Inactivation of food borne pathogens by lipid fractions of culinary condiments and their nutraceutical properties

Ayeza Naeem,1 Tanveer Abbas,1 Tahira Mohsin Ali,2 Abid Hasnain2
1Department of Microbiology; 2Department of Food Science and Technology, University of Karachi, Karachi, Pakistan

Abstract
Lipid fraction from four different culinary condiments namely black seed (Nigella sativa), fennel seeds (Foeniculum vulgare), bay leaf (Laurus nobilis) and coriander seeds (Coriandrum sativum) were investigated for total phenolic content, antioxidant activity, total flavonoid content, total flavonol content and antibacterial attributes. Antimicrobial properties were determined against food-borne bacteria through agar well diffusion, drop agar diffusion, macrobroth dilution with simultaneous determination of their minimum inhibitory concentrations and changes in cellular morphology was analyzed through Scanning electron microscopy. Generally, ethanolic lipid fractions were more effective bioactively as compared to methanolic LFs. Parallel results were obtained for antioxidant activities with the highest antioxidant activity exhibited by ethanolic LFs. The results positively support the use of these lipid fractions in generating new systems to inhibit bacterial growth, extend the shelf life and enhance the safety of the packaged food product. The examined oils can also be used for therapeutic purposes.

Introduction
Food poisoning is still a major apprehension simultaneously for consumers and the food industry regardless of the use of numerous conservation procedures. Due to the resistance that pathogens build to counter antibiotics, there is an increasing awareness to make use of natural antibacterial derivatives for food preservation and safety, like extracts of culinary herbs and condiments.1

Lipid fractions have since quite a while ago served as enhancing flavors in food and drinks, and because of their versatile composition of antimicrobial complexes, they have potential for food preservation.2 Various pharmaceutical and biological activities like, antibacterial, antifungal, antitumor, antimutagenic, antiobesity, antiviral, anti-inflammatory, and antiprotozoal properties are assigned to them.3 The antimicrobial activity of lipid fractions is allotted to many terpenoid and phenolic compounds, which also in crude form have been shown to possess antibacterial or antifungal activity.4 The antibacterial properties of these compounds are in part related to their lipophilic attribute, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion.5 However, there are often large variances in the stated antibacterial activity of oils from the same source. The justification for this diversity can be due to the geographical sources, the harvesting seasons, the genotype, the climate, the drying and the distilled part of the plant which are significant factors influencing the chemical composition and relative magnitudes of the individual elements in the lipid fractions of the plant. Also, several lipid fraction components show noteworthy antimicrobial characteristics when tested discretely.6 However, there is confirmation that lipid fractions are more intensely antimicrobial than their major antimicrobial constituents.7

The present study deals with the isolation of lipid fractions of Nigella sativa Linn. (Family: Ranunculaceae), Coriandrum sativum L. (Family: Apiaceae Umbelliferae), Foeniculum vulgare Miller (Family: Apiaceae) and Laurus nobilis L. (Family: Lauraceae) and to compare these lipid fractions in terms of their antioxidant, total phenolic, total flavonoid and total flavonol contents. Moreover, the antimicrobial activities of these fractions were also investigated using different assays against five food borne pathogens.

Materials and Methods
Chemicals
All chemicals used in this research were of analytical grade and were obtained from Sigma Aldrich (Sigma Aldrich GmbH, Sternheim, Germany). Mueller Hinton agar and broth were purchased from Thermo Scientific™ Oxoid™. Extract of analytical grade and were obtained from Sigma Aldrich (Sigma Aldrich GmbH, Sternheim, Germany). Mueller Hinton agar and broth were purchased from Thermo Scientific™ Oxoid™.

Seed material
Four different dried spices i.e. black seeds, fennel seeds, coriander seeds and bay leaf were purchased from a local market during the month of February 2015. The spices were ground to fine powder using a Waring® Spice Grinder WSG60K and preserved in zip-lock® bags and stored at freezing temperature until analyzed.

