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Combinatorial effect of probiotics and some medicinal oils on pathogenic bacteria

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Abstract

Probiotics (PB) and medicinal oils (MO) have beneficial effects against microbial gut infections. The present study aimed to evaluate the *in vitro* combined antibacterial activities of PB and MO against pathogenic strains. Antibacterial activities of three probiotic strains (*Lactobacillus plantarum, Lactobacillus casei, and Lactobacillus acidophilus*) and five MO (Bitter almond, Clove, Eucalyptus, Peppermint and Thyme oils) were investigated using agar well diffusion method. Cell-free supernatant (CFS) from PB combined with MO showed different effects against the tested pathogenic strains. Thyme and clove oils showed the highest activity. The combinations of CFS from PB and MO were tested by agar well diffusion method. These combinations showed synergistic activity against *Staphylococcus aureus* especially combinations containing clove, peppermint and thyme oils. Combinations were investigated also by Checkerboard dilution method. Most of CSF significantly lowered the minimum inhibitory concentrations (MICs) of MO against the tested bacteria and fractional inhibitory concentration indices (FICIs) for these combinations ranged from 0.12 to 0.75. Most of the tested CFS-MO combinations showed marked synergistic activity against *S. aureus, E. coli and K. pneumoniae*. In a conclusion, *in vitro* antibacterial study for CFS-MO combinations in our ongoing work suggests the possibility of using these combinations in clinical urinary and gastrointestinal tract infections.

Key words
probiotics, essential oils, gut infection, antibacterial, checkerboard

1. Introduction

Probiotics are living microorganisms, that, when taken in appropriate quantities, provide a health benefit to the human body [1]**.** Most ordinary used probiotics are obtained from the genera *Bifidobacterium and Lactobacillus*. [2]. Supernatant of most *Lactobacillus* species contains several antimicrobials including hydrogen peroxide, organic acids, fatty acid, aroma constituents, and low-molecular-mass compounds that kill pathogens. Strains of *Lactobacillus* species can produce organic acids, and these acids might interact with cell membrane, resulting in induction of protein denaturation and intracellular acidification. Hydrogen peroxide probably acts as a precursor for production of free radicals, which can cause damage of DNA similarly as peroxidation of membrane lipids leading to an elevation to the membrane permeability [3]. Medicinal oils (MO) contain mixtures of volatile constituents naturally synthesized by plants [4]. Probiotics are long recognized for their antioxidant, antibacterial, antiviral and antifungal properties, so they are widely utilized in food and medicine. [5]. The recurrent interest in new alternative natural antimicrobial substances is directing the researchers to reach out new applications of these products to decrease the infections caused by drug-resistant pathogens and to reduce the hazards caused by antibiotics [6]. Our study aims to evaluate the bacterial effect of *Lactobacillus* strains and some MO on the growth of pathogens and to estimate the possible synergistic interactions between CFS and MO.

2. Materials and Methods

Bacterial strains and culture conditions

Three lactobacillus strains [*Lactobacillus plantarum* (ATCC 8014), *Lactobacillus casei* (DSM 20011), and *Lactobacillus acidophilus* (DSM 20079)] and 5 standard strains of *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739) *K. pneumoniae* (ATCC 10031), *P. mirabilis* (ATCC 29906), *Ps. aeruginosa* (ATCC 10145), were obtained from Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. *Lactobacillus* strains were cultured in De Man, Rogosa and Sharpe (MRS) broth anaerobically at 37 °C. Clinical pathogenic bacteria were isolated from patients suffering from acute gastroenteritis and urinary tract infection. The patients were attending Minia University Hospital, Minia, Egypt. These isolates were authenticated according to standard laboratory procedures [7].

Medicinal oils

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Five medicinal oils (Bitter almond, Clove, Eucalyptus, Peppermint and Thyme oils) were purchased from El-gomhoria Company for trading chemicals & medical appliances, Cairo, Egypt. The oils were stored in tightly closed glass bottles at 4 °C until use.

Screening for acid and bile tolerance of *Lactobacillus* strains

Resistance to acidic pH (pH = 3) was measured after centrifuging the overnight cultures of *Lactobacillus* strains at 6000 rpm for fifteen min at 4 °C then re-suspending the produced pellets in the same volume of isotonic 0.9 % (w/v) NaCl, pH 3.0. Afterwards, Suspensions were incubated at 37 °C for 3 h. The resulting pellets were then plated onto MRS agar and incubated for 48 h at 37 °C. Bile tolerance was estimated by culturing *Lactobacillus* strains on MRS agar containing 2 % (w/v) bile salt. The cultures were checked after incubation for 48 h at 37 °C [8].

