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Bioceutical Properties of Culinary Herbs, Spices and Their Possible Outcomes with Standard Antibiotics

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Abstract

Culinary condiments have been used for centuries to treat several types of ailments. Four ethanolic lipid fractions including *Nigella sativa*, *Foeniculum vulgare*, *Laurus nobilis*, and *Coriandrum sativum* were selected to assess their antimicrobial potential alone and combined with antibiotics. Antibacterial activity was determined by various conventional procedures such as aromatogram test, well diffusion, macro-broth dilution, disc diffusion assay. The tested lipid fractions exhibited antibacterial activity against selected bacterial strains. Antibacterial activity of lipid fractions was also seen within two hours of incubation. These lipid fractions attacked the cell wall *i.e.* the penetrability of bacterial cell and hence intracellular contents released in the environment which was detected at 260 nm of absorbance and verified via scanning electron microscopy. Antagonistic effect was mostly found by the combination of antibiotics and lipid fractions. Though, synergistic effect was obtained with beta-lactam drugs when combined with lipid fraction of *Laurus nobilis*. Hence the lipid fractions may be used to treat various communicable diseases. However antagonistic outcomes of the combinations suggested that herbal and allopathic therapies should not consume concurrently.

Keywords

Antagonism, Synergism, Antibiotics, Lipid Fractions, Antimicrobial Activity

1. Introduction

Lipid fractions (LFs) have been utilized for a considerable length of time for the treatment of human beings. They produce positive therapeutic, restorative and mental impact on human body. These fragrant plants have multiple uses and were considered as the core part of witchcraft, healing and holy practice in Egypt

[1].

Food associated illnesses are of great concern. Not only foodborne pathogens but commensal microorganisms are huge menace to public health therefore, cannot be neglected. Studies affirmed that commensal bacteria transfer resistance genes to other bacteria faster than pathogenic microbes [2] [3]. Such commensal species for example *Citrobacter* can easily affect the respiratory tract, enter the circulatory system, upset digestion and cause gastric-related problems like gastroenteritis caused by verotoxigenic *C. freundii* recouped from butter parsley sandwiches [4] [5]. Likewise, *Serratia* was additionally viewed as innocuous for humans [6]. But later the contribution of *Serratia* in histamine-producing foodborne poisoning was discovered [7]. Most of the bacteria of Enterobacteriaceae family have enzyme named histidine decarboxylase responsible for histamine production which mounts various allergic responses [8] [9].

On account of enormous expansion of resistance developed against antimicrobial agents, high dose of antibiotics and SOS drugs (last resort) are given to treat infections. These two possible options for recovery are responsible for serious side effects and sometimes produce irreversible damage to the body. Therefore, to consider the consumer's choice towards natural remedy over synthetic drugs which cause minimum to no after effects. Researchers are paying more attention to nature's blessing in the avatar of LFs that possess several benefits and have great antimicrobial potential. LFs used in this research were *Laurus nobilis*, *Foeniculum vulgarae*, *Coriandrum sativum*, and *Nigella sativa* that possessed remarkable antimicrobial and antioxidant activities [10]. Their activities have never been tested against commensal strains. These commensal strains were recovered from street foods of Karachi city and had a great potential to transfer resistant genes to the pathogenic microbes [11]. The focus of the research was to assess the antimicrobial potential of selected lipid fractions against bacterial strains isolated from street foods, Karachi, and to analyze the possible outcomes produced by the combination of lipid fractions and antibiotics.

2. Materials and Methods

2.1. Tested Microorganisms

Four gram negative and one gram positive bacterial strains namely *Serratia liquefaciens*, *Serratia fonticola*, *Escherichia coli*, *Citrobacter freundii* and *Staphylococcus aureus* respectively were selected. These tested microorganisms were recovered from street foods vended in the city of Karachi [11].

2.2. Lipid Fractions

Four ethanolic lipid fractions namely *Nigella sativa* (black cumin), *Coriander sativum* (coriander), *Foeniculum vulgare* (fennel), and *Laurus nobilis* (bay leaf) were selected to assess their antimicrobial potential against selected strains [12]. These lipid fractions were collected from Food Science and Technology, University of Karachi by solvent extraction procedure [13].

