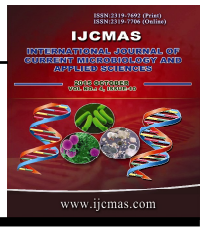
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 507-526

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 507-526**
http://www.ijcmas.com



Original Research Article

Effect of Different Plant Oils on *Escherichia coli* O157:H7 and *Staphylococcus aureus* Isolated from some Egyptian Fresh Juices

Rania M.M. Abdel-Baki[1]*, Galal M. Khalafalla[1], Basita A. Hussein[2] and Olfat S. Barakat[1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Genetic, Faculty of Agriculture, Cairo University, Giza, Egypt
**Corresponding author*

Keywords

E. coli O157:H7, Staphylococcus aureus, Plant oils, Egyptian fresh juices, Shiga toxins (stx1 and stx2)

A B S T R A C T

Fresh juices are the best way to get raw liquid nutrients into the body, because of their highly content of minerals and vitamins. They widely consumed by millions of people and highly susceptible to spoilage, so it is important from public health point of view to evaluate physical, chemical, and microbial characteristics of fresh juices. This study aims to determine total bacterial counts, total coliforms, fecal coliforms as well as presence-absence of *E. coli* O157:H7, total fungi, total yeast, *Staphylococcus aureus*, and total spore forming bacteria in common Egyptian fresh juices. During this study 259 bacterial strains (159 *E. coli* O157:H7 and 100 *Staphylococcus aureus*) were isolated from different Egyptian fresh juices (2 samples in both of winter and summer of each sugar cane, strawberry, orange, guava, banana, cocktail, carrot, mango, sobia, and tamarind. *E. coli* O157:H7 isolates were identified using classical as well as molecular diagnosis methods. The highest total viable bacterial count (6.2 cfu/ml) was found in carrot sample, and the lowest(2.5 cfu/ml) was found in Sobia sample in winter, while in summer, cocktail recorded the highest total count (7.29 cfu/ml) and the lowest one (5.21cfu/ml) was found in banana juice. Out of 159 isolated *E. coli* O157:H7 strains, 23 isolates were subjected to PCR analysis for the presence of *E. coli O157:H7* using specific primers to shiga toxins (stx1 and hylA) and haemolysin gene (hlyA). The isolates revealed a positive result for the presence of *E. coli O157:H7*. Out of 159 isolated *E. coli* O157:H7 strains, 23 isolates were subjected to PCR analysis for the presence of *E. coli O157:H7* using specific primers to shiga toxins (stx1 and stx2) and haemolysin gene (hlyA). The isolates revealed a positive result for the presence of *E. coli O157:H7*. Of the 23 *E. coli* O157:H7 positive samples by PCR, five isolates showed stx1, five isolates showed hylA, and 9 isolates showed both stx1 and hylA, 11 isolated strains of *E. coli* O157:H7 strains and 3 isolated strains of *Staphylococcus aureus* were used in addition of the control strain. Microbial groups as well as microbial load obtained for different juices differed according to their physical and chemical characteristics. Obtained results also showed that Clove oil has a great effect in the inhibition of all isolated strains of *E. coli O157:H7* with low concentrations (20, 25, 50, 75, 100 µl), while lemon cinnamon, marjoram, black seed, peppermint, and thyme give good results with higher concentrations (100, 150, 200, 250, 300, 400, 600, 800 µl). On the other hand, basil, sage, caraway, rosemary, fennel, dill, and anise oils has no effect on isolated strains even with the concentration of 800 µl

Introduction

Fresh juices, are highly susceptible to spoilage, in fact more so than whole fruit. Unprotected by skin or cell walls, fluid components are thoroughly mixed with air and microorganisms from the environment. Thus, unheated juices are subject to rapid microbial, enzymatic, chemical and physical deterioration (Bates *et al.,* 2001). In developing countries, fruit and vegetable juices sold by street vendors are widely consumed by millions of people. These juices provide a source of readily available and affordable source of nutrients to many sectors of the population, including the urban poor. Unpasteurized juices are preferred by the consumers because of the "fresh flavor" attributes and hence, in recent times, their demand has increased. They are simply prepared by extracting the liquid and pulp of mature fruit and vegetables usually by mechanical means. The final product is an unfermented, clouded, untreated juice, ready for consumption (Durgesh *et al.,* 2008). Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Victorian Government Department of Human Services, 2005; Oliveira *et al.,* 2006; Nicolas *et al.,* 2007; Durgesh *et al.,* 2008).

