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Open Access Journal of Microbiology & Biotechnology
ISSN: 2576-7771

Application of Lipid Fractions of Indigenous Spices in Unpasteurized Fruit Juices System: Increase Storage Expectancy

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Research Article

Volume 6 Issue 1

Received Date: December 26, 2020

Published Date: February 04, 2021

DOI: 10.23880/oajmb-16000182

Abstract

Because of rise in the demand of fresh produce and their products, consumers select safe and natural preservatives over chemical ones. Hence the point of this examination was to investigate the antimicrobial analysis of lipid fractions against microbial contaminants in fruit juices vended in University of Karachi. Eight different juices were collected and ethanolic and methanolic extract of four different lipid fractions were used to test the antimicrobial activity to ensure the food safety. The uncountable microbial load (log 2.653) were decreased to less than half of its initial count with a significant difference ($P<0.05$). Ethanolic and methanolic lipid fractions showed promising results in tested juice environment with as high as 99.775% reduction. The tested lipid fractions exhibited significant antimicrobial activity on the selected fruit juices that have acidic pH. Therefore, we propose the application of culinary lipid fractions in fruit juices as natural alternatives to extend their shelf lives.

Keywords: Antimicrobial Activity; *Coriandrum Sativum; Foeniculum Vulgare; Laurus Nobilis; Nigella Sativa;* Preservatives

Abbreviations: TNTC: Too Numerous to Count; LFs: Lipid fractions

Introduction

Prepared-to-drink fresh juices are exceptionally delectable and easily available at any open spot and roadside shops close to our proximity [1]. Fresh fruits juices are very beneficial for human health as these products are enriched with vitamins, minerals, antioxidants and fibers [2]. Freshly squeezed juices of fruits and vegetables are very popular among all age groups especially in youngsters because of its dietary significance and reviving taste. Because of such characteristics, there is an increased demand and strong preference for juices made from fresh fruits and vegetables over canned or artificially flavored juices [3,4].

Fruits can be spoiled through poor hygiene of street vendors. Surface of fruits and vegetables are damaged or punctured during post-harvest handling. Microbes introduce their selves via filthy equipment used for cutting chopping, and mixing [5]. The use of unsatisfactory methods of extraction (juicers, peelers) and handling of fruit and vegetables during preparation of fresh juices are the potential risk factors associated with foodborne diseases [6]. Ecological pollution is one of the chief culprits to sully fruits and fruit juices via dust particles, swarming flies, and other insects that will act as vectors for transmission of microorganisms. Some of these food borne microorganisms isolated from contaminated juices are responsible for food borne diseases mainly upsetting gastrointestinal system as reported in the previous studies [2,7,8]. It is noted that freshly squeezed fruit juices cannot be stored for a longer period and have short shelf lives therefore, fresh and natural products are the potential vehicles for transmitting pathogenic bacteria and among the key cause of food-related

diseases [9,10]. *E. coli*, *S. aureus*, *V. cholerae*, *Klebseilla*, and *C. albicans* are the major contaminants [11] and *Salmonella*, *Streptococcal spp*, and *Proteus* spp are the minor contaminants of juice [12]. Besides, fresh fruit juices are always in demand during all seasons especially in midsummer. In summers, temperature can reaches to 30-45°C in Pakistan, a range that is ideal for fostering the growth of foodborne pathogens and dangerous for human health. Some pathogenic strains such as *Escherichia coli, Salmonella* and *Shigella* have very low infective dose to cause serious foodborne diseases. Therefore, research into alternate means (other than the thermal ones) is needed to not only effectively preserve the nutritional value of these juices, as well as making them safe and healthy for consumption [13].

Lipid fractions are well- known for their broad spectrum inhibitory potentials against microorganisms [14-19]. US Food Drugs and Administration also recognizes edible oils obtained from routinely used culinary herbs and spices in food industries as well as in domestic kitchens as relatively safe for antimicrobial preservation [20]. Several studies have reported LFs derived from clove, cinnamon, geraniol, lemongrass and palmarosa are exhibited *in vitro* inhibitory effects against food borne bacterial pathogens [15,21-25]. Hence, the goal of this investigation was to assess the antimicrobial potential against microbial contaminants through direct incorporation of lipid fraction into fresh fruit juices.