Susceptibility of *Lactobacillus* strains to tested MO

The possibility of using PB-MO combination was emphasized by agar well diffusion assay [9]. A culture containing 10^8 CFU/ml of *Lactobacillus* strains was plated on MRS plates. Wells of 7 mm diameter were punched in the agar plates and filled with 100 µl oil stock solution (50 mg/ml). The plates were then incubated for 24 h at 37 °C and the zones of inhibition around the wells were measured.

Inhibitory effect of *Lactobacillus* CFS and MO on the growth of the tested pathogens

Lactobacillus strains were grown in MRS broth for 48 h at 37 °C. Cell-free supernatant(CFS) was obtained by centrifuging the culture at 15000 rpm for 15 min at 4 °C and then filtered through 0.45 µm filters [10]. Antibacterial activities of CFS of *Lactobacillus* strains and MO were determined by the agar well diffusion method against some clinical isolates. Bacterial inoculum colonies from overnight nutrient agar were used to make suspension of the tested pathogenic microorganisms (10^6 CFU/ml). Wells with a 7-mm diameter were punched in the agar plates and were filled with 100 µl of CFS or 100 µl of 50 mg/ml concentration of oils. The plates were then incubated at 37 °C for 24 h and the zones of inhibition diameters were measured [9, 11].

Assessment of combination between the MO and CFS of the tested PB using agar well diffusion method

Combination effectiveness was assessed by the agar well diffusion method [9] as described above. Wells were filled with 50 µl of 50 mg/ml concentration of each oil with 50 µl of CFS of *Lactobacillus* strains. After incubation, the diameter zones of inhibition were measured.

Evaluation of Combinations between MO and CFS using checkerboard microdilution method

Firstly, MICs of oils were performed by micro broth dilution assay [12]. The MICs were defined as the lowest concentration of MO inhibiting visible growth after 24 h incubation. After determining the individual MICs of MO and CFS, their MICs in combination were investigated by checkerboard micro dilution method [13]. The concentrations of MO and CFS were ranged from 4 or 5 folds below the expected MIC. The interactions were evaluated using two-fold dilutions of each antibacterial agent. Bacterial inoculum of standard strains, corresponding to 10^6 CFU/ml, was prepared. Each micro dilution was included 100 µl of the two-fold diluted concentrations of both MO and CFS was inoculated with 10 µl of the inoculum suspension. The plates were incubated for 24 h at 37 °C, and the results were read visually.

The Fractional Inhibitory Concentration (FIC) index for drugs A and B was then calculated using the following equation:

FIC index = FICA + FICB = MIC *(*A in presence of *B)* / MIC (A alone) + MIC *(*B in presence of A*)* / MIC (B alone)

3. Results

3.1. Prevalence of different microorganisms

A total of 187 pathogenic strains were isolated from 180 urine and stool specimens. It was found that *E. coli* was the most prevalent pathogen isolated from urine and stool specimens followed by *K. pneumoniae*, while no *S. aureus* or *Ps. aeruginosa* strains were isolated from stool samples (**Figure1**).

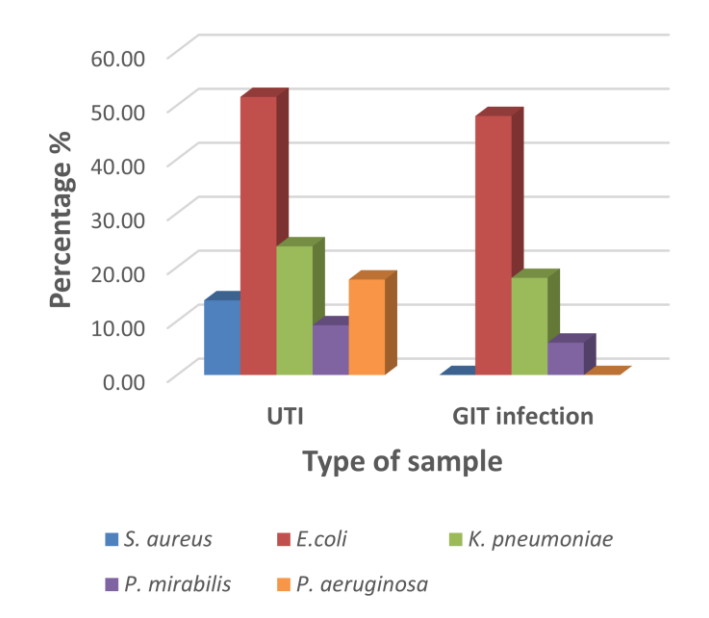


Figure (1): Prevalence of different microorganisms among clinical specimens

3.2. Bile salt and acid tolerance

The 3 tested *Lactobacillus* strains were able to grow on MRS agar containing 2 % bile salts and resist the acidic pH (pH=3).