E. coli strains producing toxin are called Shiga toxin (Stx) -producing *E. coli* (STEC).S higa-toxin producing shiga toxin 1 (stx-1) and shiga toxin 2 (stx-2) (Manna, 2006). *E. coli* (STEC) also referred to as Verocytotoxic *E. coli* (VTEC) are currently considered as important emerging Food borne bacterial pathogens of public health concern (Jordan, 2010). Since the mid 1980s, genome identification and selection have progressed rapidly with the help of PCR technology. Specific primers for hlyA, stx1 and 2 genes were used for the detection of *E. coli* O157:H7 strain in beef, mutton and chicken (Kiranmayi and Krishnaiah, 2010). Therefore, molecular characterization of shiga toxigenic *E. coli* associated with raw meat and milk samples collected from Riyadh, Saudi Arabia (Al-Zogibi *et al.,* 2015) and unpasteurized fruit and vegetable juices (Hyun *et al.,* 2014).

During the past 30 years *Escherichia coli* in addition to other enteric pathogens, has emerged as one of the most important human diarrheal pathogens; several outbreaks of food-borne infections due to *E. coliO157:H7* have been reported in different parts of the world, following consumption of unpasteurized apple cider and orange juices (Cheng *et al.,* 2002). Unpasteurized juice can be a vehicle for food borne diseases (Bull *et al.,* 2004). Fresh fruits are prone to fungal contamination in the field, during harvest, transport, marketing, and with the consumer. It is important to identify fungal contaminants in fresh fruits because some moulds can grow and produce mycotoxins on these commodities while certain yeasts and moulds can cause infections or allergies (Tournas and Eugenia Katsoudas, 2005).

Condiments, and plant extracts have strong medicinal, preservative, and antioxidant properties. The antimicrobial activity of these ingredients is attributed to their essential oils, which are lipophilic and penetrate through the membrane to the interior of the cell and perform the inhibitory activity at the target site (Zaika, 1998., Moushumi Ghosh *et al.,* 2007). Antimicrobials of animal (lactoperoxidase,

lysozyme, and chitosan), plant (essential oils, aldehydes, esters, herbs, and spices), and microbial origin (nisin) can be used to effectively reduce pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices (Rosa *et al.,* 2009), so we can use plant oils as a food additives which has an antimicrobial effect against microorganisms. Therefore this study aims to (a) determine total bacterial counts, total coliforms, fecal coliforms as well as presence-absence of *E. coli* O157:H7, total fungi, total yeasts, *Staphylococcus aureus*, and total spore forming bacteria in some Egyptian fresh juices. (b) Identify isolated strains of *E. coli* O157:H7 as well as *S. aureus* isolates using specific culture media and classical methods. (c) the Control of *E. coli* O157:H7 and *Staphylococcus aureus* (as pathogenic strains) using some plant oils.

Materials and Methods

Sampling

A total of 40 different fresh juices samples (2 samples each of sugar cane, strawberry, orange, guava, banana, cocktail, carrot, mango, sobia, and tamarind) were collected from different sites in Giza area in both winter and summer.

Microorganisms: *E. coli* O157:H7 (ATCC 35150) and *Staphylococcus aureus* (ATCC 13565) as a positive control strains were obtained from The National Research Center, Dokki, Giza, Egypt

Physical, chemical, and microbiological evaluation of juice samples

Physical and chemical characteristics of fresh juices

Temperature and pH value were measured by Engineered system temperature electrode, using Cole Parmer bench-top, high accuracy pen type pH meter with temperature display model pH – 009(111). While total soluble sugars were determined using portable refractometer model RHB 0-90.

Microbiological analysis

Different microbiological analysis and physical characteristics were determined in all juices samples i.e. determination of total bacterial counts (T.C), presence-absence of *E. coli* O157:H7, total fungi (F), total yeast (Y), *Staphylococcus aureus* (Staph), and total spore forming bacteria (S.F) using plate count, while MPN technique was used for determination of total coliforms and fecal coliforms. In addition, the effect of some plant oils on *E. coli* O157:H7 as well as on *S.aureus* was investigated.