2.3. Aromatogram Test

Volumetric measurement was performed to find out the direct correlation between LFs and antibacterial activity against selected food borne bacteria. 18 hour's old cultures were standardized in physiological saline and matched with 0.5 concentration of McFarland index. 100 µL bacterial inoculum was added and make thin and uniform bacterial lawn on Mueller Hinton agar plates with the help of sterile cotton swabs. Five sterilized filter paper disc of 0.6 cm soaked in 0.5, 1, 2, 5, and 7.5 µL concentrations of LFs were positioned onto MHA plates and incubated at 37˚C for 18 - 24 hours. Next day observed zone of inhibition around each disc [14] [15] [16].

2.4. Drop Agar Diffusion Method

This assay was used to examine the antibacterial activity of four ethanolic LFs in their concentrated form [17] [18]. The Mueller Hinton agar plates were formerly inoculated with test organisms with 0.5 McFarland concentration. 10 µL drop of LFs was placed on MHA plates and left untouched for proper dispersion at ambient temperature. After a while, plates were incubated at 37˚C ± 1˚C for 24 hours. Zone of inhibition around each drop was measured in millimeters.

2.5. Agar Well Diffusion Method

Antibacterial activity of ethanolic LFs at different concentrations (1000 µg/mL, 500 µg/mL, and 250 µg/mL) prepared in DMSO was determined by agar well diffusion method as described by Martins *et al.*, 2013 and Mehboob *et al.*, 2020. To analyze the combined effects of ethanolic LFs were also evaluated by this method [2] [19].

2.6. Broth Dilution Method

To determine the minimal inhibitory concentrations of ethanolic lipid fractions in liquid media, broth dilution method was performed as described Weerakkody *et al.*, 2010 at selected concentrations e.g. 1000, 500, 250, 125, 62.5, and 15.625 µg/mL prepared in DMSO Antibacterial activity of ethanolic lipid fractions based on the occurrence and absence of bacterial growth in the form of turbidity [2] [20] [21].

2.7. Loss of Absorbance

To measure the intracellular contents released from bacterial cell, loss of absorbance was estimated at 260 nm [22]. Working bacterial culture was centrifuged at 13,000 rpm for 15 minutes. 3x washing was done and cells were transferred to phosphate buffer saline. The concentrations of LFs used were same as broth dilution method and supplemented in bacterial cell suspension. Tested and control tubes were incubated for 2 hours in shaking incubator (CBM-Scientific.ES.20) at 150 rpm with incubation temperature 35˚C ± 2˚C. 2 hours later, 2 mL samples were filter sterilized by 0.2 µm pore sized filter membrane (Sigma Aldrich). The absorbance of the filtrate was measured at 260 nm via UV-V spectrophotometer Shimadzu, Japan 1240 Mini.

2.8. Antibiotics

Levofloxacin (5 µg), streptomycin (10 µg), gentamicin (10 µg), amoxicillin (10 µg), tetracycline (30 µg) chloramphenicol (30 µg), and oxacillin (1 µg). The following antibiotics were purchased from Thermo Fischer Scientific Oxoid Ltd.

2.9. 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 hrs. old) were standardized with 0.5 McFarland index. 10 µl of each selected bacterial suspension was inoculated into the micro centrifuge tube containing Mueller Hinton broth and MIC of LFs and incubated at 37˚C for 18 hours. Control tubes having bacterial suspension were also run alongside. After overnight incubation, 10 µL crystal violet was added in microcentrifuge tubes and let it stand for 1 minute. The tubes were centrifuged at 11,000 rpm for 10 minutes. 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.

2.10. Evaluation of the Combined Impact of Ethanolic Lipid Fractions and Standard Antibiotics

According to the method proposed by Moussaoui & Alaoui, 2016 & Toroglu, 2007 combined effects of selected ethanolic LFs and antibiotics were examined through disc diffusion method [23] [24]. 10 µL drop of undiluted LFs were soaked on promptly accessible antibiotics disc, then plates were permitted to dry for proper diffusion, incubated at 37˚C ± 1˚C for 24 hours to find out the zone of clearance around each circle. The gained results were differentiated and tabulated between antibiotics + LFs and antibiotics alone by similar strategy as disk diffusion method.

