Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 20 April 2016 • Accepted: 01 September 2016

Research

doi:10.15412/J.JBTW.01050901

Inhibitory Effects of *Aloe Vera* Gel Aqueous Extract and *L. casei* Against *E. coli* in Yoghurt

Zahra Nemati Niko[1], Peyman Ghajarbeygi[2], Razzagh Mahmoudi[2*], Shaghayegh Mousavi[3], Karim Mardani[4]

[1] Department of Food Hygiene and Safety, School of Health, Qazvin University of Medical Sciences, Qazvin, Iran

[2] Health Products Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

[3] Microbiology, Department of Health, Qazvin University of Medical Sciences, Qazvin, Iran

[4] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

*Correspondence should be addressed to Razzagh Mahmoudi, Health Products Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran; Tel: +989127868571; Fax: +989127868571; Email: r.mahmodi@yahoo.com.

ABSTRACT

Chemical preservatives are usually used to reduce or eliminate pathogenic or spoilage microorganisms, So many researches have been done to substitute the chemicals with naturally occurring compounds, especially plant essential oils. In this study the growth and survival of *E.coli* as a pathogen agent were investigated under the synergistic effects of simultaneous presence of *Aloe Vera* gel aqueous extract and *Lactobacillus casei*. For this purpose, an amount of 10^8-10^9 cfu/ml of *L. casei*, 10^3 CFU/ml *E.coli*, and two different concentrations of *Aloe Vera* gel aqueous extract (5 and 10%) were added to yoghurt. The samples were kept for 10 days in 4°C and the survival of *E.coli* was evaluated. The presence *E. coli* was determined by culture in selective media and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Aloe Vera gel aqueous extract against *E.coli* was investigated by Micro-well dilution assay. The MIC and MBC values ranged 20% and 40%. The highest antibacterial activity was seen at the end of the storage period and in the samples containing 10% extract (2.33±0.24 $\log_{10}$ cfu/g). *E. coli* count in samples containing extract and in probiotic yogurt were significantly decreased in comparison with the control group at the end of storage period. however, there was no significant difference in *E. coli* count between probiotic and non-probiotic yogurt containing extract and According to the results of this study *L.casei* and *Aloe vera* gel aqueous extract could be used as natural preservative agents in the dairy products.

Key words: Aloe Vera gel, Aqueous extract, Probiotic Yoghurt, E.coli.

Copyright © 2016 Zahra Nemati Niko et al. This is an open access paper distributed under the Creative Commons Attribution License.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

Antibiotic resistance has become a global concern. In recent years, there is increasing incidence of multiple resistances in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. In addition, concerns over the inevitable side effects of chemical food preservatives, these had forced scientist to search for new antimicrobial substances from various sources like medicinal plants (1). Despite the strong antimicrobial activity of Essential oils (EOs) against foodborne pathogens and spoilage micro-organisms, their application as preservatives in food is currently limited due to the unfavorable changes they may cause in the taste of food products (2, 3). Recently, various natural compounds like spices are preferred and used as food preservatives (4). The EO are aromatic and volatile oily liquids obtained from plant material. They are normally formed in groups of cells or specific cells , found in stems and leaves, and usually concentrated in one particular region such as bark, leaves, or fruit (5). *Aloe vera (Aloe barbadensismiller)* is a plant, which belongs to the family of *Liliaceae* and is mainly lush with a whorl of elongated, pointed leaves (6, 7). *Aloe vera* grows in arid climates and is widely distributed in India, Africa and other arid areas. The species is frequently cited as being used in herbal medicine (8). The Leaves have high capacity to retain water in dry and very hot weather conditions and can survive in very difficult circumstances. The gel contains 99.3% of water, the remaining 0.7% is made up of solids mainly carbohydrates (8). Furthermore, activity against a variety of infectious agents has been attributed to *Aloe vera*; for instance, antiviral, antibacterial and antifungal (9-11). Specific plant compounds such as