Total bacterial counts and total spore forming bacteria were determined using nutrient agar medium at 30°C for 24–48 hr (Downes and Ito, 2001),Yeasts were determined using Potatoes dextrose agar medium at 25°C for 5 days (Barnett *et al.,* 2000), Fungi were determined using Sabouraud Dextrose Agar medium at 25°C for 5 days (Jarett and Sonnenwirth, 1980), *Staphylococcus aureus* was determined using Baird-Parker Agar medium at 37 °C for 24–48 hr (Horwitz, 2007). According to Bergey's Manual of Systematic Bacteriology (Brenner *et al.,* 2005), biochemical tests were performed to confirm and identify *Staphylococcus aureus* i.e. Gram staining, Catalase test, Simon citrate agar, Gelatin Hydrolysis Test, litmus milk, starch hydrolysis, various sugar fermentation tests (lactose, fructose, sucrose, mannitol, sorbitol, and mannose), while Coagulase test were performed according to MacFaddin (2000), and total coliforms and fecal colifoms were determined in

MacConkey broth medium (Holt and Krieg, 1994) using the most probable number technique (MPN) at 37°C for 24–48 hr for total coliforms while, were incubated at 44.5°C for 24–48 hr for total fecal coliforms.

Morphological characteristics and biochemical tests for *S. aureus*

Gram staining for isolated strains was carried out according to (Syndney and William, 1982). Catalase production was carried out according to (Clarke and Cowan**,** 1952), while carbohydrates fermentation was carried out according to (Harrigan, 1998).

Isolation and identification of *E. coli* O157:H7 from Juices

Procedure for isolation and identification of *E. coli* O157:H7 from Juices

The bacteria isolates used in this study were collected from different juice samples. Each juice sample (25 ml) was enriched in 225 ml of modified tryptone soya broth medium (mTSB) (Doyle and Schoeni, 1987; Hill *et al.,* 1998) and incubated with agitation (120 r.p.m.) for 18–24 h at 37°C. After 24 h enrichment aliquots of 100 µl were plated on to Eosine Methylene Blue agar medium (EMB) (Cunnif, 1995) to presumptively identify isolates as Gram-negative enteric bacteria and *E. coli* (green-metallic colonies) and on to sorbitol MacConkey agar (SMA) to test for sorbitol non-fermenting bacteria (colorless colonies) (Abdul-Raouf and Ammar, 1996; Mabrouk, 2001). After 18 to 24 h at 37°C, characteristic colonies from EMB agar (green-metallic colonies), and SMA agar were transferred onto Sorbitol MacConkey agar medium supplemented with cefixime tellurite (Zadik *et al.,* 1993). Sorbitol non-fermenting isolates which give colorless colonies on MacConkey agar supplemented with cefixime tellurite after 18 to 24 h at 37°C were presumptively identified as *E. coli* O157:H7 and were examined by gram staining and catalase test and subjected to PCR analysis using primers specific.

Molecular identification of *E. coli* O157:H7

Preparation of DNA samples for PCR

DNA was isolated from different samples according to Kiranmayi and Krishnaiah (2010). The isolates positive for *E. coli O157:H7* by PCR method were further examined for the presence of shiga toxins (stx1 and stx2) using specific primers (Table 1). An *E. coli* O157:H7 (ATCC 35150) strain, obtained from National Research Center, Dokki, Giza, Egypt was used as known positive strain in PCR analysis. The amplification reaction was carried out in 20 µl total volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 2.5 U Taq DNA polymerase (all reagents from Promega Corp., USA), 10mM primer and 25 ng template. Amplification was carried out in a Biometra thermal cycler.

The amplification program was given in table 2. The amplification products were resolved by electrophoresis in a 1% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TAE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD) (Kiranmayi and Krishnaiah, 2010).