2.11. Statistical Analysis

For statistical evaluation, post hoc Duncan test was applied to find out the significant difference at $p < 0.05$ between control and tested sets of ethanolic LFs. All the tests were performed 3 times and results were presented as Mean ± SD by using SPSS software (version 24, SPSS Inc., USA).

3. Results

3.1. Aromatogram Test

All the four selected ethanolic LFs demonstrated no antibacterial activity at 0.5, 1, 2, 5, and 7.5 µl against all the tested bacterial strains.

3.2. Drop Agar Diffusion Method

Antimicrobial strength of concentrated ethanolic LFs was clearly explained with the aid of drop agar diffusion method. LF of FE exhibited significant antibacterial activity against all the tested bacteria and produced highest zone of inhibition ranges in between (11 ± 0 to 29.66 ± 0.57) mm among all the selected lipid fractions. Followed by LF of CE gave good competition and secured second position producing inhibition zone between (9 ± 0 to 24.33 ± 1.15) mm. While moderate antibacterial activity showed by LF of LE against all the tested bacterial isolates. However, LF of NE failed to retard the multiplication of *S. fonticola*, and *C. freundii* but demonstrated average antibacterial activity against *E. coli*, *S. liquefaciens*, and *S. aureus* with inhibition zone (9.93 ± 0.11) mm, (8.25 ± 0.4) mm, and (15 ± 0) mm respectively.

3.3. Agar Well Diffusion Method

Antibacterial strength was also determined by agar well diffusion method (Table 1). All four ethanolic lipid fractions failed to inhibit the growth of *S. fonticola* and *S. liquefaciens* at all the chosen concentrations. While all of the lipid fractions showed antimicrobial potential against *S. aureus* and *C. freundii*. The lowest MIC of LF of NE and LE against *S. aureus* and *C. freundii* were 250 µg/mL. Correspondingly, the growth of *S. aureus* was inhibited by the LF of FE and CE at 1000 µg/mL. The MIC values of FE and CE were 500 µg/mL against *C. freundii* as shown in Table 2.

Table 1. Antibacterial activity of ethanolic lipid fractions of *Nigella sativa*, *Foeniculum vulgare*, *Coriandrum sativum*, and *Laurus nobilis* by agar well diffusion method[a].

Bacteria	*Nigella sativa*			*Foeniculum vulgare*			*Coriandrum sativum*,			*Laurus nobilis*		
	Essential oils concentration in (µg/mL) Zone of inhibition in mm											
	1000	500	250	1000	500	250	1000	500	250	1000	500	250
E. coli	11.53 ± 0.80	10.4 ± 0.52	9.13 ± 0.80	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
S. fonticola	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
S. liquefaciens	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
S. aureus	12.53 ± 1.50	9.67 ± 0.58	8.67 ± 0.58	8.73 ± 0.30	N/D	N/D	9.5 ± 0.5	N/D	N/D	10 ± 1	9.4 ± 0.52	7.27 ± 0.64
C. freundii	11.33 ± 1.15	10.6 ± 0.52	9.33 ± 0.58	12.67 ± 3.21	7.83 ± 0.29	N/D	19.33 ± 0.58	9.6 ± 0.69	N/D	11 ± 1.01	11 ± 1.73	10 ± 0

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

Table 2. Minimum Inhibitory concentration determined by agar well diffusion method[a].

Bacterial strains	MIC of Essential oils (µg/mL)			
	Nigella sativa	*Foeniculum vulgare*	*Coriandrum sativum*	*Laurus nobilis*
E. coli	250	N/D	N/D	N/D
S. fonticola	N/D	N/D	N/D	N/D
S. liquefaciens	N/D	N/D	N/D	N/D
S. aureus	250	1000	1000	250
C. freundii	250	500	500	250

[a]N/D = No detection of antibacterial activity.