anthraquinones, dihhydroxyanthraquinones, and saponins have been proposed to have direct antimicrobial activity (12-15). Yogurt is one of the traditional fermented dairy products and one of the most popular milk products produced by two lactic acid bacteria as fermentation starter (*streptococcus thermophilus and lactobacillus delbrueckii subsp. Bulgaricus)*. Dairy products, especially yogurt, are the best well known carrier for transmission of probiotic organisms to the consumers (16). Probiotics have been defined as "live micro-organisms confer a health benefit on the host" when administered in enough amounts (17). *L. casei* belongs to *Lactobacillaceae* family. The morphology and properties of *L. casei* are rod-shaped colonies about 1 mm diameter (white, shiny and smooth), negative-catalase, mesophyl, gram positive, micro aerophilic. The addition of *L. casei* into yogurt as starter can promote physiological values and extra nutritional and improve the nutritional and technological properties of the product as a probiotic functional food (18, 19). Antimicrobial effect of *Lactic Acid Bacteria* (LAB) is mainly due to *lactic acid* and other organic acid production, which results in decreasing the pH of the growth environment (20). *E. coli* is a pathogen, which causes hemolytic uremic syndrome, hemorrhagic colitis and thrombotic thrombocytopenic purpura in humans and can through milk and other dairy products, contaminated water and also meat be transmitted to humans (16, 21). Due to the high consumption of dairy products, especially yoghurt in Iran, also the possibility of secondary pollution and survival *E. coli* in yoghurt (22). The aim of this study was to evaluate the survivability of *E. coli* during the 10 days of preservation of yoghurt prepared with *Aloe vera* gel aqueous extract and probiotic bacteria *L. casei* Alone and in combination with each other by culture in selective media.

2. MATERIALS AND METHODS

2.1. Extraction of Aloe Vera gel

Aloe Vera plant originally purchased from Qazvin market. The fully expanded leaves of *Aloe vera* were selected, washed with distilled water and were subjected to surface sterilization with 70% ethyl alcohol followed by 0.1% $HgCl_2$. The parenchymatous covering of the leaves were peeled and the gel drained out. Slurry was formed with the help of pestle and mortar (23).

2.2. Preparation of aqueous extract

Sufficient amount of crushed plant (50 g) was poured into the extraction container and 200 cc distillated water was added. The Container was placed on medium heat, stirred constantly until the first signs of boiling was seen. After boiling the solution for 15 minutes, the extract was filtered through Whattman filter paper (No. 1), then it was kept in sterile glass containers and dark and in the refrigerator (24).

2.3. Bacterial strains

Lyophilized *E. coli* was obtained from the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. Subcultivation and preparation of the inoculantnts were conducted according to Parsaeimehr et al. (25).

2.4. Starter and probiotic bacteria

Freeze dried yoghurt inoculantntnt (Christian Hansen Co., R 704, Denmark) containing *Streptococcus salivarius ssp. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (1:1) was used as a starter. A commercial lyophilized culture of the probiotic *L. casei* ATCC 3939 was obtained from the Iranian Organization of Industrial Research. Subcultivation and preparation of the probiotic bacteria were conducted according to standard method (26).

2.5. Preparation and inoculantnttion of yoghurt

Raw cow milk was subjected to a heat treatment at 90°C for 20 min, followed by cooling to 40 – 45°C. It was inoculantntted with the test organisms at 10^3 CFU/mL in separate groups, the *Aloe Vera* gel aqueous extract was added to the milk before processing with different concentrations (5 and 10 %) followed by mixing. As yoghurt starter culture (*L. bulgaricus* and *S. thermophilus*) (1.5%) was added to the milk, followed by mixing, finally *L. casei* (10^8-10^9 Cfu/ml) added. Samples were packed in sterilized 250 mL capped glass, followed by incubation at 40°C for 3 hours till gel forms (pH 4.5). Freshly yoghurt was cooled and stored at 4°C for 10 day (27).

2.6. E. coli enumeration

(An amount of 10 g**)** from yoghurt samples were pooled in 90 mL of sterile 0.1% (w/v) peptone water (Merck, KGaA) in sterile 500-mL stomacher bags. Samples were blended in a Stomacher 400 (Interscience, Saint-Nom-La- Breteche, France) for 3 min. *E. coli* counts were determined on EMB agar (Merck) after incubation at 37°C for 48 h (5).

2.7. Micro-well dilution assay

The MIC and MBC values were studied for the bacterial strains in microplate. The inoculant of the bacterial strains was prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Then *Aloe Vera* gel aqueous extracts were prepared in 50, 40, 30, 20, 10, 5 and 2.5 % concentrations. MIC values of extract against pathogenic bacteria strains were determined based on a microwell dilution method. The 96-well plates were prepared by dispensing into each well 80 µl of nutrient broth and 20 µl of the inoculant and 100 ppm aliquot from different concentrations of *Aloe Vera* gel aqueous extracts were added to the wells. The last well containing 180 µl of nutrient broth without compound and 20 ppm of the inoculant on each strip was used as the negative control. The final volume in each well was 200 ppm. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The *Aloe Vera* gel aqueous

extract tested in this study was screened two times against *E. coli* (28).