Antimicrobial activity of plant oils against *E. coli* O157:H7 and *S. aureus*

The antibacterial activity of 14 types of

plant oils (clove, lemon, thyme, Cinnamon, Marjoram, Black seed, Peppermint, Basil, Sage, Caraway, Rosemary, Fennel, Dill, and Anise) obtained from Orlane Egypt Company, Beni-Suef, Egypt, with different concentrations 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 600, 800 µl) against *E. coli O157:H7* and *S. aureus* was performed by agar well diffusion method (Bauer *et al.*, 1996). For inoculums preparation and assay of antibacterial activity, nutrient agar medium was used. Each of the bacterial strains was inoculated in nutrient broth and the cultures were incubated for 24h. Inoculums of each test strain was later mixed thoroughly to provide a homogenous liquid suspension (Sivakami *et al.*, 2013).

The respective bacterial cultures were poured into the nutrient agar medium and poured respectively, for uniform distribution of microorganisms. Wells were made on each agar plates using the sterile well puncture or cork borer. Different concentrations diluted with water ethanol solution 10% (1:1) 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 600, 800 µl of plant oils was loaded in the wells using a sterile micropipette. Respective ethanol solution10% (1:1) was used as a negative control for each plate. The plates were incubated for 24 hours at 37°C for each isolated strain. At the end of incubation period, the zone of inhibition was measured (Neelam *et al.*, 2012).

Statistical analysis

The data recorded in triplicate while presented as the means of the three replicates and were subjected to ANOVA test. The statistical analyses of the data were carried out by following two and three factors factorial experiment. Data analysis was preceded by Assistat software (Silva and Azevedo, 2009).

Results and Discussion

Physical, chemical, and microbiological evaluation of fresh juice samples

In the present investigation, different Egyptian fresh juices of sugar cane, strawberry, orange, guava, banana, cocktail, carrot, mango, sobia, and tamarind) were microbiologically analyzed for different microbial groups included total bacterial counts, total coliforms, fecal coliforms as well as presence-absence of *E. coli* O157:H7, total fungi, total yeasts, *Staphylococcus aureus*, and total spore forming bacteria. Data obtained were shown in table 3 for summer and table 4 for winter respectively. The high microbial load could be attributed to improper washing of fruits leading to contamination of extracted juices. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of such microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis *et al.*, 2006; Durgesh *et al.*, 2008).

Tamarind juice gives a negative result for both total and fecal coliforms, perhaps the lowest value of pH (2.8) in summer and (2.9) in winter is the reason. However, gives a positive result for *E. coli* O157:H7 after enrichment of sample with modified Tryptic Soya broth medium. The highest staphylococcal count was found in Sobia juice (4.9 CFU /ml) in summer, while in winter the highest staphylococcal count (4.3 CFU /ml) was found in carrot and banana juices. During summer months, the highest total viable bacterial count (7.29 CFU /ml) was recorded for cocktail juice, while the lowest total bacterial count was recorded as

(5.23 CFU /ml) for orange juice. The maximum value of pH (5.42) was in sobia juice, the minimum value of pH (3.77) was in Strawberry juice.

Table 4 shows the microbiological quantity of Egyptian fresh juices in winter.

The highest total viable bacterial count (6.2 CFU /ml) was recorded for Carrot juice sample, while the lowest total bacterial count was recorded as (2.5 CFU /ml) for Sobia sample. The maximum value of pH (6.51) was in carrot juice, this was in accordance with the maximum value of total count. The minimum value of pH (2.90) was in tamarind which may cause total count to decrease to 4.9cfu/ml. *E. coli O157:H7* was proved its presence in all samples except for mango and tamarind. Data obtained from tables 3 and 4 showed that the total count recorded for juices samples in summer was higher than that recorded in winter, which indicates to the effect of high temperature on total bacterial count.

All types of juices give a positive result with total coliforms as well as fecal coliforms except on tamarind juice. This may indicate to unsanitary conditions, unhygienic practices during or after production as well as poor quality of water source used. Durgesh *et al.* (2008) showed occurrence of high microbial loads consisting of number of pathogens like coliforms, fecal coliforms, *E. coli*, *S. aureus* and *Vibrio cholerae* in freshly squeezed juices of sugarcane, lime and carrot. Sugarcane juice followed by carrot juice showed high microbial counts consistent with pH values of 5.4 and 6.2 which do not affect the survival of pathogens adversely. In contrast, lime juice with pH 2.3 showed much lower total viable count ranging between logs 0–8.2.