Analytical methods
Antioxidant activity
The free radical scavenging ability of

Extraction of lipid fractions
Lipid fractions were extracted by the solvent extraction method as proposed by Cheikh-Rouhou et al. 20078 using methanol and ethanol as an extractant. Dried spice powder (50g) was extracted separately with 250ml of each solvent. After mixing in a shaking water bath for four hours at 40°C, the mixture was centrifuged for 15 minutes at 1000g. The supernatant was filtered through a Whatman® No. 2 filter paper. The extraction procedure was repeated twice and the solvent was removed using a rotary evaporator (Rotavapor R-210, Buchi laboratories, Switzerland) at 40°C. The concentrated lipid fraction was pooled in an amber colored bottle and tightly sealed and stored at freezing temperature until analyzed. Extraction yield of each ethanolic and methanolic lipid fraction was calculated in terms of percent extraction yield and tabulated by the formula:

\[
\text{Extraction yield of lipid fraction (\%) = } \frac{\text{Mass of essential oil (g)}}{\text{Total mass of spice powder (g)}} \times 100
\]

Copyright A. Naeem et al., 2018
Licensee PAGEPress, Italy
Microbiology Research 2018; 9:7465
doi:10.4081/mr.2018.7465
the lipid fractions was determined using the method as described by Han, Weng, & Bi, 2008. Two hundred microliters of different concentrations (10µg/ml, 100µg/ml and 250µg/ml) of lipid fractions was mixed with 2.7ml of 0.06mM methanolic solution of DPPH (2,2-diphenyl-1-pircyyl - hydrazyl). The absorbance of the resulting mixture was measured after 15 minutes at 517 nm using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan).

Determination of total phenolic content
The concentration of total phenols in lipid fractions was analysed using Folin-Ciocalteu Micro method; Waterhouse 2002; and calibrating with standard curve of gallic acid. Briefly, 20 µL of lipid fraction was mixed with 1.58ml of distilled water and 100 µL of Folin-Ciocalteu reagent. The mixture was homogenized completely and incubated for 8min. Subsequently, 300 µL of aqueous 15% sodium bicarbonate was added, and the mixture was allowed to stand for 120 minutes with intermittent shaking. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan). Total phenolic concentration was expressed as gallic acid equivalent in mg per gram of lipid fraction.

Determination of total flavonoid content
Total flavonoid content was dictated by a colorimetric technique portrayed beforehand by Hajlaoui et al., 2009. Lipid fraction (250µL) was diluted with 1250 µL of distilled water followed by the addition of 75 µL of a 5% NaNO2 (w/v) solution. After 6 minutes, 150 µL of a 10% AlCl3,6H2O (w/v) solution was added, and the blend was permitted to remain for another 5 minutes. Five hundred microliters of 1 M NaOH was added, and the aggregate was made up to 2.5 ml with distilled water. The absorbance was measured against the blank at 510 nm utilizing a UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan) in correlation with known Quercetin standard.

Total flavonol content
Total flavonols in the lipid fractions were evaluated utilizing the strategy reported beforehand by Hajlaoui et al., 2009. To 2000 µL of lipid fraction at different concentrations (10µg/ml,100 µg/ml and 250 µg/ml), 2000 µL AlCl3 (2% w/v in ethanol) and 3000 µL (50 g/L) sodium acetic acid solution was included. The blend was shaken and heated for 2.5 hours at 20°C. Absorbance was measured at 440 nm. Total flavonols were communicated as mg of quercetin counterparts per gram of dry weight (mg QE/ml of lipid fraction) utilizing the calibration curve with quercetin.

Determination of antibacterial activity

Bacterial cultures
Five food borne pathogens (Escherichia coli ATCC 8739, Vibrio parahaemolyticus ATCC 17802, Listeria monocytogenes ATCC 13932, Bacillus cereus ATCC 11778 and Vibrio alginolyticus ATCC 17749) were selected as test microorganisms. The cultures were grown overnight on nutrient agar plates for 16 hours and next day a loopful of each test bacteria were inoculated in 3ml of Mueller Hinton broth and were incubated at 37°C until turbidity of 0.5 (1.5×10^8 CFU/ml) Mcfarland index was achieved.

Lipid fraction dilutions
Lipid fractions of black seeds, fennel, bay leaf and coriander seeds were diluted in 40% DMSO according to the method of Martins et al. 2013. The concentrations of the lipid fractions used were 1000µg/ml, 500µg/ml, 250µg/ml,125µg/ml and 62.5µg/ml. For the bioassay, the stock solutions of lipid fractions were sterilized by filtration using sterile membrane filters.

Antibacterial test using the agar well diffusion method
The antibacterial activity of the lipid fractions was determined by the agar well diffusion method proposed by Martins et al. 2013.

Drop agar diffusion method
Antibacterial activity of the selected lipid fractions was determined by drop agar diffusion method as previously described by Lopes-Lutz, Alviano, Alviano, & Kolodziejczyk, 2008.

Determination of minimum inhibitory concentration
Minimum inhibitory concentration is generally considered as a measure of antimicrobial performance of LFs.

