*; SF = *Serratia fonticola*, SL = *Serratia liquefaciens*, CF = *Citrobacter freundii*; SA = *Staphylococcus aureus*; N/D = no antibacterial activity detected; s = Synergism effect; e = Additive effect; a = Antagonistic effect; i = Indifference; R = resistant; Values are represented as Mean ± SD).

Table 4. Antibacterial activity of essential oil of *Lavandula angustifolia* and its combined effects with standard antibiotics[a]

Bacterial strains	Zone of inhibition in millimeters (mm)														
	A				B									C	
	V	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	13.06± 0.11	29.5± 0.7	R	R	24.3± 0.5	17±0	26±0	11.93± 0.11	30±0a	a	21.9± 0.17s	28±0a	25±0a	26.1± 0.23I	20.1± 0.17a
SF	9.9± 0.17	26.5± 0.7	R	R	24.2± 0.4	16.3± 0.5	28.2± 0.40	10.5± 0.70	a	a	a	7.9± 0.17a	a	a	9 ± 0 a
SL	11.67± 0.57	25.6± 0.5	R	R	21.9± 0.05	14.1± 0.28	25.8± 0.28	13.1± 0.17	18.1± 0.17a	a	5±0a	22±0a	22.73± 0.46a	18±0a	20±0a
SA	N/D	26±0	R	R	22.1± 0.1	22± 0.2	26.1± 0.17	17.2± 0.34	21.9± 0.17a	N/D	10±0a	12±0a	17.86± 0.23a	15±0a	10±0a
CF	14.83± 0.28	22±0	R	R	22.3± 0.5	16±0	23.7± 0.57	12±0	25±0a	a	10±0a	18±0a	18.06± 0.11a	21.83± 0.28a	11±0a

[a]A = Inhibition zones that occurred with essential oil (essential oil of *Lavandula*: 10 µL); B = Inhibition zones that occurred with standard antibiotic disc, levofloxacin (L), oxacillin (O), amoxicillin; (M), chloramphenicol (C), gentamicin (CN), tetracycline (T), and streptomycin (P); C = Inhibition zones that occurred when essential oil and standard antibiotic were used together, (essential oil of *Lavandula* 10 µL/disc); (V = *Lavandula angustifolia*; EC = *Escherichia coli*; SF = *Serratia fonticola*; SL = *Serratia liquefaciens*; CF = *Citrobacter freundii*; SA = *Staphylococcus aureus*; and N/D = no antibacterial activity detected; s = Synergism effect; e = Additive effect; a = Antagonistic effect; i = Indifference; R = resistant; Values are represented as Mean ± SD).

freundii and adversarial impact on rest of the isolates. The combination of EO of *Lavandula angustifolia* with all the tested antibiotics showed antagonistic effects against all five bacterial strains however, synergistic effect was acquired with amoxicillin against *Escherichia coli*. Most of the synergistic effect was found with the combination of gentamicin with *Elettaria cardamomum* EO against *Serratia liquefaciens*, *Escherichia coli* and *Staphylococcus aureus*. Phenomena of synergism was also observed against *Staphylococcus aureus*, while the combination of *Elettaria cardamomum* EO and tetracycline and streptomycin were applied. Most of the synergistic outcomes were obtained from EO of *Prunus dulcis* was alone unable to show their antibacterial potential against these food isolates. Of the combination of *Prunus dulcis* EO and streptomycin against *Escherichia coli*, *Serratia liquefaciens* and *Citrobacter freundii* synergistic effect was observed. Also the mix of EO of *Prunus dulcis* with levofloxacin and gentamicin demonstrated synergistic effect against *Staphylococcus aureus* and *Citrobacter freundii* respectively. Against *Escherichia coli*, the previously mentioned mix with both the antibiotics produced synergistic effect. Synergistic effect of at least two drugs produced surpass results than the sum of their individual effects is called synergism (Singh *et al*., 2000). Phenomena of synergism can be obtained in multiple circumstances for instance one antibiotic may prevent the inactivation of a second antimicrobial compound by microbial enzyme. Synergistic effect can likewise be seen in such case in which a drug like penicillin inhibit the cell wall synthesis may increase the rate of entrance of aminoglycosides into the bacterial cell therefore, two drugs consecutively obstruct the metabolic pathway of microbes (Brooks *et al*., 1995).