3.4. MIC of Lipid Fractions in Broth Dilution Method

In comparison to the well diffusion results, ethanolic lipid fractions demonstrated and produced enhanced antibacterial potential was detected in broth dilution method (Table 3). The highest and lowest MIC values of LF of FE were 1000 µg/mL for *S. fonticola* and 125 µg/mL for *S. aureus*, *E. coli*, and *S. liquefaciens* respectively. The least MIC value of LF of LE detected at 62.5 µg/mL against all the tested bacterial strains except *C. freundii*. Likewise, 125 µg/mL MIC value of LF of NE was noted against *E. coli*, *S. liquefaciens*, *S. aureus*, and *C. freundii* but for *S. fonticola*, 125 µg/mL MIC was observed. Furthermore, the MIC values of *S. liquefaciens* and *S. fonticola* were 500 µg/mL and 125 µg/mL against *S. aureus* and *C. freundii* while no activity was detected against *E. coli*.

3.5. Loss of Absorbance

Antimicrobial activity of lipid fractions was significant in broth medium therefore, antimicrobial potential was evaluated by estimating loss of absorbance at 260 nm. All the chosen LFs demonstrated significant difference between the tested and control group. The values were noted after 2 hours of incubation period. All four LFs specifically NE and FE at all the selected concentrations exhibited tremendous activity against *S. aureus*. The highly effected LFs were NE and CE against *E. coli* at 500 µg/mL. Similarly, highest destruction of bacterial cells of *S. liquefaciens* noted at 1000 µg/mL of CE. In case of *S. fonticola*, LF of NE at 250 µg/mL, LE at 500 µg/mL, FE at 1000 µg/mL and 500 µg/mL produced irreversible destruction to the bacterial cells. While LFs of NE, CE, and FE were effective against *C. freundii* at 1000 µg/mL, and 500 µg/mL respectively (Table 4).

3.6. Combined Effects

Combined effects of standard antibiotics and ethanolic LFs were evaluated [25]. All the selected ethanolic LFs produced antagonistic effect against the tested food isolates when combined with selected antibiotics (Tables 5-8). Only LF of *L. nobilis* (LE) showed synergistic effect when combined with oxacillin and amoxicillin against *C. freundii* in Table 7. Also, synergistic effect was seen against *S. liquefaciens* when combination of LF of *N. sativa* (NE) and levofloxacin applied (Table 8).

Selected LFs combined to determine the antimicrobial strength against five tested food isolates. Noticeable antibacterial activity was not found against any bacterial strains at selected concentrations as demonstrated in Table 9. However, all the possible combination of ethanolic LFs proved significant activity against *S. aureus* with zone of inhibition ranges between (10 ± 0 to 15 ± 0) mm. Among them the most noteworthy and the minimal inhibition zone produced with the blend of N + L and F + L respectively.

3.7. Detection of Cellular Alterations

Significant morphological and physiological changes appeared on bacterial cells after treated with ethanolic LFs. Cell wall became disrupted and it will lead to

enhance the permeability of cell membrane. Due to increase in permeability, phenomena of cytolysis were seen and cell became swollen up and burst and all the internal contents released outside the cell (Figure 1).

4. Discussion

Plants are the great and natural source of obtaining substances that can be used to develop or recreate new germicidal agents. These natural substances have superlative properties alone and in combination and can facilitate the therapeutic process and increase the effectiveness of treatments. To achieve this objective, *In vitro* examinations are required to find the potentials and competency of natural substances [26]. Antibacterial activity of four ethanolic LFs and their combined impact with commercial drug showed in tables and figure against five food borne isolates. All four ethanolic LFs demonstrated exquisite effects in together agar well and macro-broth techniques. However, some of the LFs unable to show their full antibacterial competency on solid medium as compared to broth medium. This is because of the indirect relation of viscosity and diffusive nature of