Table 1. Percentage yield of lipid fractions from dried condiments.a

<table>
<thead>
<tr>
<th>Condiments</th>
<th>Percentage yield (weight of lipid fraction: weight of dried plant material w/w)</th>
<th>Methanol %</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black seeds</td>
<td>11.15±1.00^a</td>
<td>43.11±1.00^d</td>
<td></td>
</tr>
<tr>
<td>Fennel</td>
<td>30.46±1.00^a</td>
<td>26.70±1.00^d</td>
<td></td>
</tr>
<tr>
<td>Coriander seeds</td>
<td>20.86±1.00^a</td>
<td>33.42±1.00^d</td>
<td></td>
</tr>
<tr>
<td>Bay leaf</td>
<td>12.22±1.00^a</td>
<td>12.93±1.00^c</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicates ± SD. Values in the same column with different superscripts are significantly different at P<0.05.

Analysis of disruption in cellular morphology using Scanning electron microscopy
Selected bacteria were standardized to a 0.5 Mcfarland scale and incubated for 18h at 35°C. Same strains were exposed to lipid fractions at MIC overnight at 35°C. After 18h of incubation, 10 µl of crystal violet was added to Eppendorf tubes and left for 1min. Subsequently, the content of the tubes was washed with 70%, 80% and 90% ethanol and centrifuged at 13000 rpm for 10min each. The bacterial cells were coated up to 300^A with gold and viewed under SEM (JSM-6380A) at Centralized Science Laboratories, University of Karachi.

Statistical analysis
Analysis of variance was employed to compute significant differences between the means, and Duncan’s test at P<0.05 was used to separate means using SPSS software (version 24, SPSS Inc., USA). Adobe photoshop was used to create canvas of SEM images.

Results and Discussion

Extraction yield of lipid fractions
Table 1 shows the respective percentage yield of different lipid fractions extracted from spices. The highest yield was observed for Nigella sativa lipid fraction with the value of 43%. Lipid fractions were extracted at a fixed temperature by methanol and ethanol solvents. These two solvents differ in their relative abilities to extract different bioactive constituents from the herbs and spices. On general basis, percentage yield of ethanolic lipid fractions was higher as compared to their counterparts. This signifies that the solvent ethanol has a better capability to percolate in the lipid rich cells and to extract out the lipid fraction (Table 2).
Quantification of bioactive compounds

Biologically active compounds namely phenolics, antioxidants, flavonoids and flavonols were estimated (Table 3).

Estimation of total phenolic content

Total phenolic content in lipid fractions prepared from above mentioned herbs was determined by Folin-Ciocalteu Micro method. The total phenolic content ranged from 0.82-5.40 mg/L gallic acid equivalents (Table 3). The phenolic content of methanolic LFs was higher for all the selected herbs except for fennel.

Total antioxidant activity

Antioxidant activity was determined using DPPH assay. DPPH (2,2-diphenyl-1-picyl-hydrazyl) is a stable free radical and accepts an electron of hydrogen radical to become a stable diamagnetic molecule. When lipid fractions comprising of different proportions of antioxidants are reacted with the DPPH radical, the absorbance of the reaction mixture is decreased at 517nm. The highest radical scavenging activity was observed for ethanolic lipid fraction of coriander seeds. It was also observed that the percent scavenging activity also increased with the increasing concentration of lipid fractions (Table 2). The TPC showed a strong correlation with the DPPH scavenging abilities. The LFs which yielded higher TPC also had higher values for percent inhibition. Antioxidant activity was also evaluated as ascorbic acid equivalents for ethanolic lipid fraction of bay leaf.

Table 2. Determination of percent DPPH scavenging effect of lipid fractions.

<table>
<thead>
<tr>
<th>Spices</th>
<th>Solvents</th>
<th>Percent DPPH scavenging effect (percent inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 µg/ml 100 µg/ml 250 µg/ml</td>
</tr>
<tr>
<td>Black seeds</td>
<td>Methanol</td>
<td>64.67±0.94c 87.60±1.11b 97.62±1.10b</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>61.60±1.05a 80.54±1.06a 90.45±1.01a</td>
</tr>
<tr>
<td>Fennel</td>
<td>Methanol</td>
<td>60.47±1.11b 68.27±0.94c 76.21±1.03c</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>93.40±1.13b</td>
</tr>
<tr>
<td>Coriander seeds</td>
<td>Methanol</td>
<td>50.36±1.23a 68.38±0.71a 74.70±1.01a</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>64.55±1.15b</td>
</tr>
<tr>
<td>Bay leaf</td>
<td>Methanol</td>
<td>87.49±0.83d 84.89±1.03b 99.38±1.07b</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>33.89±0.98c 64.67±0.94a</td>
</tr>
</tbody>
</table>

Values are means of triplicates±SD. Values in the same column with different superscripts are significant at P<0.01.