Materials and Methods

Sample Collection

Total eight fruit juice (unpasteurized) samples namely apple (*Pyrus malus*), pineapple (*Ananas comosus*), pomegranate (*Punica granatum*), orange (*Citrus reticulata*), sweet orange (*Citrus sinensis*), grape fruit (*Citrus paradisi*), sugarcane (*Saccharum officinarum*), and sapodilla (*Manilkara zapota*) were purchased from different canteens located in the premises of University of Karachi. Sterile strainers were used to remove fleshy portion from fruit juice samples and the clear juice was collected in sterile bottles. The pH of fruit juices was also measured (Table 1).

English name	Common name	Scientific name	pH
Apple	Saib	*Pyrus malus*	3.3
Pineapple	Ananas	*Ananas comosus*	3.5
Orange	Kino	*Citrus reticulata*	2
Sweet orange	Mosambi	*Citrus sinensis*	3
Sugarcane	Ganna	*Saccharum officinarum*	7.5
Pomegranate	Anaar	*Punica granatum*	3
Sapodilla/Nose berry	Sapodilla	*Manilkara zapota*	5.3
Grapefruit	Chakotra	*Citrus paradisi*	3

Table 1: Name of the tested fruits and pH examined for the microbial nature in fruit juices vended in University of Karachi.

Lipid Fractions

Four methanolic and four ethanolic LFs of *Nigella sativa* (black cumin), *Foeniculum vulgare* (fennel), *Laurus nobilis* (bay leaf) and *Coriandrum sativum* (coriander seed) were collected from Food Science and Technology, University of Karachi [26] to determine their inhibitory effects against microbial load present in the fruit juices. These LFs were extracted through solvent extraction procedure [27] and the procedure was clearly described by Naeem, Abbas, Ali, & Hasnain [28].

Total Bacterial Count

The total microbial load in fresh fruit juices before and after treated with LFs was analyzed according to Siddiqua, et al. [29] with certain alterations. Initially, 1 mL of each fruit juice was dispensed into 1.5 mL sterile micro centrifuge tubes. Then, 100 µL of different concentrations of LFs (250, 500, and 1000 µg/mL) prepared in DMSO were added in each tube and incubated at 35 ± 1°C for 2 hours. The same procedure was performed with control groups without incorporation of LFs at 0 hour. After respective incubation, 20 µL drop of each tested and control groups were positioned on nutrient agar plates. After the drops were appropriately assimilated, the plates were incubated for 24 hours at 37°C. The method was repeated 3x and the TBC was recorded following day.

Statistical Analysis

Investigation of variance was utilized to process significant contrasts between the mean with standard deviation by utilizing one-way ANOVA, and Duncan's test was utilized to compute the noteworthy distinction at P <

0.05 among test and control by utilizing SPSS programming (version 24, SPSS Inc., USA).

Results

All the fresh fruit juices had uncountable microbial colonies in their undiluted and diluted form at 0 hour. But after treated with LFs, remarkable decreased up to (0.66 ± 0.47) and (1 ± 0) CFU/mL in the colonial count was observed after two hours of incubation respectively as shown in Table 2 and 3. The total bacterial count which exceed from countable range represented as too numerous to count (TNTC) in the tables and in the graphs showed as 2.653 log CFU/mL value. It was observed that all tested LFs significantly reduced microbial load ($p<0.05$) present in the fruit juices, albeit varying in their degrees.