3.3. Susceptibility of *Lactobacillus* strains to the tested MO

All tested *Lactobacillus* strains exhibited complete resistance to the 5 MO when the susceptibility of *Lactobacillus* strains to the tested MO was examined.

3.4. Antibacterial effect of CFS of *Lactobacillus* strains against the clinical pathogenic strains

The *Lactobacillus* CFS of the 3 strains showed good antagonistic effects against most of the tested clinical pathogens (**Table 1**). CFS of *L. casei* exhibited the highest % of inhibition against nearly all the tested clinical strains, while *L. plantarum* showed the best activity against *K. pneumoniae* strains.

3.5. Screening of antibacterial activity of MO against clinical pathogenic strains

Our results revealed that different clinical strains had different susceptibilities to the same EO. Thyme and clove oils completely inhibited *S. aureus* clinical strains. Also, these oils inhibited most of *K. pneumoniae* strains. Peppermint oil showed moderate effect followed by bitter almond oil while eucalyptus oil showed the least inhibitory activity (**Table 2**).

3.6. Assessment of the combination between tested CFS and MO

3.6.1. Screening of antibacterial activity of combination using agar well diffusion method

Sixty two strains sensitive to both tested CFS and MO were selected. The combinations were tested to estimate the possible synergistic effects against the selected clinical pathogenic strains (**Tables 3-7**) and (**Figure 2**).These combinations showed synergistic activity with large number of the tested *S.aureus* isolates especially combinations containing thyme, clove and peppermint. Eucalyptus and bitter almond oils showed the lowest activity against *K. pneumoniae* isolates. Most of the tested PB combinations with MO showed low synergistic activity against *P. mirabilis* and *P. aeruginosa*.

3.6.2. Evaluation of combinations between CFS of *Lactobacillus* strains and MO using Checkerboard Method

Results of MICs were determined (**Table 8**).The interaction between the tested MO and CFS of *Lactobacillus* strains was investigated against the standard strains. Most of the tested CFS significantly lowered the MICs of MO against the tested microorganisms and FICIs for these combinations ranged from 0.12 to 0.75. Antagonism was defined as a FIC index of 2.0, additively as a FIC index of 1.0 and synergism was defined as an FIC index of 0.5. Synergy was further subclassified as marked (FIC $\leq$ 0.5) and weak (FIC index, between 0.5 and 1.0).
Checkerboard assays of *S. aureus* and *E.coli* strains showed marked synergistic profiles when CFS of *L. plantarum*, *L. casei*and *L. acidophilus* were combined with different MO. FICIs ranged from 0.19-0.38 for *S. aureus* and 0.12-0.38 for *E.coli* strain. Regarding *S. aureus*, combination of peppermint oil with CFS of *L. acidophilus* and *L. plantarum*strains showed weak synergism. Combinations of the 3 CFS of *Lactobacillus* strains with most tested MO showed marked synergistic effects in case of *K. pneumoniae* strains, but also weak synergistic behavior with the bitter almond oil.
All antibacterial combinations demonstrated synergistic actions against *P. mirabilis* Combination of CFS and both thyme and bitter almond oils demonstrated additive activity.The combined effects of the 3 CFS with peppermint oil and combination between *L. acidophilus* and thyme oil showed weak synergistic activities, while the remaining oils had marked synergistic effect on *P. aeruginosa*. (**Figure 3**). The effect of combinations of CFS and MO on different clinical strains by Checkerboard method was determined **(Table 9)**.