Studies of Shakir *et al.* (2009) on mango juice showed that the mean total viable count (8.00×10^3 -8.05×10^8 CFU /ml), total coliforms (1100 - >2400 MPN/100 ml), fecal coliforms (7 - >2400 MPN/100 ml), and total fungi (1.05×10^2 - 8.05×10^4 CFU /ml). The investigation of Joy *et al.* (2006) on mango and orange juices indicated that pathogenic bacterial counts were significantly high in orange followed by mango. Perhaps attributable to the quantity of water used for dilution. They reported the mean total viable count (24.4 CFU /ml), total coliforms (9.48MPN/100 ml), and Pathogenic *E. coli* (3.9 CFU /ml) in orange juice, while in mango juice was total viable count (10.6 cfu/ml), total coliforms (7.15 MPN/100 ml), and pathogenic *E. coli* (2.2 CFU /ml). Studies of Rashed *et al.* (2013) reported that the highest bacterial load ($2.8x10^7$ CFU /ml) from vendor fruit juice sample was found in a sugarcane juice, while found the highest total staphylococcal count ($8.99x10^5$ CFU /ml) was recorded for packed orange fruit juice and in two Strawberry juices (4.5×10^3 and 1.45×10^4 CFU /ml). Many of juices showed the presence of coliforms, since the highest coliform count ($1.58x10^6$ CFU /ml) was recorded for sugarcane and ($3.6x10^4$ CFU /ml) in orange juice samples. Also they reported the presence of fecal coliform ($7.95x10^2$ and $1.95x10^2$ CFU /ml) in two Sugarcane juices samples. Studies of Ankur *et al.* (2009) showed that the total viable count of 38 samples of juices of pineapple, sweet lime and vegetable juices (carrots) were in the range of $2.0x10^4$–$4.6x10^6$ CFU/ml. The total coliforms count was in the range of log of 3-4 in almost all the samples tested. The presence of fecal coliforms in range of log of 3 indicates use of contaminated water during handling and washing etc. Studies of Andres *et al.* (2004) reported the presence of coliform in fruit

juice which is not allowed by safe food consumption standard. Water used for juice preparation can be a major source of microbial contaminants including coliforms, faecal coliforms, faecal streptococci, etc (Tasnim *et al.,* 2010). Fruit juices contaminated at any point of processing could be the source of infections pathogens (Tsiga *et al.,* 2008).

Proposed sources of contamination of fruit used for juice have included the use of fallen fruit that has been in contact with contaminated soil, water, sewage or manure, use of contaminated water in washing or processing fruit, and contamination at the point of consumption (Vojdani *et al.,* 2008).

Bello *et al.,* (2014) studied different juices and showed that Yeast count was $3.5x10^4$ CFU /ml in orange juice. Mean total coliform count $1.5x10^4$ CFU /ml was in orange juice. The investigation of Javid *et al.,* 2013 showed that total count were in the range of 9×10^9–4×10^4 CFU /ml, while the total coliform were in the maximum value 210 ml as MPN index for sugarcane juice and lowest 9.0 ml as MPN index were calculated for orange juice. Total coliform Bacteria were absent in orange and lemon Juice, while apple, banana, mango and sugarcane juices were positive for presence of total coliforms 15, 23, 9.0 and 93 ml as MPN index respectively. *E. coli* were present in apple, banana, mango, and sugarcane juice, while it was absent in orange and lemon juices. The yeast and mould 4×10^5, 3×10^6, 3×10^4, 7×10^5, 6×10^4 and 45×10^8 CFU /ml were found of Apple, Banana, Mango, Orange, Lemon and Sugarcane juice respectively. Studies of Javed *et al.* (2015) on sugarcane juice showed that in all the localities the street vended sugar cane juices remained hygienically poor as indicated through high bacterial load i.e. 4×10^2–3×10^7 CFU/ml. All samples were contaminated with coliform bacteria ranged from 46 to 1100 MPN/ml. Seventy five percent of samples were contaminated with confirmed *E. coli*. All the examined samples were contaminated with yeast and mould. Total coliforms were present in all analyzed ice samples whereas confirmed *E. coli* was present in 37% of samples.