TVC without lipid fraction (control at 0 hour)			Total number of colonies after treated with methanolic lipid fractions after two hours of incubation											
			LM			CM			FM			NM		
Fruit juices	Conc. form	10^{-6}	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml
P. malus	TNTC	328x10^{6}	TNTC	TNTC	TNTC	68.7±2.30	1.33±0.6	1.67±0.5	73.3±6.5	17±3	1±0	22±7.54	72.7±14	TNTC
p. granatum	TNTC	TNTC	1±0	1.33±1.15	2.33±2.52	16.3±4.04	2.7±0.57	3±1	14±5.29	30±10	26.7±3.51	1.33±0.57	1±0	38.3±7.64
C. paradisi	TNTC	TNTC	26±4.48	40±3.51	49.3±3.06	24.7±6.11	26.33±4.04	37±4.36	175.33±25	534.66±5.03	TNTC	35±5	28.7±1.53	15.3±4.51
C. sinensis	TNTC	36x10^{7}	TNTC	TNTC	TNTC	185.6±12.5	TNTC	TNTC	35.7±6.03	TNTC	TNTC	TNTC	TNTC	TNTC
C. reticulata	TNTC	TNTC	91±4.58	132.6±4.73	99±3	71±9.64	97.3±7.51	73±8.72	124.7±4.73	142.3±10.79	82.7±10.01	54.7±5.03	67±2.65	92.7±6.25
M. zapota	TNTC	TNTC	TNTC	TNTC	TNTC	9.33±2.08	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
S. officinarum	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
A.comosus	TNTC	4x10^{8}	1±0	1.33±0.57	30.7±2.08	TNTC	TNTC	54.3±4.51	23.7±8.1	1.33±0.57	1±0	TNTC	TNTC	4±4.24

Table 2: Total bacterial counts [Mean CFU/mL ± standard deviation for fresh fruit juices samples][a].
[a]TNTC = Too numerous to count; Conc. = concentrations; LM = Methanolic lipid fraction of *L. nobilis;* FM = Methanolic lipid fraction of *Foeniculum vulgare*; CM = Methanolic lipid fraction of *Coriandrum sativum*; NM = *Nigella sativa*

TVC without lipid fraction (control at 0 min)			Total number of colonies after treated with ethanolic lipid fractions after two hours of incubation											
			LE			CE			FE			NE		
Fruit juices	Conc. form	10^{-6}	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml
P. malus	TNTC	328x10^{6}	1±0	1.33±0.57	1.66±1.15	25.66±4.04	TNTC	TNTC	11.33±16.2	23.66±5.51	44.66±5.03	1.33±0.57	1.66±1.15	47.33±12.50
p. granatum	TNTC	TNTC	TNTC	11.66±1.53	25±5	1.66±1.15	30±10	40±10	1±0	1.66±0.57	15.6±4.51	10±5	15±5	32.33±3.21
C. paradisi	TNTC	TNTC	168.3±10	215±6.3	318.7±9.8	236.66±9	309±3	TNTC	229±17.52	302.66±2.08	306±7.94	97.66± 11.68	168.33± 12.58	221.33± 12.06
C. sinensis	TNTC	36x10^{7}	TNTC	TNTC	TNTC	31±1.73	TNTC	TNTC	46±4	TNTC	TNTC	TNTC	TNTC	TNTC
C. reticulata	TNTC	TNTC	35.7±4.0	72±5.56	49±1.73	88±2	101±4	67.66±5.0	31.7±2.8	41.66±2.1	51.7±2.1	86.7±41.3	80.33±2.5	79±1.7
M. zapota	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
S. officinarum	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
A. comosus	TNTC	4x10^{8}	TNTC	TNTC	25±5	TNTC	49.3±2.1	25.3±4.2	TNTC	13±2	10±2.6	48.33±2.1	1±0	1.3±0.6

Table 3: Total bacterial counts [Mean CFU/mL ± standard deviation for fresh fruit juices samples][a].
[a]TNTC = Too numerous to count; Conc. = concentrations; TVC = Total viable count; LE = Ethanolic lipid fraction of *L. nobilis*; FE = Ethanolic lipid fraction of *Foeniculum vulgare*; CE = Ethanolic lipid fraction of *Coriandrum sativum*; NE = *Nigella sativa*