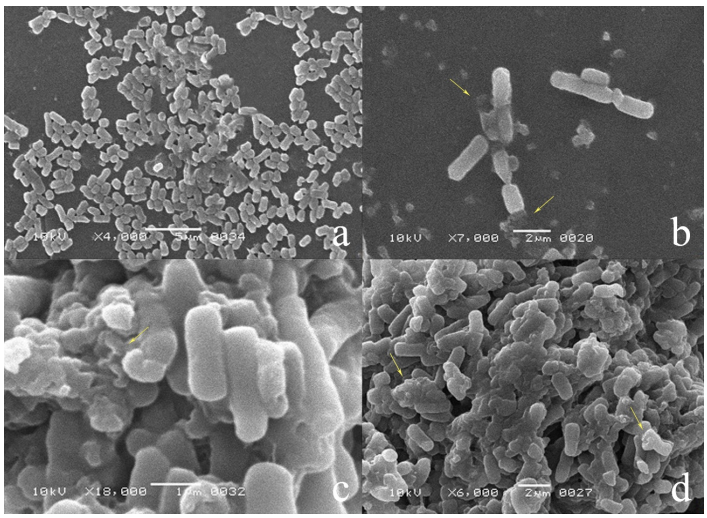

Figure 1. Scanning electron microscopic images of tested bacteria when treated with lipid fractions. *Escherichia coli* (a) control (b) treated with *Nigella sativa* at 250 µg/mL (c) *Foeniculum vulgare* at 250 µg/mL; (d) treated with *Laurus nobilis* at 250 µg/mL.

Table 3. Minimum Inhibitory concentration determined by broth dilution method[a].

Bacterial strains	MIC of Essential oils (µg/mL)			
	Nigella sativa	*Foeniculum vulgare*	*Coriandrum sativum*	*Laurus nobilis*
E. coli	125	125	N/D	62.5
S. fonticola	62.5	1000	500	62.5
S. liquefaciens	125	125	500	62.5
S. aureus	125	1000	125	62.5
C. freundii	125	125	125	125

[a]N/D = No detection of antibacterial activity.

Table 4. Bactericidal activity of ethanolic lipid fractions measured at 260 nm on intracellular constituents of foodborne bacteria[a].

Food isolates	Control	NE1	NE2	NE3	BE1	BE2	BE3	CE1	CE2	CE3	FE1	FE2	FE3
S. aureus	2.873 ± 0^{a}	3.466 ± 0.014^{c}	3.049 ± 0^{b}	3.475 ± 0^{d}	2.98 ± 0.02^{b}	3.171 ± 0.04^{c}	2.96 ± 0.03^{b}	3.495 ± 0.018^{d}	$3.2 \pm 0^{c,d}$	2.87 ± 0.017^{a}	3.5 ± 0.01^{e}	3.253 ± 0.055^{e}	3.2 ± 0^{c}
E. coli	2.873 ± 0^{a}	2.98 ± 0.026^{b}	3.049 ± 0^{c}	3.475 ± 0.0^{e}	3.197 ± 0^{e}	3.12 ± 0^{d}	3.073 ± 0.038^{c}	3.08 ± 0^{d}	3.475 ± 0^{e}	3.173 ± 0.001^{d}	3.015 ± 0.029^{c}	2.938 ± 0.023^{b}	3.03 ± 0.29^{b}
S. liquefaciens	3.0 ± 0^{a}	2.953 ± 0.029^{a}	$3.09 \pm 0.038^{b,c}$	3.03 ± 0.022^{c}	3.14 ± 0.04^{b}	3.17 ± 0.04^{d}	$3.031 \pm 0.03^{b,c}$	3.253 ± 0^{c}	$3.138 \pm 0.05^{c,d}$	2.985 ± 0.036^{a}	3.118 ± 0.013^{b}	$3.05 \pm 0^{a,b}$	3.05 ± 0^{c}
S. fonticola	$3.11 \pm 0^{b,c}$	2.998 ± 0^{a}	2.936 ± 0.022^{a}	3.253 ± 0^{d}	3.138 ± 0.05^{c}	3.419 ± 0^{c}	2.978 ± 0.03^{b}	3.1 ± 0^{c}	2.903 ± 0.084^{a}	2.84 ± 0^{a}	3.63 ± 0.05^{d}	3.5 ± 0.02^{d}	3.351 ± 0.002^{e}
C. freundii	$2.99 \pm 0^{a,b}$	3.127 ± 0.006^{c}	$2.998 \pm 0^{a,b}$	3.04 ± 0.034^{c}	$2.978 \pm 0.031^{a,b}$	2.993 ± 0^{a}	2.993 ± 0^{b}	3.157 ± 0^{d}	3.048 ± 0.063^{b}	2.838 ± 0^{a}	3.007 ± 0.013b	3.049 ± 0^{b}	2.998 ± 0^{b}

[a]Values are means of triplicates ± SD. Different superscript mentioned on the values such as[a,b,c,d] are significantly different at $P < 0.05$. NE1, NE2, NE3, LE1, LE2, LE3, CE1, CE2, CE3, and FE1, FE2, and FE3 are the selected concentrations of *Nigella sativa* at 1000, 500, and 250 µg/mL, *Laurus nobilis* at 1000, 500, and 250 µg/mL, *Coriander sativum* at 1000, 500, and 250 µg/mL, and *Foeniculum vulgare* at 1000, 500, and 250 µg/mL respectively.