Table 1: Antibacterial effect of CFS of *Lactobacillus* strains on the growth of clinical strains

No. of Isolated strains	No. of strains inhibited [Inhibition percentage (%)]		
	CFS (*L. plantarum*)	CFS *(L. casei)*	CFS *(L. acidophilus*)
S. aureus* (18)	14 (77.8%)	16 (88.9%)	13 (72.3%)
***E.coli* (91)**	38 (41.8%)	45 (49.5%)	37 (40.7%)
***K. pneumonia* (40)**	29 (72.5%)	26 (65%)	25 (62.5%)
***P. mirabilis* (15)**	9 (60%)	10 (66.7%)	9 (60%)
***P. aeruginosa* (23)**	10 (43.5%)	12 (52.2%)	11 (47.8%)

* No of the isolated strains

Table 2: Screening of antibacterial activity of various MO against clinical strains using agar well diffusion method

Isolated strains	No. of strains inhibited				
	Bitter almond oil	Clove oil	Eucalyptus oil	Peppermint oil	Thyme oil
S. aureus* (18)	11(61.1 %)	18 (100 %)	10 (55.6 %)	16 (88.9 %)	18 (100 %)
***E.coli* (91)**	41(45.1 %)	78 (85.7 %)	28 (30.8 %)	75 (82.4 %)	84 (92.3 %)
***K. pneumoniae* (40)**	28 (70 %)	38 (95 %)	19 (47.5 %)	33 (82.5 %)	39 (97.5 %)
***P. mirabilis* (15)**	9 (60 %)	13 (86.7 %)	9 (60 %)	5 (33.3 %)	13 (86.7 %)
***P. aeruginosa* (23)**	8 (34.8 %)	14 (60.9 %)	7 (30.4 %)	11 (47.8 %)	15 (65.2 %)

* No. of isolated strains

Table 3: The effect of CFS combination with the tested MO on *S. aureus* isolates

	% of clinical *S. aureus* isolates inhibited by CSF/ MO combination (Total no.=9)				
Oils / CFS	**Bitter Almond Oil**	**Clove Oil**	**Eucalyptus Oil**	**Peppermint Oil**	**Thyme Oil**
CFS (*L. plantarum*)	44.4	88.9	33.3	88.9	88.9
CFS *(L. casei)*	44.4	88.9	55.6	88.9	77.8
CFS *(L. acidophilus*)	55.6	88.9	44.4	88.9	88.9

Table 4: The effect of CFS combination with the tested MO on *E.coli* isolates

	% of clinical *E.coli* isolates inhibited by CSF/ MO combination (Total no.=25)				
Oils / CFS	**Bitter Almond Oil**	**Clove Oil**	**Eucalyptus Oil**	**Peppermint Oil**	**Thyme Oil**
CFS (*L. plantarum*)	44	64	36	76	84
CFS *(L. casei)*	28	68	32	80	80
CFS *(L. acidophilus*)	32	60	40	68	72

Table 5: The effect of CFS combination with the tested MO on *K. pneumoniae* isolates

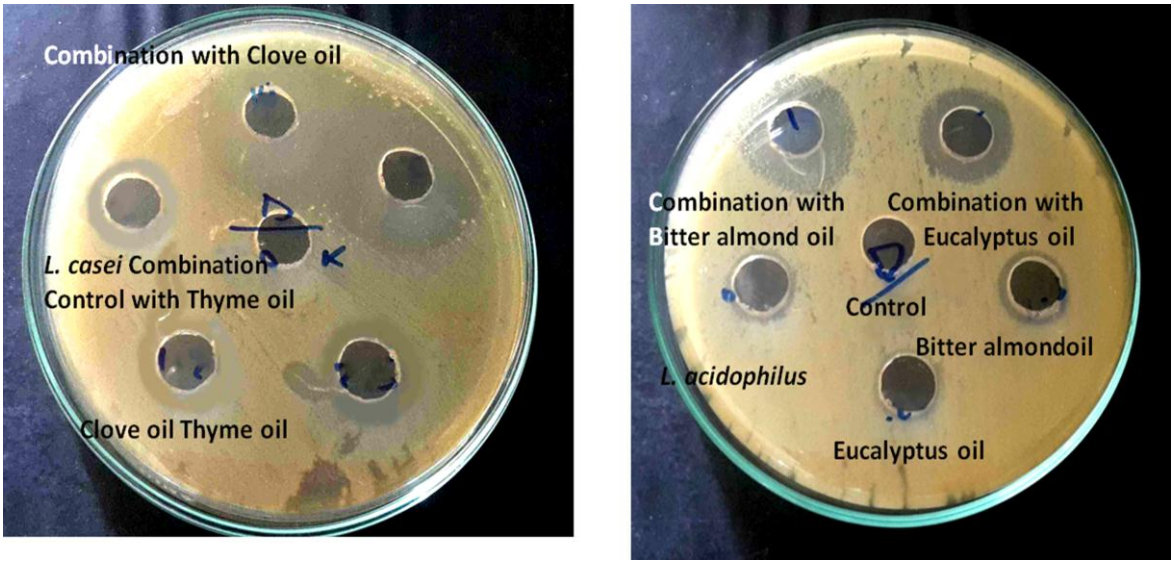
	% of clinical *K. pneumoniae* isolates inhibited by CSF/ MO combination (Total no.=16)				
Oils / CFS	**Bitter Almond Oil**	**Clove Oil**	**Eucalyptus Oil**	**Peppermint Oil**	**Thyme Oil**
CFS (*L. plantarum*)	31.3	50	25	75	68.8
CFS *(L. casei)*	31.3	56.3	25	75	56.3
CFS *(L. acidophilus*)	37.5	50	31.3	68.8	50