Ethanolic Lipid Fractions

The most inhibitory ethanolic lipid fractions were NE and LE against the microbial contaminants present in the *Pyrus malus* juice (Figure 1b). The LE at all the three concentrations particularly at 1000 µg/ml exhibited significant antimicrobial activity and reduced microbial load from log 2.653 to log 0. Similarly, the NE decreased the microbial count to log 0.1 and log 0.159 at 1000 µg/ml and 500 µg/ml respectively. Reduction in the microbial count was also observed at 1000 µg/ml of FE and CE to log 0.653 and log 1.405 respectively. In the juice of *Ananas comosus*, NE was the most effective LF and inhibited microbial count from log 2.653 to log 0 and log 0.1 at 500 µg/ml and 250 µg/ml respectively followed by FE, LE, and CE also reduced the microbial count at 250 µg/ml (Figure 2b). LF of FE at 500 µg/ml and CE at 1000 µg/ml demonstrated antimicrobial activity to an extent that restrained the microbial development to log 0.2 and log 0.159 respectively in the juice of *Punica granatum* (Figure 3b). However, in the juice of *Citrus sinensis*, only LF of FE and CE at 1000 µg/ml inhibited the bacterial count from log 2.653 (TNTC) to log 1.66 and log 1.49 respectively, while rest of the LFs failed to inhibit the microbial growth (Figure 4b). LF of FE and LE at 1000 µg/ml inhibited bacterial enumeration up to log 1.5 found in the juice of *Citrus reticulata* (Figure 5b).On the contrary, reduction in the microbial count was not as such observed with all the four ethanolic LFs in the juice of *Citrus paradisi* (Figure 6b) and all of them at selected concentrations were ineffective against microbial load present in the juices of *Manilkara zapota* and *Saccharum officinarum* (Figure 7b and 8b).

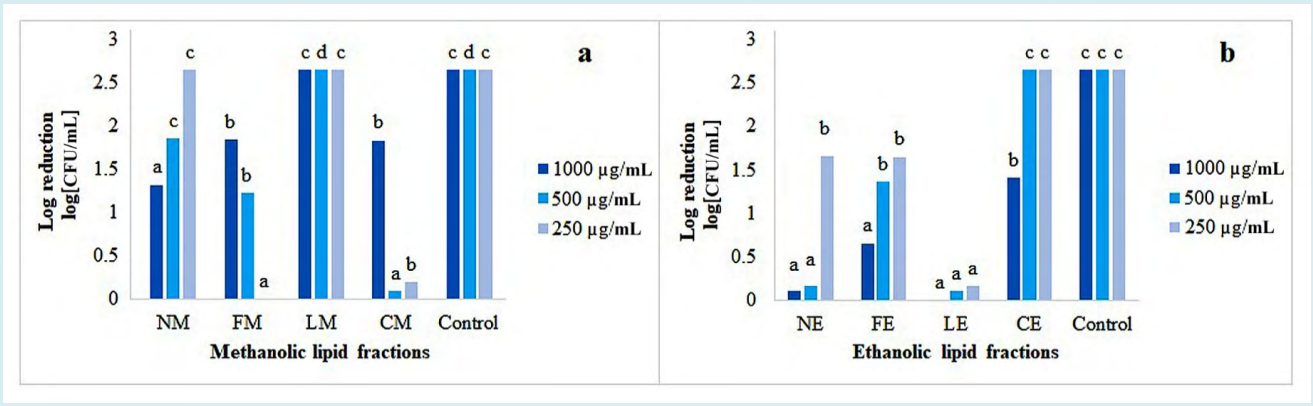

Figure 1: Antibacterial effect of LFs against microbial contaminants in *Pyrus malus* juice: NM; methanolic LF of *Nigella sativa*, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