Table 5. Antibacterial activity of ethanolic lipid fraction of *Foeniculum vulgare* and its combined effects with standard antibiotics[a].

Bacteria	Zone of inhibition in millimeters (mm)														
	A	B							C						
	FE	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	29.66 0.57	29.5 ± 0.7	R	R	24.3 ± 0.5	17 ± 0	26 ± 0	11.93 ± 0.11	30 ± 0A	R	7 ± 0A	26 ± 0A	20.1 ± 0.11A	22 ± 0A	10 ± 0A
SF	11 ± 0	26.5 ± 0.70	R	R	24.2 ± 0.4	16.3 ± 0.51	28.2 ± 0.40	10.5 ± 0.70	20 ± 0A	7 ± 0A	8 ± 0A	20 ± 0A	13 ± 0A	7 ± 0A	12 ± 0A
SL	12.33 ± 2.51	25.6 ± 0.46	R	R	21.9 ± 0.05	14.1 ± 0.28	25.8 ± 0.28	13.1 ± 0.17	30 ± 0A	7 ± 0A	R	26 ± 0A	18.06 ± 0.11A	R	17 ± 0A
SA	16.3 ± 1.52	26 ± 0	R	R	22.06 ± 0.11	22.1 ± 0.23	26.1 ± 0.17	17.2 ± 0.34	24.9 ± 0.05A	7.93 ± 0.11A	8 ± 0A	25.06 ± 0.11A	21 ± 0A	12.6 ± 0.57A	16.7 ± 0.51 I
CF	12.33 ± 0.57	22 ± 0	R	R	22.3 ± 0.5	16 ± 0	23.66 ± 0.57	12 ± 0	21 ± 0A	7 ± 0A	8 ± 0A	21 ± 0A	15 ± 0A	8 ± 0A	18 ± 0A

[a]A = Inhibition zones that occurred with lipid fractions (lipid fractions *Foeniculum vulgare*: 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 lipid fractions and standard antibiotic were used together, (lipid fractions of *Foeniculum vulgare* 10 µL/disc); (FE = *Foeniculum vulgare*, 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).

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Table 6. Antibacterial activity of ethanolic lipid fraction of *Nigella sativa* and its combined effects with standard antibiotics[a].

Bacteria	Zone of inhibition in millimeters (mm)														
	A	B										C			
	NE	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	9.93 ± 0.11	29.5 ± 0.7	R	R	24.3 ± 0.5	17 ± 0	26 ± 0	11.93 ± 0.11	26 ± 0A	R	R	25.1 ± 0.17A	18 ± 0A	R	R
SF	15 ± 0	26.5 ± 0.7	R	R	24.2 ± 0.4	16.3 ± 0.51	28.2 ± 0.40	10.5 ± 0.70	23 ± 0A	R	R	22 ± 0A	10 ± 0A	R	R
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	**32 ± 0S**	R	R	24 ± 0A	16 ± 0A	R	10A
SA	15 ± 0	26 ± 0	R	R	22.06 ± 0.11	22.1 ± 0.23	26.1 ± 0.17	17.2 ± 0.34	25.13 ± 0.23A	11 ± 0A	10 ± 0A	26 ± 0A	16.06 ± 0.11A	18.66 ± 0.57A	15 ± 0I
CF	N/D	22 ± 0	R	R	22.3 ± 0.5	16 ± 0	23.66 ± 0.57	12 ± 0	21.06 ± 0.11A	R	R	22.2 ± 0.40 I	11 ± 0A	R	R

[a]A = Inhibition zones that occurred with lipid fractions (lipid fractions 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 lipid fractions and standard antibiotic were used together, (lipid fractions of *Nigella sativa* 10 µL/disc); (NE = *Nigella sativa*, 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).