Table 6: The effect of CFS combination with the tested MO on *P. mirabilis* isolates

	% of clinical *P. mirabilis* isolates inhibited by CSF/ MO combination (Total no.=6)				
Oils / CFS	**Bitter Almond Oil**	**Clove Oil**	**Eucalyptus Oil**	**Peppermint Oil**	**Thyme Oil**
CFS (*L. plantarum*)	16.7	33.3	33.3	33.3	33.3
CFS *(L. casei)*	33.3	33.3	33.3	16.7	33.3
CFS *(L. acidophilus*)	33.3	33.3	16.7	16.7	33.3

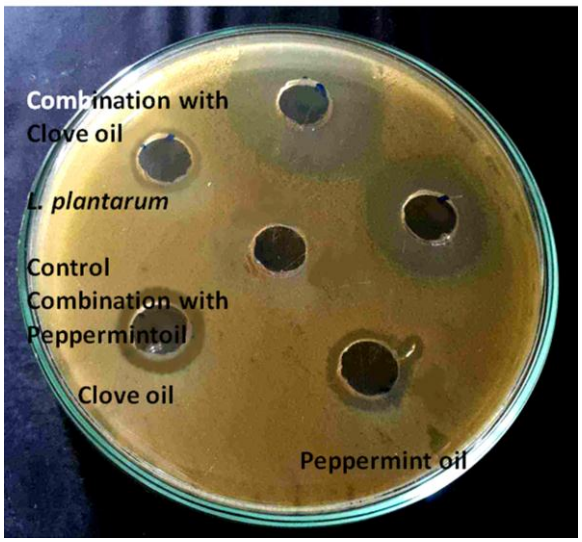
Table 7: The effect of CFS combination with the tested MO on *P. aeruginosa* isolates

	% of clinical *P. aeruginosa* isolates inhibited by CSF/MO combination (Total no.=6)				
Oils / CFS	**Bitter Almond Oil**	**Clove Oil**	**Eucalyptus Oil**	**Peppermint Oil**	**Thyme Oil**
CFS (*L. plantarum*)	33.3	33.3	33.3	50	33.3
CFS *(L. casei)*	33.3	16.7	16.7	50	33.3
CFS *(L. acidophilus*)	16.7	16.7	16.7	50	16.7



(A) **(B)**

Figure 2: Synergistic effect of PB-MO combinations on the growth of (A) *K. pneumoniae*, (B) *S. aureus* and (C) *E. coli*.



(C)

Figure 2: Synergistic effect of PB-MO combinations on the growth of (A) *K. pneumoniae*, (B) *S. aureus* and (C) *E. coli* (cont.).

Table 8: MIC of MO and CFS against the standard strains using microdilution assay:

		MIC				
Microorganism		***S. aureus*** **(ATCC 6538)**	***E.coli*** **(ATCC 8739)**	***K. pneumoniae*** **(ATCC 10031)**	***P. mirabilis*** **(ATCC 29906)**	***P. aeruginosa*** **(ATCC 10145)**
MO (mg/ml)	**Bitter Almond oil**	12.5	12.5	12.5	25	12.5
	Clove oil	1.6	3.1	6.3	6.3	12.5
	Eucalyptus oil	25	12.5	25	25	25
	Peppermint oil	3.1	6.3	6.3	12.5	6.3
	Thyme oil	0.8	1.6	1.6	3.1	3.1
CFS (µl/ml)	***Lb plantarum***	62.5	31.3	31.3	31.3	62.5
	Lb casei	31.3	15.6	31.3	31.3	31.3
	Lb acidophilus	62.5	31.3	31.3	62.5	62.5