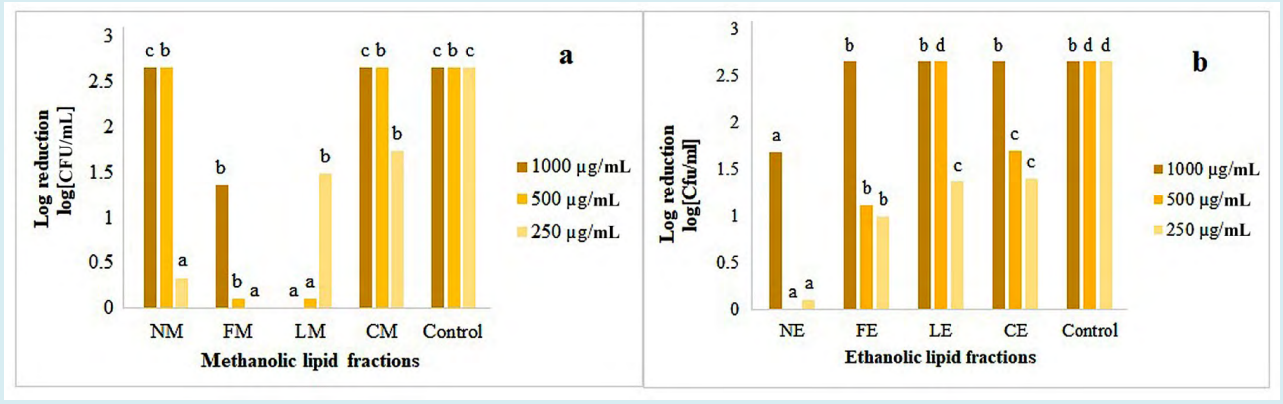

Figure 2: Antibacterial effect of LFs against microbial contaminants in *Ananas comosus* juice: NM; methanolic LF of *Nigella sativa*, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

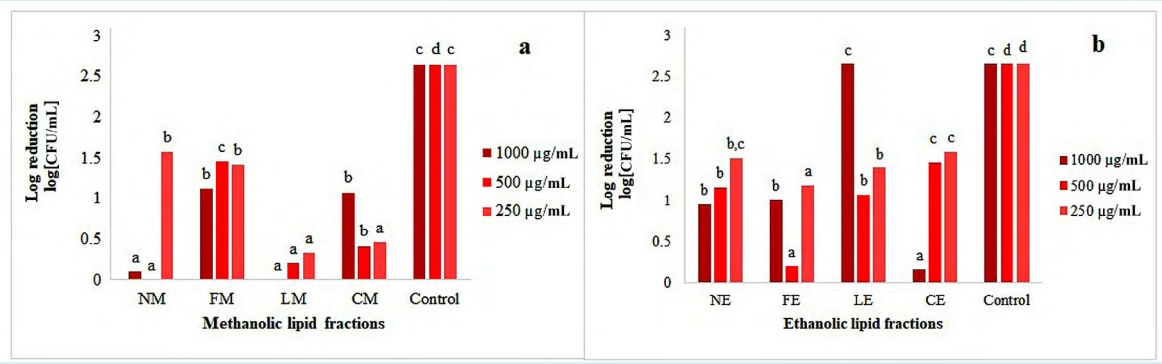

Figure 3: Antibacterial effect of LFs against microbial contaminants in *Punica granatum* juice: NM; methanolic LF of *Nigella sativa*, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

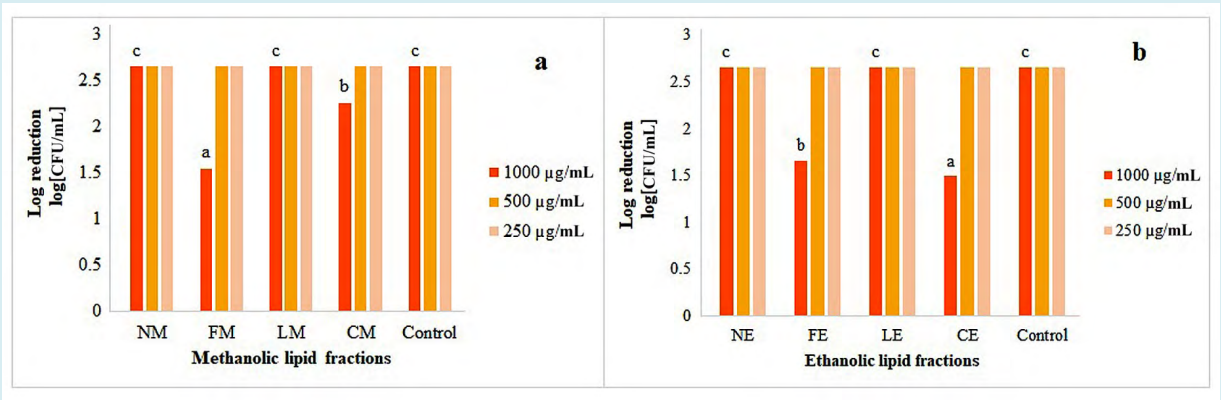

Figure 4: Antibacterial effect of LFs against microbial contaminants in *Citrus sinensis* juice: NM; methanolic LF of Nigella sativa, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of Nigella sativa, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