2.8. Statistical Analysis

All experiments were conducted in triplicate, and results were computed as mean± standard deviation and were subjected to one-way analysis of variance to establish whether the differences in experimental results were significant or not. Result were considered statically significant when P <0.05.

3. RESULTS AND DISCUSSION

The growth inhibition values of *Aloe Vera gel* aqueous extract against *E. coli* shown in Table 1.

Table 1. MIC and MBC value Aloe Vera Gel aqueous extract (%) against E.coli

Antibacterial activity	Aqueous extract of Aloe Vera Gell (%)						
	2.5	5	10	20	30	40	50
MIC (%)	-	-	-	*	-	-	-
MBC (%)	-	-	-	-	-	*	-

Based on the results the MIC and MBC values were 20% and 40% respectively, also Based on the results of this study, the survival of *E. coli* was decreased during the 10-days of preservation of different yoghurt supplemented with different concentrations of extract and probiotics separately and in combination with each other at refrigerator temperature. The lowest bacteria count was recorded for yoghurt sample containing 10% extract (2.33±0.24 $\log_{10}$ cfu/g). The growth of *E. coli* during 10 days storage of various yoghurt samples are shown in Figure 1 and

Table 2.

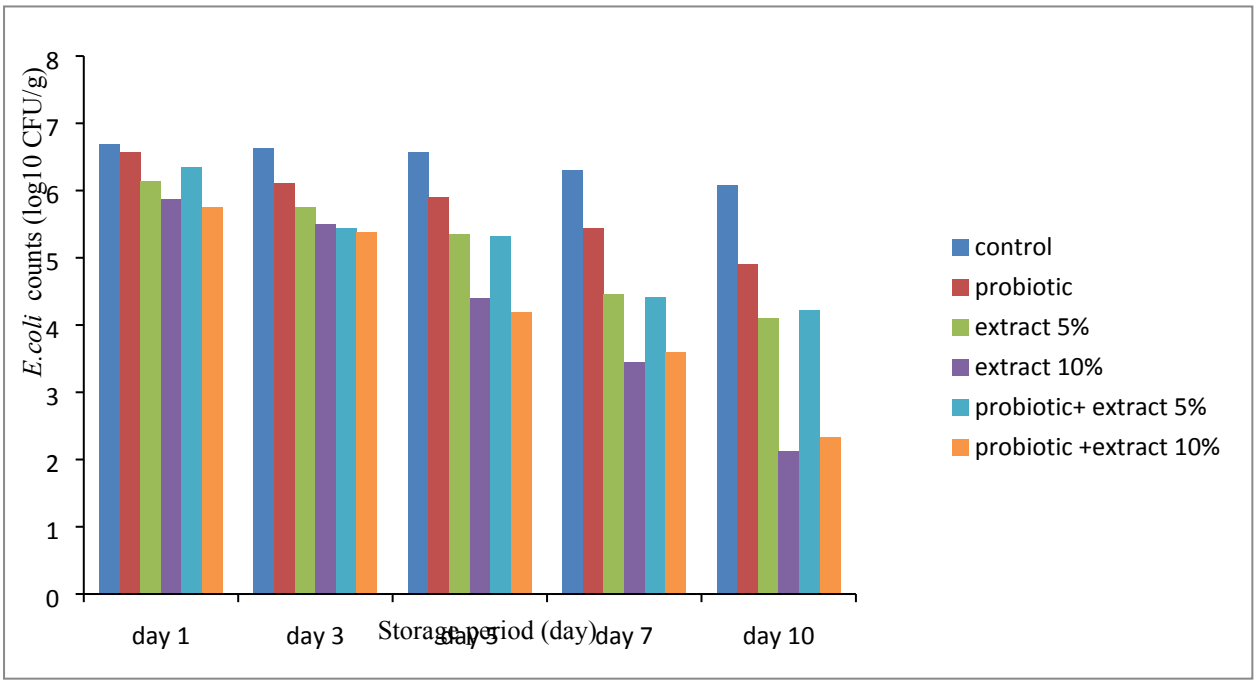

Figure 1. Survival of E.coli (Mean ±SD log10 CFU/g) in yoghurt samples during storage (10 days)