Worth mentioning of these two drugs namely oxacillin and amoxicillin (beta-lactam drugs) to which all the tested foodborne bacteria showed complete resistance but remarkable results were obtained with the combination of EO and these two antibiotics. It was reported in a study that more than 92 % of *staphylococcus* have beta-lactamase enzyme hence resistant towards both the derivatives of penicillin (Barry, 1990). The combination of EO of *Prunus dulcis* with amoxicillin showed significant antibacterial activity against all the tested bacteria. With *Elettaria*

Table 5. Antibacterial activity of essential oil of *Elettaria cardamomum* and its combined effects with standard antibiotics[a]

Bacterial strains	Zone of inhibition in millimeters (mm)														
	A	B							C						
	GC	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	N/D	29.5± 0.7	R	R	24.3± 0.5	17±0	26±0	11.93± 0.11	24.93± 0.11a	a	17.7± 0.46s	15± 0.17a	23±0s	25± 0a	a
SF	N/D	26.5± 0.7	R	R	24.2± 0.4	16.3± 0.5	28.2± 0.40	10.5± 0.70	10±0a	a	a	a	a	a	a
SL	N/D	25.6± 0.5	R	R	21.9± 0.05	14.1± 0.28	25.8± 0.28	13.1± 0.17	21.13± 0.23a	a	a	14± 0a	18± 0s	17± 0a	a
SA	N/D	26±0	R	R	22.1± 0.1	22± 0.2	26.1± 0.17	17.2± 0.34	25± 0A	19.9± 0.05s	27.06± 0.11s	19.8± 0.23a	25±0s	31.9± 0.17s	20± 0s
CF	11.33± 0.57	22±0	R	R	22.3± 0.5	16±0	23.7± 0.57	12±0	26.06± 0.11a	a	a	26±0a	19.1± 0.28a	22.9± 0.05a	13± 0a

[a]A = Inhibition zones that occurred with essential oil (essential oil of *Elettaria cardamomum*: 10 µL); B = Inhibition zones that occurred with standard antibiotic disc, levofloxacin (L), oxacillin (O), amoxicillin; (M), chloramphenicol (C), gentamicin (CN), tetracycline (T) and streptomycin (P); C = Inhibition zones that occurred when essential oil and standard antibiotic were used together, (essential oil of *Elettaria cardamomum* 10 µL/disc); (GC = *Elettaria cardamomum*; EC = *Escherichia coli*; SF = *Serratia fonticola*; SL = *Serratia liquefaciens*; CF = *Citrobacter freundii*; SA = *Staphylococcus aureus*; N/D = no antibacterial activity detected; s = Synergism effect; e = Additive effect; a = Antagonistic effect, i = Indifference; R = resistant; Values are represented as Mean ± SD).

Table 6. Antibacterial activity of essential oil of *Prunus dulcis* and its combined effects with standard antibiotics[a]

Bacterial strains	Zone of inhibition in millimeters (mm)														
	A	B							C						
	PD	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	N/D	29.5± 0.7	R	R	24.3± 0.5	17±0	26±0	11.93± 0.11	30±0s	a	24±0s	17.9± 0.05a	23±0s	13.2± 0.3a	20.0± 0.1s
SF	N/D	26.5± 0.7	R	R	24.2± 0.4	16.3± 0.5	28.2± 0.40	10.5± 0.70	9±0a	a	6.1± 0.17s	a	a	a	a
SL	N/D	25.6± 0.5	R	R	21.9± 0.05	14.1± 0.28	25.8± 0.28	13.1± 0.17	21±0a	a	20.1± 0.17s	a	14.06± 0.11I	-A	18±0s
SA	N/D	26±0	R	R	22.1± 0.1	22± 0.2	26.1± 0.17	17.2± 0.34	29.06± 0.11s	19.1± 0.23s	34±0s	a	-A	20±0A	14.9± 0.05a
CF	N/D	22±0	R	R	22.3± 0.5	16±0	23.7± 0.57	12±0	21.9± 0.17a	N/D	24±0s	a	23±0S	13.1± 0.17a	18.8± 0.23s

[a]A = Inhibition zones that occurred with essential oil (essential oil of *Prunus dulcis*: 10 µL); B = Inhibition zones that occurred with standard antibiotic disc, levofloxacin (L), oxacillin (O), amoxicillin (M), chloramphenicol (C), gentamicin (CN), tetracycline (T), and streptomycin (P); C = Inhibition zones that occurred when essential oil and standard antibiotic were used together, (essential oil of *Prunus dulcis* 10 µL/disc); (PD = *Prunus dulcis*; EC = *Escherichia coli*; SF = *Serratia fonticola*; SL = *Serratia liquefaciens*; CF = *Citrobacter freundii*; SA = *Staphylococcus aureus*; and N/D = no antibacterial activity detected; s = Synergism effect; e = Additive effect; a = Antagonistic effect; i = Indifference R = resistant; Values are represented as Mean ± SD).