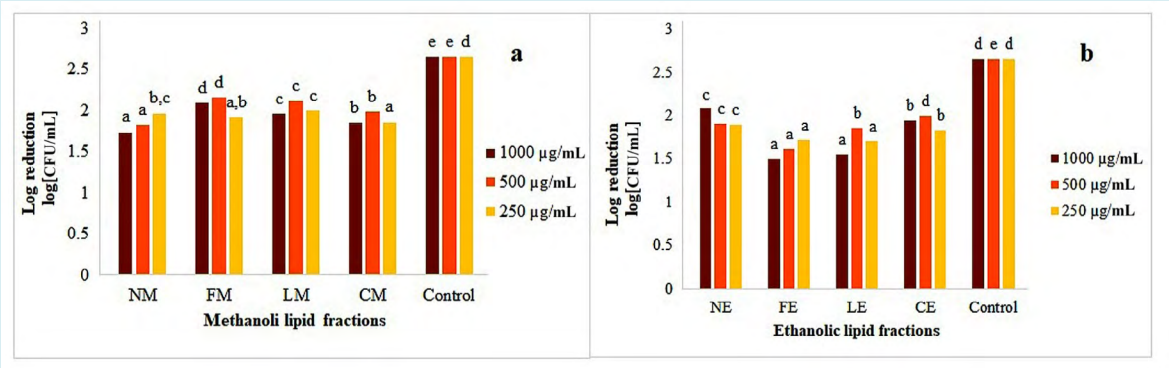

Figure 5: Antibacterial effect of LFs against microbial contaminants in *Citrus reticulata* juice: NM; methanolic LF of *Nigella sativa*, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

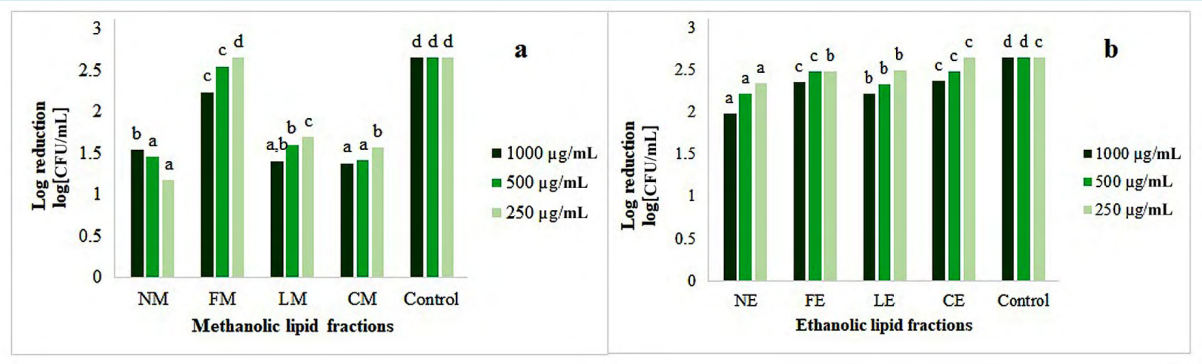

Figure 6: Antibacterial effect of LFs against microbial contaminants in *Citrus paradisi* juice: NM; methanolic LF of Nigella sativa, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of Nigella sativa, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