CFS: Cell-Free Supernatant, MIC: Minimum Inhibitory Concentrations

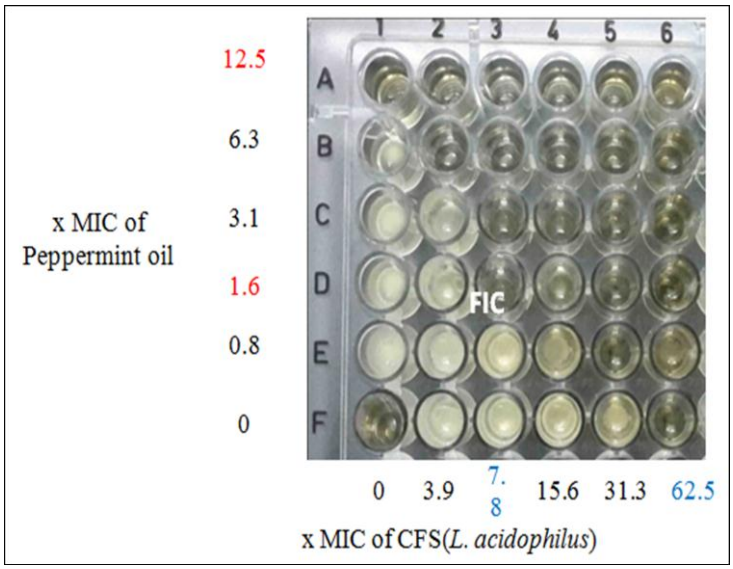


Figure 3: Checkerboard of *P. mirabilis* growth inhibition with different concentrations of CFS *(L. acidophilus)* and/or peppermint oil (FIC= 7.8/ 62.5 + 1.6 / 12.5 = 0.25).

4. Discussion

There are several advantages of utilizing natural products as antimicrobial compounds such as better patient tolerance, relatively inexpensive, fewer adverse effects, and wide acceptance due to their better biodegradability, renewability, and their traditional applications. [14].

In the present study, *Lactobacillus* strains (*L. plantarum*, *L.casei* and *L.acidophilus*) showed acceptable resistance to the effect of bile salts and acidic pH. Resistance to the low pH of the gastric juice in the stomach and the bile salt in the small intestine is one of the important selection criteria for probiotic as they are usually administrated orally [15]. Bile tolerance can be due to expression of bile resistance related proteins in the bacterial cells [16]**.** PB has a known antibacterial effect. There are many proposed mechanisms for the antibacterial action of the PB. Organic acids, bacteriocins, hydrogen peroxide and other inhibitory chemicals are released by the PB [17]. Bacteriocins are toxic chemicals which are highly potent against most of the bacteria. However, the most feasible mode of action is by lowering of pH with the release of organic acids [18].

In this study, CFS of *Lactobacillus* strains showed good antibacterial activity against the pathogenic strains. Zavisic et al. [19] proved that the 2 *Lactobacillus* isolates exhibited antagonistic action towards some pathogenic organisms. In contrary to our findings, Shanthya et al. [20] revealed that lactobacillus has strongest antagonistic activity against *P. aeruginosa*. Supernatant of most *Lactobacillus* bacteria contains many antibacterials including hydrogen peroxide, organic acids and other compounds that kill pathogens. These antimicrobials did not have any inhibitory effect on PB [3].

The antimicrobial activity of MO has long been recognized and they have been tested *in vitro* against a wide range of pathogenic bacteria [21]. In our study, the previous MO was screened for their antibacterial activity against the clinical pathogenic strains. Clove and thyme oils showed the best antibacterial activity against most of the tested strains and eucalyptus oil showed the lowest inhibitory activity, even though earlier studies have reported better antibacterial activity for this oil [22**,** 23]. Most of the antibacterial activity in MO is found in the oxygenated terpenoids, while some hydrocarbons also exhibit antibacterial effects [24]. It seems evident that there is a relationship between the high activity of thyme oil and the presence of phenol components [25]. The antibacterial activity of clove oil could be associated with eugenol which is known to exhibit antibacterial activity [26]. The antibacterial activity of peppermint oil observed in this study was comparable with other reports [27]. The antibacterial activity of peppermint oil is due to the presence of terpenoides, menthone, menthol and many other components [28].

To test the combination between PB and MO, firstly, *Lactobacillus* strains must be resistant to that oil to avoid direct killing of the probiotic strain. After determination of resistance of the 3 *Lactobacillus* strains and pathogenic strains to MO as shown above, the combinations of CFS from the 3 and the 5 oils were tested to estimate the possible synergistic effect against pathogenic strains by both agar well diffusion and checkerboard methods. Our results showed that synergistic effect was verified from the combination of different PB with the tested MO. The advantage of utilizing such a combination is its beneficial effect with its antibacterial property. The PB may help in improving the gut epithelial conditions while MO act on killing the pathogens present in the human body [14]. MO with known antibacterial activity will produce beneficial or harmful effects when combined with conventional antibacterials, depending on the ratio in which the 2 components exist [29].