cardamomum EO, produced synergistic effect against *Escherichia coli* and *Staphylococcus aureus*. Combination of amoxicillin and EO of *Nigella sativa* and *Lavandula angustifolia* exhibited synergistic impact against *Escherichia coli*. Synergy was also seen against *Escherichia coli*, *Serratia fonticola* and *Staphylococcus aureus* with the combination of amoxicillin and EO of *Citrus limon*. Then again, EO of *Prunus dulcis*, *Elettaria cardamomum* and *Citrus limon* produced synergistic effect against *Staphylococcus aureus* when combined with oxacillin. This research was supported by the former study conducted on tea tree (*Camellia sinensis*). EO of tea tree are very effective against pathogenic microbes including methicillin-resistant *Staphylococcus aureus*. Synergistic effect was noticed against *Staphylococcus aureus*, while the combination of EO of *Camellia sinensis* and oxacillin were applied (Abascal and Yarnell, 2002).

EO of *Citrus limon* and *Lavandula angustifolia* gave proficient outcomes in their undiluted form against all the tested bacteria except for *Staphylococcus aureus* demonstrated by drop agar diffusion technique. These two EO were picked to check the consolidated impact in their neat form and at different concentrations obtained by agar well diffusion method. CCL (combination of *Citrus limon* and *Lavandula angustifolia*) EO at all the tested concentrations displayed noteworthy synergistic effect against *Serratia fonticola*. While the additive result was seen against *Escherichia coli* (24.8 ± 0.28 mm) and *Serratia liquefaciens* (15 ± 0 mm) at 500 µg/mL and no antibacterial activity was observed against *Citrobacter freundii* at all the tried concentrations as shown in (Table 8). Synergistic effect was spotted against *Staphylococcus aureus* at all the tested combinations to which these EO alone were unable to inhibit the growth of *Staphylococcus aureus*. Scanning electron microscopy was performed to verify antibacterial activity and mode of action of selected EO. Scanning electron microscopic images revealed the alteration in cellular morphology of bacterial cell after treated with EO at the MIC as shown in (Fig. 1). Image (a) and (c) were the control images of *Escherichia coli* and *Staphylococcus aureus* respectively which were not exposed to the EO hence the morphology and arrangement of bacterial cells remain unchanged. On the other hand, bacterial cells treated with EO responsible for bulging of cells, disruption of the cell wall and the

arrangement of bacterial cells were distorted. Components present in the EO for example, thymol, carvacrol, and eugenol are responsible for the disruption of cell membrane. Furthermore, inactivates the microbial enzymes by reacting its active sites was accounted in few studies (Guynot *et al*., 2003). It can also be hypothesized that the possible mechanism of action of these EO may involve termination of N-acetyl muramic acid linkages, which would subsequently cease the cell wall synthesis (Ginsberg *et al*., 2006). Therefore, according to the obtained results, it can be hypothesized that the mode of action of tested EO was the cell wall and cell membrane of bacteria. Briefly, all five EO displaced noteworthy results against all five bacterial strains. EO of *Citrus limon* and *Lavandula angustifolia* showed their best antibacterial efficiency in solid agar medium while EO of *Prunus dulcis*, *Elettaria cardamomum*, and *Nigella sativa* proved their antibacterial strength in broth medium. In combination with antibiotics, EO of *Prunus dulcis* was the best of all to produce synergistic effect followed by *Citrus limon*, *Elettaria cardamomum*, *Nigella sativa*, and *Lavandula angustifolia*. Although antagonistic and additive outcomes were seen as well.