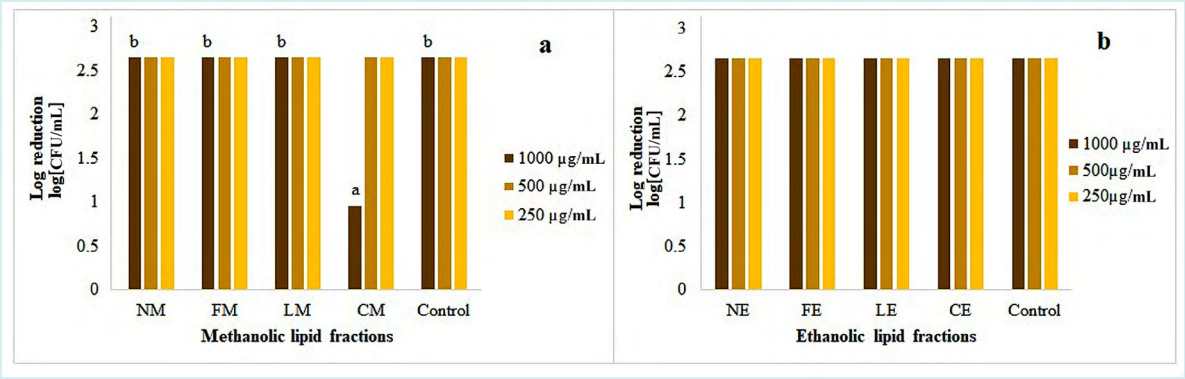

Figure 7: Antibacterial effect of LFs against microbial contaminants in *Manilkara zapota* juice: NM; methanolic LF of Nigella sativa, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of Laurus nobilis CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

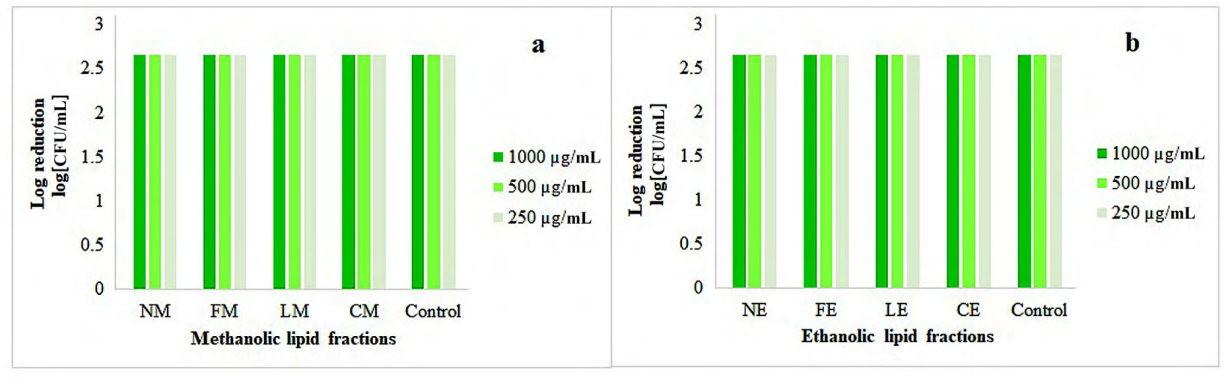

Figure 8: Antibacterial effect of LFs against microbial contaminants *in Saccharum officinarum* juice: NM; methanolic LF of *Nigella sativa*, FM; methanolic LF of *Foeniculum vulgare*, LM; LF of *Laurus nobilis* CM; methanolic LF of *Coriandrum sativum*, NE; ethanolic LF of *Nigella sativa*, FE; ethanolic LF of *Foeniculum vulgare*, BE; ethanolic LF of *Laurus nobilis* CE; LF of *Coriandrum sativum*. Data represent mean log [CFU/mL] values of triplicate measurements. While different lowercase letters (a, b, c, d) represent significant differences ($P < 0.05$) between tested and control groups.

Methanolic Lipid Fractions

In the juice of *Pyrus malus*, LF of FM at 250 µg/ml and CM at 500 µg/ml efficiently decreased the microbial count to log 0 and log 0.1 respectively from log 2.653. Correspondingly, microbial count was effectively inhibited more than half of its initial count by the LF of NM at 1000 µg/ml. However LF of LM was unable to inhibit the bacterial growth (Figure 1a). LFs of NM at 250 µg/ml, FM at 500 µg/ml, 250 µg/ml and LM at 1000 µg/ml and 500 µg/ml showed highest antimicrobial activity against the bacterial count present in the juice of *Ananas comosus* (Figure 2a). All the four methanolic LFs exhibited antimicrobial potential towards the microbial count present in the juice of *Punica granatum* (Figure 3a). Specifically, the NM at 500 µg/ml and LM at 1000 µg/ml were the most effective methanolic LFs. On the contrary, all the four methanolic LFs were unable to retard the replication of microbes except FM at 1000 µg/ml and reduced the total bacterial count from log 2.653 to log 1.548 in the juice of *C. sinensis* (Figure 4a). While all the methanolic LFs showed more or less similar antimicrobial effect against the bacterial count in the juice of *Citrus reticulata* at all the three concentrations (Figure 5a). Although, LF of NM, LM, and CM at all the three concentrations showed good antibacterial activity apart from the LF of FM in the juice of *Citrus paradisi* (Figure 6a). On the other hand, there was no demonstration of antibacterial activity at all the three concentrations of any methanolic LFs against the bacterial count found in the juices of *Manilkara zapota* and *Saccharum officinarum* as sown in figure 7a and 8a. Except CM at 1000 µg/ml inhibited microbial count to log value 0.962.