Table 7. Antibacterial activity of ethanolic lipid fraction of *Laurus nobilis* and its combined effects with standard antibiotics[a].

Bacteria	Zone of inhibition in millimeters (mm)														
	A	B										C			
	LE	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	10.33 ± 0.57	29.5 ± 0.7	R	R	24.3 ± 0.5	17 ± 0	26 ± 0	11.93 ± 0.11	31 ± 0 A	R	R	25 ± 0A	20 ± 0A	R	R
SF	8.33 ± 0.57	26.5 ± 0.7	R	R	24.2 ± 0.4	16.3 ± 0.51	28.2 ± 0.40	10.5 ± 0.70	27 ± 0A	R	R	25 ± 0A	15 ± 0A	R	12 ± 0A
SL	9.66 ± 0.57	25.6 ± 0.5	R	R	21.9 ± 0.05	14.1 ± 0.28	25.8 ± 0.28	13.1 ± 0.17	26.2 ± 0.3A	R	R	25 ± 0A	16 ± 0A	R	17 ± 0A
SA	8 ± 1.73	26 ± 0	R	R	22.06 ± 0.11	22.1 ± 0.23	26.1 ± 0.17	17.2 ± 0.34	18 ± 0A	8 ± 0I	8.06 ± 0.11I	21 ± 0 A	17 ± 0A	10 ± 0A	19.2 ± 0.3A
CF	9.0 ± 0.0	22 ± 0	R	R	22.3 ± 0.5	16 ± 0	23.66 ± 0.57	12 ± 0	29 ± 0A	10 ± 0S	10 ± 0S	16 ± 0A	16.1 ± 0.11A	10 ± 0A	10.1 ± 0.2A

[a]A = Inhibition zones that occurred with lipid fractions (lipid fractions of *Laurus nobilis*: 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 lipid fractions and standard antibiotic were used together, (lipid fractions of *Laurus nobilis* 10 µL/disc); (LE = *Laurus nobilis*, 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).

Table 8. Antibacterial activity of ethanolic lipid fraction of *Coriandrum sativum* and its combined effects with standard antibiotics[a].

Bacteria	Zone of inhibition in millimeters (mm)														
	A	B							C						
	CE	L	O	M	C	CN	T	P	L	O	M	C	CN	T	P
EC	24.33 ± 1.15	29.5 ± 0.7	R	R	24.3 ± 0.5	17 ± 0	26 ± 0	11.93 ± 0.11	31 ± 0A	R	8 ± 0A	22 ± 0A	17 ± 0 I	8 ± 0A	11 ± 0I
SF	12.66 ± 2.08	26.5 ± 0.7	R	R	24.2 ± 0.4	16.3 ± 0.51	28.2 ± 0.40	10.5 ± 0.70	25 ± 0A	8.06 ± 0.11A	8 ± 0A	28 ± 0A	19 ± 0A	9.06 ± 0.11A	16 ± 0A
SL	9 ± 1	25.6 ± 0.5	R	R	21.9 ± 0.05	14.1 ± 0.28	25.8 ± 0.28	13.1 ± 0.17	30 ± 0A	7 ± 0A	7 ± 0A	25 ± 0A	18.2 ± 0.3A	7 ± 0A	17 ± 0A
SA	16 ± 1	26 ± 0	R	R	22.06 ± 0.11	22.1 ± 0.23	26.1 ± 0.17	17.2 ± 0.34	25 ± 0A	8 ± 0A	7.1 ± 0.11A	22 ± 0 I	25 ± 0A	10.5 ± 0.70A	10 ± 0A
CF	10.66 ± 1.15	22 ± 0	R	R	22.3 ± 0.5	16 ± 0	23.66 ± 0.57	12 ± 0	27 ± 0A	8 ± 0A	8 ± 0A	22 ± 0 I	18 ± 0A	6.2 ± 0.40A	13.1 ± 0.2A

[a]A = Inhibition zones that occurred with lipid fractions (lipid fractions of *Coriandrum sativum*: 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 lipid fractions and standard antibiotic were used together, (lipid fractions of *Coriandrum sativum* 10 µL/disc); (CE = *Coriandrum sativum*, 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).