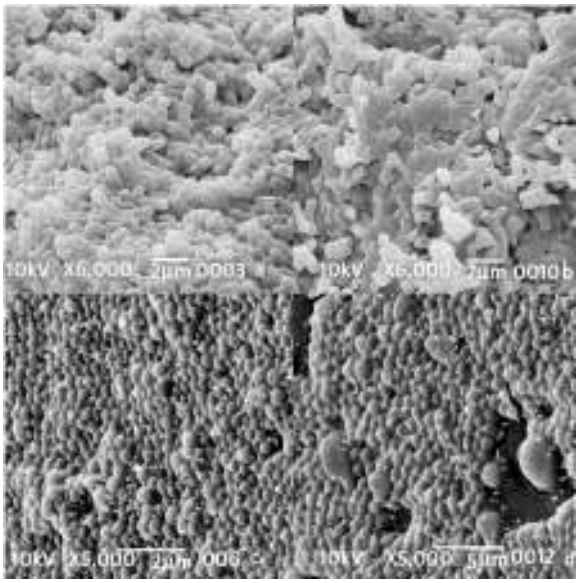

Fig. 1. Scanning electron microscopic images of tested bacteria when treated with EO at MIC. *Escherichia coli* (a) control (b) treated with EO of *Citrus limon* at 500 µg/mL; *Staphylococcus aureus* (c) control (d) treated with CCL at 1000 µg/mL.

Table 7. Antibacterial activity of essential oil of *Nigella sativa* and its combined effects with standard antibiotics[a]

Bacterial strains	Zone of inhibition in millimeters (mm)														
	A				B							C			
	NS	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	N/D	29.5± 0.7	R	R	24.3± 0.5	17±0	26±0	11.93± 0.11	23.96± 0.05a	a	23±0s	30±0s	15±0a	21.96± 0a	11.96± 0.05i
SF	N/D	26.5± 0.7	R	R	24.2± 0.4	16.3± 0.5	28.2± 0.40	10.5± 0.70	13±0a	a	a	a	a	10±0a	a
SL	N/D	25.6± 0.5	R	R	21.9± 0.05	14.1± 0.28	25.8± 0.28	13.1± 0.17	20±0a	a	a	20±0a	17.9± 0.17s	14.9± 0.17a	a
SA	N/D	26±0	R	R	22.1± 0.1	22± 0.2	26.1± 0.17	17.2± 0.34	25±0a	a	a	A	18.9± 0.17a	14.86± 0.23a	10±0a
CF	N/D	22±0	R	R	22.3± 0.5	16±0	23.7± 0.57	12±0	30.06± 0.11s	a	a	21.9± 0.17a	23±0s	25±0s	15.06± 0.11s

[a]A = Inhibition zones that occurred with essential oil (essential oil of Nigella sativa: 10 µL); B = Inhibition zones that occurred with standard antibiotic disc, levofloxacin (L), oxacillin (O), amoxicillin (M), chloramphenicol (C), gentamicin (CN), tetracycline (T), and streptomycin (P); C = Inhibition zones that occurred when essential oil and standard antibiotic were used together, (essential oil of *Nigella sativa* 10 µL/disc); (NS = *Nigella sativa*; EC = *Escherichia coli*; SF = *Serratia fonticola*; SL = *Serratia liquefaciens*; CF = *Citrobacter freundii*; SA = *Staphylococcus aureus*; N/D = no antibacterial activity detected; s = Synergism effect; e = Additive effect; a = Antagonistic effect; i = Indifference; R = resistant; Values are represented as Mean ± SD).

Table 8. Antibacterial activity of combined effects of essential oils *Citrus limon* and *lavandula angustifolia* (CCL) by agar well diffusion method[a]

EO conc.	Bacterial strains				
	Zone of inhibition in millimeter (mm)				
	Escherichia coli	*Citrobacter freundii*	*Serratia liquefaciens*	*Staphylococcus aureus*	*Serratia fonticola*
CCL 1000 µg/mL	15 ± 0	N/D	N/D	15 ± 0	30.33 ± 0.57
CCL 500 µg/mL	24.8 ± 0.28	N/D	15 ± 0	15 ± 0	35.06 ± 0.11
CCL (undiluted)	15.1 ± 0.23	N/D	N/D	15 ± 0	40.4 ± 0.50

[a]N/D = No antibacterial activity detected.

Conclusion

The fundamental reason of this study is to decrease drug resistance by combining classical antibiotics and essential oils. Association of essential oils and antibiotics and the combination of essential oils exhibited synergistic effect which is beneficial for making new antimicrobial agents that have great potential to fight against infectious diseases. This synergistic effect also enhances the antibacterial activity of the respective antibiotics which become ineffectual due to increase in the drug resistance. It can be used as antibacterial supplement in the developing countries in the direction of the development of new therapeutic agents. Acquired antagonistic results due to the interaction of antibiotics and essential oils can cause side effects. This investigation propose the advanced studies on molecular level in a manner that the interactions of antimicrobial agents increased the selective toxicity of drugs, therefore *in vivo* studies would be necessary to evaluate the antibacterial potential of these essential oils.

Conflict of Interest. The authors declare no conflict of interest.

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