Discussion

Culinary condiments are known to possess antimicrobial traits in their DNA thus they are rich in bioactive compounds [30-32]. These bioactive compounds contain phenolics, subclasses of polyphenols, flavanoids, carotenoids, and anthocyanin that are very proficient against disease causing germs. Cookery herbs and spices are not only utilized as aromatic and flavoring agent but also maintain the quality of food as preservative agent [33]. Various studies have been reported on the germicidal properties of lipid fractions. Non-synthetic substances are always in demand and now a days there has been drastically increased in their demand due to food safety concern. Heavy microbial count encountered in the juices might be dangerous and unsafe for human consumption. According to various standards, microbial limit in fresh fruit juices and nectars is maximum of 10^3 cfu/g (mL) total plate count and 30 cfu/gm for yeast and moulds [34]. While total viable count higher than 4 log 10 is responsible for food spoilage according to Codex standard [35] and Gulf Standard [36].

Outnumbered investigation has been done in the past and currently on antimicrobial attributes of lipid fractions. However, antimicrobial effectiveness was not fully understood in food system till date and very little data present on it. Lipid fractions have given very competent results in *in-vitro* examination but the situation is opposite in case of *in-vivo* investigation because of the complex nature of food that act as a buffering agent and may goes favor to microbes. Hence to accomplish the identical results as *in-vitro*, higher concentrations of LFs are utilized to create a similar antimicrobial impact. Increase in the concentrations of lipid fractions will directly affect sensory palate that will change the original essence of food. In this study, effective results were obtained against the microbial contaminants in fresh fruit juices at minimum concentrations. Antimicrobial activity of selected LFs was previously tested against the ATCC culture of *E. coli* (8739), *L. monocytogenes* (13932), *V. parahaemolyticus* (17802), *V. alginolyticus* (17749), and *B. cereus* (11778) via agar well diffusion method. Among them, LFs of *L. nobilis* (bay leaf) was the most effective against all the tested foodborne pathogens [37]. Antimicrobial action of the tested LFs was also assessed against commensal and foodborne pathogens recovered from famous street food of Karachi [38]. Effective outcomes were obtained from all the tested LFs in both agar and broth media. To our best knowledge little or no data have documented on the direct application of these selected LFs into fruit juices to evaluate the microbial reduction before and after treatment. However many researches have been done in which the inoculum of bacteria were prepared in appropriate growth media and inoculated into artificially prepared food system. Earlier study demonstrated the antimicrobial potential of *Origanum vulgare* and *Rosmarinus officinalis* alone and in blend against *L. monocytogenes*, *Y. enterocolitica*, *A. hydrophila* and *P. fluorescens* in vegetable soup and in experimentally inoculated fresh-cut vegetables [39].

LFs work best at acidic environment because low pH increases their hydrophobicity thereby permitting easier dissolution in the lipopolysaccharides of the bacterial plasma membrane [40]. Our study also supports the previous reported findings. LFs were found to be more effective in the juice with lower pH as compared to the one with higher pH value like *Saccharum officinarum* (sugarcane) i.e. pH 7.5 as shown in Figures 7 and 8. Therefore, it could be stated that LFs of herbs and spices have good selective toxicity and utilized as natural-borne supplements to extent the expectancy of minimum processed foods [41].

Conclusion

These lipid fractions obtained from herbs and spices containing antimicrobial properties could be an ideal

substitute over chemical preservatives. Incorporation of lipid fractions in the food system should be done in a manner that it does not amend the original taste, aroma and appearance of food. Organoleptic assessments are therefore required for the application of lipid fractions in various food matrices for antimicrobial preservation.

Acknowledgement

The authors desire to express her gratefulness to Ms. Ayeza Naeem for providing lipid fractions from Department of Food Science and Technology, University of Karachi.

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