Table 3. Total phenolic, total antioxidant, total flavonoid and total flavonol contents of lipid fractions of condiments.

<table>
<thead>
<tr>
<th>Spices</th>
<th>Solvents</th>
<th>Total phenolic content (mg/L)</th>
<th>Total antioxidant content (Ascorbic acid equivalent)</th>
<th>Total flavonoid content (mg/L)</th>
<th>Total flavonol content (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total phenolic content (mg/L)</td>
<td>Total antioxidant content (mg/L)</td>
<td>Total flavonoid content (mg/L)</td>
<td>Total flavonol content (mg/mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Un-diluted</td>
<td>10 µg/ml 100 µg/ml 250 µg/ml</td>
<td>10 µg/ml 100 µg/ml 250 µg/ml</td>
<td></td>
</tr>
<tr>
<td>Black seeds</td>
<td>Methanol</td>
<td>3.12±0.20c</td>
<td>15.40±0.37c 15.72±0.10b 14.82±1.10d</td>
<td>0.82±0.002a 23.62±1.22c 25.70±1.10d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>2.15±0.60b</td>
<td>20.12±1.02c 20.53±0.39c 20.43±0.77c</td>
<td>5.09±0.20c 20.62±1.41b 22.63±1.06b</td>
<td></td>
</tr>
<tr>
<td>Fennel</td>
<td>Methanol</td>
<td>1.78±0.09b</td>
<td>17.96±0.99b 18.98±1.00d 18.94±1.00d</td>
<td>2.15±0.66b 20.12±1.02c 22.63±1.06c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>5.09±0.20c</td>
<td>20.62±1.41b 22.63±1.06b 22.68±0.94d</td>
<td>8.83±0.002c 23.62±1.22c 25.70±1.10d</td>
<td></td>
</tr>
<tr>
<td>Coriander seeds</td>
<td>Methanol</td>
<td>3.33±0.15c</td>
<td>17.34±0.09c 17.42±0.72c 17.65±1.41c</td>
<td>1.20±0.05c 21.52±0.35c 31.49±0.88c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>1.89±0.40c</td>
<td>16.37±0.38c 21.62±1.12b 22.68±1.06c</td>
<td>0.83±0.002c 23.62±1.22c 25.70±1.10d</td>
<td></td>
</tr>
<tr>
<td>Bay leaf</td>
<td>Methanol</td>
<td>3.12±0.20c</td>
<td>15.40±0.37c 15.72±0.10b 14.82±1.10d</td>
<td>0.83±0.002c 23.62±1.22c 25.70±1.10d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>2.15±0.60b</td>
<td>20.12±1.02c 20.53±0.39c 20.43±0.77c</td>
<td>5.09±0.20c 20.62±1.41b 22.63±1.06b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicates±SD. Values in the same column with different superscripts are significantly different at P<0.05.

Table 4. Antibacterial activity of lipid fractions of black seeds, fennel, coriander seeds and bay leaf (un-diluted) by drop agar diffusion method.

<table>
<thead>
<tr>
<th>Food pathogens tested</th>
<th>Black seeds</th>
<th>Fennel</th>
<th>Coriander seeds</th>
<th>Bay leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Methanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739</td>
<td>7.32±1.14</td>
<td>10.52±1.98</td>
<td>10.55±0.62</td>
<td>9.27±1.12</td>
</tr>
<tr>
<td>Listeria monocytogenes ATCC 13302</td>
<td>9.06±1.03</td>
<td>17.37±1.34</td>
<td>11.29±0.81</td>
<td>15.56±1.25</td>
</tr>
<tr>
<td>Vibrio parahemolyticus ATCC 1769</td>
<td>24.54±1.34</td>
<td>26.13±0.30</td>
<td>11.69±0.60</td>
<td>24.57±1.00</td>
</tr>
<tr>
<td>Vibrio alginitolyticus ATCC 17749</td>
<td>7.53±1.19</td>
<td>N/D</td>
<td>11.27±0.88</td>
<td>N/D</td>
</tr>
<tr>
<td>Bacillus cereus ATCC 11778</td>
<td>11.17±1.16</td>
<td>14.6±0.75</td>
<td>12.23±1.20</td>
<td>11.56±1.39</td>
</tr>
</tbody>
</table>