Table 2. Growth response of *E. coli* affected by various concentrations of extract, probiotic (P) and their combinations in Yoghurt over a 10-day storage period

Group No	Extract (%)	P (10^8 - 10^9 CFU/ml)	Log_{10}(CFU/g) ± SD on sampling days				
			1day	3day	5day	7day	10day
1	0	0	6.69±0.46	6.62±0.41	6.56±0.39	6.30±0.47	6.07±0.53
2	0	+	6.57±0.39	6.11±0.07	5.90±0.26	5.43±0.25[a]	4.90±0.18[a]
3	5%	+	6.35±0.28	5.43±0.25[a]	5.31±0.25[a]	4.41±0.38[ab]	4.21±0.21[a]
4	10%	+	5.75±0.26[a]	5.37±0.21[a]	4.18±0.09[abc]	3.59±0.12[ab]	2.33±0.24[abc]
5	5%	0	6.13±0.02	5.75±0.22[a]	5.35±0.22 [ad]	4.45±0.15 [abd]	4.1±0.17 [ad]
6	10%	0	5.87±0.17	5.49±0.34[a]	4.39±0.23 [abce]	3.44±0.31[abce]	2.12±0.15 [abce]

(P: *L.casei*). [a]Significant difference with data of group 1. [b]Significant difference with data of group 2. [c]Significant difference with data of group 3. [d]Significant difference with data of group 4. [e]Significant difference with data of group 5. [f]Significant difference with data of group 6 (Significant differences have been evaluated inside each column) (P < 0.05).

The results showed the antimicrobial potency of *Aloe Vera* gel aqueous extract against the *E. coli*. In yoghurt samples containing different concentrations of the extract, pathogenic bacteria were significantly decreased in comparison with the control group ($p<0.05$), also *E. coli* count in samples containing extract and in probiotic yogurt were significantly decreased in comparison with the control group at the end of storage period ($p<0.05$), but there was no significant difference in *E. coli* count between probiotic and non-probiotic yogurt containing extract ($p>0.05$). *Aloe Vera* gel aqueous extract in high concentration had better effect on reduction of *E. coli* bacteria count than its low concentration. However, probiotic yoghurt was more effective on *E. coli* in comparison with the control group And pathogenic bacteria *E. coli* were significantly decreased in days 7(5.43±0.25 $\log_{10}$ cfu/g) and 10(4.90±0.18 $\log_{10}$ cfu/g). In order to improving the quality of life, extensive investments have been made on therapeutic applications of plants. Herbal products have been known to treat infectious diseases throughout the history of mankind (29). Essence and extracts of medicinal plants and herbs play important roles in human life and have been very popular for long time among the Iranians (30). In recent years, probiotic bacteria, as the food additives, have been introduced into many foods, of which the dairy products have played a main role in carrying these bacteria (such as *B.bifidum and L. acidophilus*). One of the most accepted ways to extend the shelf life of perishable food products is through the use of biopreservatives. It has long been recognized that some EOs and probiotic bacteria have antimicrobial properties and that they can be used as food flavoring agents or preservatives, and for medicinal purposes (31, 32). The presented results show that the survival of pathogenic bacteria during storage period was reduced, so that the lowest count of bacteria was observed at day 10 (6.07±0.53 Log cfu/g). The number of pathogenic bacteria in yogurt samples containing extract was significantly decreased in comparison with the control sample during storage period. The lowest bacteria count was observed in the end of the preservation period in yoghurt sample containing10% extract (2.12±0.15 Log cfu/g). The pathogenic bacteria in probiotic yogurt was significantly lower than control group at the end of storage period. Elbandy et al., (2014) studied the processing of fruit nectars enriched with *Aloe vera* gel. The aim of their study was to produce therapeutic and high nutritional mango nectar by supplementation of mango pulp with *Aloe vera* gel. They showed that high concentrations of *Aloe vera* gel (20 and 25%) resulted in a dramatic fall in the levels of total bacterial counts. Total bacterial counts decreased from log10 3.9 ± 0.06 $\log_{10}$CFU/ml in control treatment at zero time point to 2.05 $\log_{10}$CFU/ml as a result of *Aloe vera* gel addition (33). The strong antibactericidal activity of *Aloe vera* gel may be attributed to a number of pharmacologically active compounds including alkaloids, tannins; flavonoids, as well as saponins which have a direct antimicrobial activity (13, 34). Agarry et al., (2005) compared the antimicrobial activities of the gel and leaf of *Aloe vera.* They reported that leaf extracts had antibacterial activity against bacterial species such as *S. aurous*, *Klebsiella pneumoniae*, and *E. coli* (35). Ibrahim et al., (2011) investigated the antimicrobial activity and phytoconstituents of aqueous, ethanol and acetone extracts of the *A. vera* gel against