Table 9. Antibacterial activity of combined effects of ethanolic lipid fractions by agar well diffusion method[a].

LFs conc.	Bacterial strains Zone of inhibition in millimeter (mm)				
	E. coli	*C. freundii*	*S. liquefaciens*	*S. aureus*	*S. fonticola*
N + F	N/D	N/D	N/D	12.8 ± 0.01	N/D
N + L	N/D	N/D	N/D	15 ± 0	N/D
N + C	N/D	N/D	N/D	10.04 ± 0.3	N/D
F + L	N/D	N/D	N/D	10 ± 0	N/D
F + C	N/D	N/D	N/D	13 ± 0.01	N/D
L + C	N/D	N/D	N/D	14.2 ± 0.04	N/D

[a]N/D = No antibacterial activity detected; N + F = (*Nigella sativa* + *Foeniculum vulgare*); N + B = (*Nigella sativa* + *Laurus nobilis*); N + C = (*Nigella sativa* + *Coriandrum sativum*); F + L = (*Foeniculum vulgare* + *Laurus nobilis*); F + C = (*Foeniculum vulgare* + *Coriandrum sativum*); and L + C = (*Laurus nobilis* + *Coriandrum sativum*); conc. = concentrations.

LFs. Higher the thickness of LFs, the less it diffused through agar medium [27]. The least MIC was noted at 62.5 µg/ml in macro-broth dilution method. Among all, LF of *L. nobilis* produced antibacterial effects at 62.5 µg/ml towards the selected foodborne strains except *C. freundii* at 125 µg/ml. Therefore, it could be hypothesized that ethanolic LFs are very compatible in Mueller Hinton broth and demonstrate their antibacterial potentials at their best [10].

Synergistic effects were only seen against *S. aureus* with the combination of LF of *N. sativa* and levofloxacin and secondly against *C. freundii*, when the combination of *L. nobilis* and oxacillin and amoxicillin were applied. While rest of the possible combinations exhibited inhibitory results. Therefore, it could be proposed that ethanol may have a negative impact and block the interaction of LFs and antibiotics. Natural compounds of plants and drugs interacted in same pharmacodynamics manner as drugs interacted with each other. Four different outcomes usually appeared when drug-drug interaction occurred in laboratory examination namely synergistic, indifference, additive, and antagonistic [28]. Several studies have reported the synergistic and other effects produced by the interactions of plants produced compounds and drugs [29] [30] [31]. Plants released natural substances made up of the mixture of various bioactive components. These bioactive components attach to the active spot present on the bacterial cell that determine the antimicrobial strength of substance and their effectiveness rely on the synchronous action of these functional groups present in the natural substances [32] [33].

Phenomena of synergism can be seen in the condition when two different compounds attack pathogen by using different mechanisms. While additive effect was observed when two different compounds attack the pathogen with same mechanism. For example, mode of action of one compound is cell wall inhibition and other is to stop protein synthesis. One of the study reported that the combined effect of vancomycin (cell wall inhibitor) and the extract of *Canarium odontophyllum* produced additive results because the mode of action of CO was

the same as the drug (vancomycin) [33] [34].

Such practice is adapted by various laboratory technologists and researchers to reduce the expansion of drug resistance. Secondary metabolites produced by plants have broad spectrum antimicrobial activity with minimum side effects. Therefore, they could be a perfect alternative of artificial medicines. On the other hand, the blend of natural metabolites with commercial drugs might regain the effectiveness of existing medicine that is lost due to over exploitation. Thus, the interaction of new antimicrobials and the synthetic drugs that produce synergistic and additive outcomes could ease the process and duration of treatment [35].

5. Conclusion

This research pinpoints the idea of utilizing LFs for therapeutic purpose. Ethanolic lipid fractions were phenomenal and exhibited great effort to kill and inhibit the growth of microbes. These natural constituents prove to be a good substitute of synthetic drug. Further studies are required to purify those compounds, responsible for antimicrobial activity. Unfortunately, antagonistic outcomes were found by the combination of antibiotics and LFs could suggest the idea that both allopathic and herbal treatment should not take simultaneously. However, synergistic effect was also observed with beta-lactam drugs.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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