The synergistic effect between MO and other antibacterial substances such as generally recognized as safe metabolites produced by lactic acid bacteria (e.g., nisin) was demonstrated [30], and it was noted that the activities of the MO are enhanced by the presence of nisin [31].

Many researchers evaluated the effects of MO in combination with PB. As Sadeghi et al. [32] evaluated the effect of *Cuminum cyminum* L. oil and *L. acidophilus* on growth of *S. aureus*. The significant inhibitory effects of oil and the probiotic on this microorganism were observed alone and in combination together. Also, the combination between *Mentha longifolia* L. oil and *L. casei*was tested for synergy against the growth of *Listeria monocytogenes* and *S. aureus*. The growth of the 2 pathogens was significantly reduced by both oil concentrations, probiotic and their combination. Thus, lower concentration of oilmay be used when it is combined with this probiotic [33]. The results of another study [34] showed that combination of certain concentration of *Teucrium polium* oil and *L. casei* decreased the required inhibitory concentration of *Teucrium polium* oil for *Salmonella typhimurium*.

The mechanism of action for PB-MO combination was previously illustrated [14]. The PB has health beneficiary characters. They retard the growth of the microorganisms, while MO kill them. Antibacterial properties of PB may be attributed to the production of bacteriocin-like chemicals. Also, they mostly arrest the proliferation of the microorganisms by lowering the pH in the gut. The microorganisms do not normally have any mechanism against the action of MO.

5. Conclusion

The use of PB lowers the survivability chances of pathogenic bacteria, while the MO in lower dosage ensures their complete killing inside the digestive tract of human**.** Combining the effect of PB with MO may reveal a new approach concerning their complementary antibacterial effects with few side effects. The synergistic effect of the MO and PB will be necessarily greater than utilizing them alone as health products. This combination (PB-MO) could contribute for the development of new safe and effective therapeutic and antibacterial agents.

References

[1] Reid G, Sanders ME, Gaskins HR, GibsonGR, Mercenier A. New scientific paradigms for probiotics and prebiotics. *J Clin Gastroenterol*.2003; 37: 105-118.

[2] Upadhyay N, Moudgal V. Probiotics: A Review. *JCOM*. 2012; 19: 2.