some human and plant pathogens by disc diffusion method. Among the three extracts, acetone and ethanol extracts recorded significant antimicrobial activity against all examined pathogens. Antibacterial activity of the acetone extract was found to be quite impressive as compared to ethanol and aqueous extracts (36). In another study, the susceptibility of *E. coli* and *S. aureus* to the crude extracts of *Aloe Vera* gel was determined by agar well diffusion method. The ethanol extract inhibited the growth of *E. coli* and *S. aureus* with zones of inhibition of 6 and 5 mm respectively while aqueous extract had zones of inhibition of 6 and 4 mm respectively. The methanol extract inhibited the growth of *E. coli* (3mm) only. The ethanol extract gave a better minimum inhibitory concentration (MIC) (0.125 and 0.125 mg/ml) than aqueous extract (0.25 and 0.25 mg/ml) and methanol extract (0.50, and 0.00 mg/ml) on *E. coli* and *S. aureus* respectively. The study revealed that ethanol and aqueous extracts of *Aloe Vera* gel had antibacterial effect on these two pathogens (37). Trivedi et al., (2015) evaluated the effect of *Aloe Vera* based herbal wines on common foodborne pathogens. *Aloe Vera* gel blended with amla extract and ginger and supplemented with sugar proved to be a good medium for the growth of *Saccharomyces cerevisiae.* MIC of *Aloe*-amla and *Aloe*-ginger wine agaist *S. Typhimiurum* was found to be 25% and 40% respectively. For *S. aureus,* MIC was recorded as 40% and 30% for *Aloe*-amla wine and *Aloe*-ginger wine respectively. MIC of *Aloe*-ginger wine was 50% whereas MIC for *Aloe*-amla wine was found to be 40% against *E. coli*. Therefore, *Aloe*-amla wine exhibited the highest efficacy against *E. coli* and *S.Typhimurium* while for *S. aureus Aloe*-ginger wine worked the best. The MBC of *Aloe*-amla wine was found to be 45% for both *S. aureus* and *E. coli* and 35% for *S.* Typhimurium. MBC of *Aloe*-ginger wine was more than 50% for *E. coli* and *S.* Typhimurium whereas for *S. aureus* it was found to be 40% (38). Shamlou and Yavarmanesh, (2016) investigated the antibacterial effects of ethanolic and aqueous extracts of *Aloe Vera* on pathogenic bacteria such as *S. aureus* (ATCC25923), *E. coli* (ATCC25922), *Listeria monocytogenes* (ATCC33090). Aqueous extract of *Aloe Vera* did not show any antibacterial activity. It is supposed that antibacterial compounds such as Anthraquinone, Hydroxyanthra and Saponin had the most roles for antibacterial activity in ethanolic extract of Aloe Vera (39). Pugh et al. and Lawless and Allan evaluated the antimicrobial activity of *A. vera* gel against the pathogens *S. aureus, K. pneumonia, B. subtilis, Streptococcus pyogenes, E. coli, Pseudomonas, Helicobacter pylori* and *S. typhi*. They observed the minimum inhibition activity against the pathogen *E. coli*. They observed the maximum zone of inhibition against *Bacillus* with 23 mm (40, 41). The difference observed in the antibacterial properties of *Aloe Vera* in various studies may occur because of the differences in the composition of plants (under the influence genetic, type, the harvest season) and the type of extraction method used. Singh et al., (2002) indicated that combination of *Anis* EO (1000 ppm) and oleoresin is quite effective in controlling the growth of spoilage microorganisms in yoghurt; also addition of this EO had no undesirable effect on the physicochemical properties of yoghurt (27). Mohammadi et al., (2011) evaluated the antimicrobial effect of Z. multiflora EO on *E. coli* O157:H7 in white brined cheese. The inhibitory effect of *Z. multiflora* EO at concentration of 200 ppm was higher compared to its lower concentrations and also compared to the control groups (42). Simsek et al (2007) investigated the survival of *E. coli* O157:H7 during the storage of plain Ayran which was produced with mint, thyme, garlic, salt and their mixture. During the storage period, viable counts of *E. coli* O157:H7 fall was sharp from 6.40 to 3.10 (log cfu/g) at pH 4.4 and from 6.30 to 3.10 (log cfu/g) at pH 4.6. and *E. coli* O157:117 was not present at the end of 14th day of the storage (43). The present study has shown that antibacterial activity of the yoghurt with *Aloe Vera* gel aqueous extract and *L. casei* bacteria on *E. coli* were different from control samples. The obtained results suggest that the *E. coli* population were inhibited significantly by high concentrations of the aqueous extract after 7 and 10 day. Antibacterial effect of lactic acid bacteria (LAB) on pathogenic microorganisms were observed in many works and this fact is well known and their result is similar with our result in current study. Several *in vitro* and *in vivo* experiments on antibacterial effect of different Lactobacillus on *Campylobacter jejuni*, *Clostridium difficile*, *E. coli* have been performed. The isolates of this study have no active effect and the observed ability to inhibit the growth of *Bacillus cereus*, *E. coli*, *L. monocytogenesis* and *S. entritidis* except one Isolate of lactobacillia that can inhibit growth of *L. monocytogenesis*. This isolate was *L. casei*. Other studies showed also that some strains of *L. casei* had an inhibitory effect on different indicator bacteria (44, 45). Amdekar et al (2010) determined the antibacterial activity of *L. casei* against enteropathogens *E. coli*, *K. pneumoniae*, *S. enteritidis* and *P.flourescens*. The results indicated strong antibacterial activity of *L. casei* against various enteropathogenic bacteria that are main cause of diarrhea and vomiting ($p< 0.05$) (46). Farahbakhsh et al. (2013) investigated bactericidal effects of the isolated probiotics against *E. cloi*, *S. Aureus*,*S. pyogenes*, and *P. vulgaris* using disk diffusion and well diffusion agar methods. Growth of 4 pathogenic bacteria was suppressed by all 8 lactobacilli **(***L. rhamnosus*, *L. plantarum*, *L .acidophilus*, *L. Casei*, *L. bulgaricus*, *L. delbrueckii*, *L. fermentum*, and *L. brevis*), *L. plantarum* showed the strongest bactericidal effects (47). In the present study, significant differences ($P\leq0.05$) were observed between the groups containing extract in different concentrations and probiotic bacteria with the control group with respect to the pathogenic bacterial counts. However, the finding of the current research did not show synergistic effect between aqueous extract and probiotic bacteria in inhibition growth of *E.coli* during storage period.