Values are means of triplicates±SD. Values in the same column with different superscripts are significantly different at P<0.05.
of bioactive compounds. The plant polyphenols group which also have similar antibacterial functions. The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to elic-

The subclasses in nols and phenolics have been shown to eli-
properties includes phenols, phenolic acids, flavonoids and flavonols etc. These compounds are produced by plant cells in defense to microbial invasion and therefore these elements have could kill a wide variety of microbes. The scanning electron microscopy images revealed the disruption in cellular morphology of tested pathogens when treated with LFs at MICs. Control cells were not exposed to these LFs (Figure 1). The changes in cell morphology were clearly visible in all treated pathogens with the LFs, in which formation of pores in cell wall and destruction of the bacterial cells were evident. Some researchers reported the effects of treatment with lipid fractions as cell wall disruption, damage to cellular membrane, membrane proteins, effusion of intracellular material, condensation of cytoplasmic fluid and reduction of proton motive force. Based on the results obtained from the changes in morphology, it can be postulated that the main target sites of these lipid fractions were cell wall and cell membrane of tested bacteria. It can also be hypothesized that the possible mechanism of action of these lipid fractions may involve termination of N-acetyl muramic acid linkages, which would subsequently halt the synthesis of cell wall. In the nutshell, the findings obtained in this study indicated that ethanol is a solvent of choice to extract lipid fractions that will be rich in polyphenols, antioxidant compounds and will also be effective to inhibit the selected food pathogens in lower concentrations.

Table 7. Minimum Inhibitory concentration (MIC) determined by Macrobroth dilution method.*

<table>
<thead>
<tr>
<th>Food pathogens tested</th>
<th>Black seeds</th>
<th>MIC of Lipid fractions (µg/ml)</th>
<th>Bay leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Methanol</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739</td>
<td>500</td>
<td>62.5</td>
<td>N/D</td>
</tr>
<tr>
<td>Listeria monocytogenes ATCC 13932</td>
<td>1000</td>
<td>62.5</td>
<td>N/D</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus ATCC 17802</td>
<td>1000</td>
<td>62.5</td>
<td>N/D</td>
</tr>
<tr>
<td>Vibrio alginolyticus ATCC 17749</td>
<td>N/D</td>
<td>62.5</td>
<td>N/D</td>
</tr>
<tr>
<td>Bacillus cereus ATCC 11778</td>
<td>N/D</td>
<td>62.5</td>
<td>N/D</td>
</tr>
</tbody>
</table>

* (N/D) No detection of antimicrobial activity hence no MIC.

Table 8. Minimum Inhibitory concentration (MIC) determined by well diffusion method.*

<table>
<thead>
<tr>
<th>Food pathogens tested</th>
<th>Black seeds</th>
<th>MIC of Lipid fractions (µg/ml)</th>
<th>Coriander seeds</th>
<th>Bay leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Methanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739</td>
<td>500</td>
<td>250</td>
<td>N/D</td>
<td>250</td>
</tr>
<tr>
<td>Listeria monocytogenes ATCC 13932</td>
<td>250</td>
<td>1000</td>
<td>N/D</td>
<td>1000</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus ATCC 17802</td>
<td>N/D</td>
<td>250</td>
<td>N/D</td>
<td>250</td>
</tr>
<tr>
<td>Vibrio alginolyticus ATCC 17749</td>
<td>250</td>
<td>250</td>
<td>N/D</td>
<td>500</td>
</tr>
<tr>
<td>Bacillus cereus ATCC 11778</td>
<td>250</td>
<td>250</td>
<td>1000</td>
<td>N/D</td>
</tr>
</tbody>
</table>

* (N/D) No detection of antimicrobial activity hence no MIC.
Conclusions

In this paper, original data for total phenolic and total flavonoid contents are a basis for assessment of the role of lipid fractions of black seeds, fennel, bay leaf and coriander seeds against free radicals’ effect and antibacterial activity. It could be concluded from the results that ethanolic lipid fractions had the highest of total phenolic and total flavonoid contents along with better antioxidant activity compared to methanolic lipid fractions. Moreover, the tested lipid fractions were found to be more active against Gram-negative food pathogens as compared to Gram positive bacteria. This could be a novel discovery of natural antimicrobials as in previously published literature natural products have shown to be more active against Gram positive microorganisms. Subsequently, the data found in this work might be used for further study of the selected lipid fractions’ indifferent applications, for example, food packaging and pharmaceutical products etc.

References