[3] Aminnezhad S, Kermanshahi RK, Ranjbar R. Evaluation of Synergistic Interactions Between Cell-Free Supernatant of *Lactobacillus* Strains and Amikacin and Genetamicin Against *Pseudomonas aeruginosa*. *Jundishapur J Microbiol.* 2015; 8(4):e16592.
[4] Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science*. 2006; 311: 808–811.
[5] Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties.*Molecules*. 2012; 17: 3989-4006.
[6] Liu ZH, Nakano, H. Antibacterial activity of herbs extracts against food-related bacteria. *J Fac Appl Biol Sci Hiroshima Univ.* 1996; 35:181-190.
[7] Collee JG, Fraser AG, Marmion, BP, Simmons A. Tests for identification of bacteria. In: Mackie & McCartney, practical medical microbiology, 14th ed., Churchill Livingstone In., *New York*, USA.1996.
[8] Nouri M, Rahbarizadeh F,Ahmadvand D, Moosakhani F, Sadeqzadeh E, Lavasani S, Vishteh VK. Inhibitory effects of *Lactobacillus salivarius* and *Lactobacillus crispatus* isolated from chicken gastrointestinal tract on *Salmonella enteritidis* and Escherichia coli growth. *Iran J Biotechnol*. 2010; 8:32-37.
[9] Vivek S, Nisha S, Vijaylata P, Reyaz MA, Bikram S, Raghbir GC. GC-MS analysis and anti-microbial activity of essential oil of Mentha piperita L. from Kullu-a North Indian region of higher altitude Himalayas. *Int J Drug Dev Res*. 2010; 2(4): 40-46.
[10] Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Afr J Biotechnol*.2003;2(8):219–27.
[11] Nitisinprasert S, Nilphai V, Bunyun P, Sukyai P, Doi K,Sonomoto K. Screening and identification of effective thermotolerant lactic acid bacteria producing antimicrobial activity against Escherichia coli and Salmonella sp.resistant to antibiotics. *Kasetsart J.* 2000; 34(3):387–400.
[12] Karpanen TJ, Worthington T, Hendry ER et al. Antimicrobial efficacy of chlorhexidine digluconate alone and in combination with eucalyptus oil, tea tree oil and thymol against planktonic and biofilm cultures of *Staphylococcus epidermidis. J Antimicrob Chemother*.2008; 62(5):1031-6.
[13] European committee for antimicrobial susceptibility testing (Eucast) Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. 2000.
[14] Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance. *The Open Microbiol J*.2014;8:6-14.
[15] Olejnik A, Lewandowska M, Obarska M, Grajek W. Tolerance of Lactobacillus and Bifidobacterium strains to low pH, bile salts and digestive enzymes. *EJPAU*, 2005; 8:25–32.
[16] Hamon E, Horvatovich P, Izquierdo E, Bringel F, Marchioni E, Aoud E, Werner D. Ennahar S. Comparative proteomic analysis of *Lactobacillus plantarum* for the identification of key proteins in bile tolerance. *BMC Microbiol.*2011; 11, 63.
[17] Abee T, Krockel L, Hill C. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *Int J Food Microbiol*. 1995; 28(2): 169–185.
[18] Millette M, Luquet FM, Lacroix M. In vitro growth control of selected pathogens by *Lactobacillus acidophilus* and *Lactobacillus casei*-fermented milk, *Letters in Applied Microbiol.*2007; 44(3): 314–319.
[19] Zavisic G, Petricevic S, Radulovic Z, Begovic J, Golic N, Topisirovic L, Strahinic I. Probiotic Features of Two Oral Lactobacillus Isolates. *BJM*.2012; 418-428.
[20] Shanthya R, Saranya S, Shenpagam HN. Antagonistic Effects of Lactobacilli on Gram-Negative Bacteria. *J Adv Lab Res Biol*.2011; 2(2):70-72.
[21] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 2003; 10: 813-829.
[22] Cimanga KK, Tona L, Apers S, De Bruyne T, Hermans N, Totte J, Pieters L, Vlietinck AJ.Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol*.2002; 79:213-20.
[23] Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral Microbiol Immunol*.2002; 19:61-4.
[24] Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. *J of Food Microbiol*.2004; 94, 223-253.
[25] van Vuuren SF, Viljoen AM.A comparative investigation of the antimicrobial properties of indigenous South African aromatic plants with popular commercially available essential oils. *J Essent Oil Res*.2006; 18: 66-71.
[26] Suresh P, Ingle V, Vijayalakshmi K. Antibacterial activity of eugenol incomparison with other antibiotics. *J Food Sci Tech*.1992; 29: 256–257.
[27] Gupta C, Garg AP, Uniyal RC, Kumari A. Antimicrobial activity of some herbal oils against common food-borne pathogens. *Afr J of Microbiol Research*.2008; 2: 258-261.
[28] Sartoratto A, Machado MAL, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG.Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz J Microbiol*.2004; 35: 275-280.
[29] van Vuuren SF, Suliman S, Viljoen AM.The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Letters in Applied Microbiol*.2009; 48: 440–446.
[30] Periago PM, Moezelaar R. Combined effect of nisin and carvacrol at different pH and temperature levels on the viability of different strains of Bacillus cereus. *Int J Food Microbiol*.2001; 68:141–148.
[31] Misaghi A, Basti AA. Effects of Zataria multiflora Boiss. essential oil and nisin on *Bacillus cereus* ATCC 11778. *Food Control*.2007;18:1043–1049.
[32] Sadeghi E, Akhondzadehbasti A, Noori N, Khanjari A, Partovi R. Effect of Cuminum cyminum L. essential oil and *Lactobacillus acidophilus* (a probiotic) on *Staphylococcus aureus* during the manufacture, ripening and storage of white brine cheese. *J of Food Processing and Preservation.*2013; 37: 449-455.
[33] Ehsani A, Mahmoudi R.Effects of Mentha longifolia L. essential oil and Lactobacillus casei on organoleptic properties and on the growth of *Staphylococcus aureus* and *Listeria monocytogenes* during manufacturing, ripening and storage of Iranian white-brined cheese. *Int J of dairy tech*.2012; 66(1)70-76.
[34] Mahmoudi R, Zare P, Nosratpour S. Application of *Teucrium polium* Essential oil and *Lactobacillus casei* in Yoghurt. *J Essent Oil Bearing Plants*.2015; 18(2).