4. CONCLUSION

The results obtained in this study showed significant inhibitory effects of extract concentrations, probiotic bacteria and time on the growth response of *E. coli* during storage of Yoghurt. The results indicated that *L. casei* and *Aloe vera* gel aqueous extract could be considered as natural Antibacterial agents against *E.coli* and researches must be developed in order to finding out more knowledge about the behavior of *E.coli* in acidic dairy products.

ACKNOWLEDGMENT

Authors would like to thank the dean for research of Qazvin University of Medical Sciences for the financial support of the project.

FUNDING/SUPPORT

Not mentioned any Funding/Support by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Pirbalouti AG, Malekpoor F, Enteshari S, Yousefi M, Momtaz H, Hamedi B. Antibacterial activity of some folklore medicinal plants used by Bakhtiari tribal in Southwest Iran. International Journal of Biology. 2010;2(2):55.
2. Basti AA, Misaghi A, Khaschabi D. Growth response and modelling of the effects of Zataria multiflora Boiss. essential oil, pH and temperature on Salmonella Typhimurium and Staphylococcus aureus. LWT-Food Science and Technology. 2007;40(6):973-81.
3. Yamazaki K, Yamamoto T, Kawai Y, Inoue N. Enhancement of antilisterial activity of essential oil constituents by nisin and diglycerol fatty acid ester. Food Microbiology. 2004;21(3):283-9.
4. Mahmoudi R, Zare P, Hassanzadeh P, Nosratpour S. Effect of Teucrium polium essential oil on the physicochemical and sensory properties of probiotic yoghurt. Journal of Food Processing and Preservation. 2014;38(3):880-8.
5. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food control. 2007;18(5):414-20.
6. Strickland FM, Kuchel JM, Halliday GM. Natural products as aids for protecting the skin's immune system against UV damage. Cutis. 2004;74(5 Suppl):24-8.
7. MUIR-BECKFORD M, BADRIE N. Consumer Acceptance of tropical wines from Aloe Vera (Aloe barbadensis) and cashew apples (Anacardium occidentale L.) in the british virgin islands. Foodservice Research International. 2000;12(3):185-96.
8. Foster S. Aloe vera: The succulent with skin soothing cell protecting properties. Herbs for Health magazine Health WorldOnline hhttp://www ealthy net/library/articles/hfh/aloe htm. 1999.
9. Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman SR, Stimson WH. In vitro susceptibilities of Shigella flexneri and Streptococcus pyogenes to inner gel of Aloe barbadensis Miller. Antimicrobial agents and chemotherapy. 2003;47(3):1137-9.
10. Antonisamy JMA, Beaulah N, Laju R, Anupriya G. Anti-bacterial and antifungal activity of Aloe vera gel extract. International Journal of Biomedical and Advance Research. 2012;3(3):184-7.
11. Kawai K, Beppu H, Shimpo K, Chihara T, Yamamoto N, Nagatsu T, et al. In vivo effects of Aloe arborescens Miller var. natalensis Berger (Kidachi aloe) on experimental tinea pedis in guinea-pig feet. Phytotherapy Research. 1998;12(3):178-82.
12. García-Sosa K, Villarreal-Alvarez N, Lübben P, Peña-Rodríguez M. Chrysophanol, an antimicrobial anthraquinone from the root extract of Colubrina greggii. J Mex Chem Soc. 2006;50(2):76-8.
13. Dabai Y, Muhammad S, Aliyu B. Antibacterial activity of anthraquinone fraction of Vitex doniana. Pakistan J Biol Sci. 2007:1-3.
14. Wu YW, Ouyang J, Xiao XH, Gao WY, Liu Y. Antimicrobial properties and toxicity of anthraquinones by microcalorimetric bioassay. Chinese Journal of Chemistry. 2006;24(1):45-50.
15. Reynolds T, Dweck A. Aloe vera leaf gel: a review update. Journal of ethnopharmacology. 1999;68(1):3-37.
16. Zacarchenco PB, Massaguer-Roig S. Properties of Streptococcus thermophilus fermented milk containing variable concentrations of Bifidobacterium longum and Lactobacillus acidophilus. Brazilian Journal of Microbiology. 2006;37(3):338-44.
17. Salminen S, Gibson C, Bouley M, Isolauri E, Boutron-Rualt M, Cummings J, et al. Gastrointestinal physiology and function: the role of prebiotics and probiotics. Br J Nutr. 1998;80(Suppl 1):S147-71.
18. Iyer R, Hittinahalli V. Modified PAP method to detect heteroresistance to vancomycin among methicillin resistant Staphylococcus aureus isolates at a tertiary care hospital. Indian journal of medical microbiology. 2008;26(2):176.
19. Matsuzaki T. Health properties of milk fermented with. Handbook of fermented functional foods. 2003:145.
20. Mohania D, Nagpal R, Kumar M, Bhardwaj A, Yadav M, Jain S, et al. Molecular approaches for identification and characterization of lactic acid bacteria. Journal of digestive diseases. 2008 Nov;9(4):190-8. PubMed PMID: 18959589. Epub 2008/10/31. eng.
21. ZOTTOLA EA, SMITH LB. THE MICROBIOLOGY OF FOODBORNE DISEASE OUTBREAKS: AN UPDATE1. Journal of food safety. 1990;11(1):13-29.
22. KASIMOZLU A, AKGÜN S. Survival of Escherichia coli O157: H7 in the processing and post-processing stages of acidophilus yogurt. International journal of food science & technology. 2004;39(5):563-8.
23. Lawrence R, Tripathi P, Jeyakumar E. Isolation, purification and evaluation of antibacterial agents from Aloe vera. Brazilian Journal of Microbiology. 2009;40(4):906-15.
24. Nostro A, Germano M, D'angelo V, Marino A, Cannatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in applied microbiology. 2000;30(5):379-84.
25. Parsaeimehr M, Basti AA, Radmehr B, Misaghi A, Abbasifar A, Karim G, et al. Effect of Zataria multiflora boiss. Essential oil, nisin, and their combination on the production of enterotoxin C and α-hemolysin by Staphylococcus aureus. Foodborne pathogens and disease. 2010;7(3):299-305.
26. Phillips M, Kailasapathy K, Tran L. Viability of commercial probiotic cultures (L. acidophilus, Bifidobacterium sp., L. casei, L. paracasei and L. rhamnosus) in cheddar cheese. International journal of food microbiology. 2006;108(2):276-80.
27. Singh G, Kapoor I, Pandey S, Singh U, Singh R. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. Phytotherapy research. 2002;16(7):680-2.
28. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, et al. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. Food chemistry. 2007;103(4):1449-56.
29. Wadud A, Prasad P, Rao M, Narayana A. Evolution of drug: a historical perspective. Bulletin of the Indian Institute of History of Medicine (Hyderabad). 2006;37(1):69-80.
30. Bhandari PR. Pomegranate (Punica granatum L). Ancient seeds for modern cure? Review of potential therapeutic applications. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2012;2(3):171.
31. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology. 2004;94(3):223-53.
32. Mahmoudi R, Kosari M, Zare P, Barati S. Kelussia odoratissima Essential Oil: Biochemical analysis and Antibacterial Properties against pathogenic and probiotic bacteria.
33. Elbandy M, Abed S, Gad S, Abdel-Fadeel M. Aloe vera gel as a functional ingredient and natural preservative in mango nectar. World Journal of Dairy & Food Sciences. 2014;9(2):191-203.
34. NUSRAT SI, LAJBER K, GUL A. Commercial extraction of gel from Aloe vera (L) leaves. Jour Chem Soc Pak Vol. 2000;22(1):47.
35. Agarry O, Olaleye M. Comparative antimicrobial activities of Aloe vera gel and leaf. African journal of biotechnology. 2005;4(12):1413.
36. Ibrahim M, Srinivas M, Narasu ML. Phytochemical analysis and antimicrobial evaluation of Aloe vera gel against some human and plant pathogens. Asian Journal of Current Chemistry. 2011;1(1):1-11.
37. Stanley M, Ifeanyi O, Eziokwu O. Antimicrobial effects of Aloe vera on some human pathogens. International Journal of Current Microbiology and Applied Sciences. 2014;3(3):1022-8.
38. Trivedi N, Rishi P, Soni SK. Antibacterial activity of prepared Aloe vera based herbal wines against common food-borne pathogens and probiotic strains. 2015.
39. SHAMLOU M, YAVARMANESH M. EVALUATION OF THE ANTIBACTERIAL EFFECTS OF AQUEOUS AND ETHANOLIC EXTRACTS OF ALOE VERA ON PATHOGENIC BACTERIA (STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, LISTERIA MONOCYTOGENES). 2016.
40. Pugh N, Ross SA, ElSohly MA, Pasco DS. Characterization of Aloeride, a new high-molecular-weight polysaccharide from Aloe vera with potent immunostimulatory activity. Journal of agricultural and food chemistry. 2001;49(2):1030-4.
41. Lawless J, Allan J. Aloe Vera: Natural Wonder Cure: HarperCollins UK; 2014.
42. MOHAMMADI K, KARIM G, HANIFIAN S, TARINEJAD A, GASEMNEZHAD R. ANTIMICROBIAL EFFECT OF ZATARIA MULTIFLORA BOISS ESSENTIAL OIL ON ESCHERICHIA COLI O157: H7 DURING MANUFACTURE AND RIPENING OF WHITE BRINED CHEESE. 2011.
43. Simsek B, Sagdic O, Ozcelik S. Survival of Escherichia coli O157: H7 during the storage of Ayran produced with different spices. Journal of food engineering. 2007;78(2):676-80.

44. Nikolic M, Terzic-Vidojevic A, Jovcic B, Begovic J, Golic N, Topisirovic L. Characterization of lactic acid bacteria isolated from Bukuljac, a homemade goat's milk cheese. International journal of food microbiology. 2008;122(1):162-70.
45. Terzic-Vidojevic A, Vukasinovic M, Veljovic K, Ostojic M, Topisirovic L. Characterization of microflora in homemade semi-hard white Zlatar cheese. International journal of food microbiology. 2007;114(1):36-42.
46. Amdekar S, Singh V, Roy P, Kushwaha S, Dwivedi D. In vitro antibacterial activity of Lactobacillus casei against enteropathogens. 2010.
47. Farahbakhsh M, Hakimi H, Bahram Abadi R, Zolfaghari M, Doraki N. Isolation of Probiotic Lactobacilli from Traditional Yogurts Produced in Rural Areas of Rafsanjan and their Antimicrobial Effects, 20012. Journal of Rafsanjan University of Medical Sciences. 2013;12(9):733-46.