Morphological characteristics and biochemical tests for *S. aureus*

Out of the 100 coagulase positive *S.aureus* isolates, 24 isolates were subjected to Gram staining, catalase test, gelatin hydrolysis test, litmus milk, and various sugar fermentation tests (lactose, fructose, sucrose, mannitol, sorbitol, and mannose). Obtained results are shown in table 5.

Isolation and identification of *E. coli* O157:H7 from Juices

Molecular identification

PCR amplification of the genomic DNA from the 23 *E. coli* O157:H7 isolates as well as two controls (positive and negative), 5 strains showed stx1 (Fig. 1) 5 strains showed hylA (Fig. 2), and 9 strains showed both stx1 and hylA as shown in table 6.

In this study the majority of strains of *E. coli* O157:H7 equally produce Stx1 and Stx2 compared with (Law, 2000) which reported that the majority of strains of *E. coli* O157:H7 produce Stx2, some produce both Stx1 and Stx2, and a few produce Stx1 only. Isolated strain from carrot juice showed presence of *E. coli* O157:H7 in both Stx1 and Stx2 genes which are similar to (Reza and Sakineh, 2013) investigation. It indicated that among 47 confirmed bacteria with antiserum, 2/13 %, 14/92 %, 29/57%, 53/38% were related to spinach, vegetable,

radish, and carrot juice, respectively. The result was predictable, given the fact that most collected samples were related to carrot juice with probability of carrots contamination in farm fields during harvest and due to lack of proper cleaning.

Antimicrobial activity of plant oils against *E. coli* O157:H7 *and S.aureus*

Antimicrobial activity of plant oils against *E. coli* O157:H7

Out of the tested 14 plant oils, only seven oils i.e clove, lemon**,** cinnamon, marjoram, thyme, peppermint, and black seed oils had inhibitory effect on isolated *E. coli* O157:H7 strains from some Egyptian fresh juices The antimicrobial effect of selected plant oils on *E. coli* O157:H7 with different concentrations (20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 600 and 800µl) divided into groups according to strain response for the lowest concentration of the plant oil under investigation.

Obtained results for the antimicrobial effect of clove oil on *E. coli* O157:H7 were presented in table 7. It could be noted that the antimicrobial effect showed by inhibition zone value was increased by increasing the concentration of clove oil. The highest inhibition zone (4 mm) was recorded for strain (63) with the concentration of 100µl, while the lowest one (0.2 mm) was recorded for strain (9) with the concentration of 20µl. Comparing the control strain with other strains, It is worth to mention that the inhibition zones of all the examined strains were significantly decreased except 4 strains. In this respect a significant increase for strain 63 with the concentration of 75µl, while there is insignificant increase for strain (Ks) with the concentrations of 20 and 25 µl, strain 71 with the concentration of 75µl, strain (63) with the concentrations of 25 and 50 µl and strains (71, 57, and 63) with the concentration of 100µl. In general strain No. 63 was the most sensitive strain which had affected by clove oil, while the concentration of 75µl is the best concentration which affecte only strain 63, therefore from the economic point of view; there is no need to increase the concentration above 75 µl.

The antimicrobial effect of lemon oil on *E. coli* O157:H7 with different concentrations (100, 150, 200, 250, 300, 400, 600, and 800 µl) were presented in table 8. It could be noted that the antimicrobial effect showed by inhibition zone value was increased by increasing the concentration of lemon oil. The highest inhibition zone (1.8mm) was recorded for strain 63 with the concentration of 800µl, while the lowest one (0.1mm) was recorded for strain 9 with the concentrations of 100 and 150µl. Comparing the control strain with other strains at the concentrations of 100, 150, 200, 250, and 300 µl, it could be noted that two strains (Ks and D1) and strain no. 53 at concentration of 300 µl had the same inhibition zones values of the control strain. The other examined strains were significantly decreased, while strains (53 and 63) at the concentrations of 200 and 250 µl and strains (71, 46, 63, and 10) at the concentration of 300 µl were insignificantly decreased. Lemon oil at the concentration of 400, 600, and 800 µl mostly equal on affected the control strains. It should be noted that lemon oil significantly affected on strain no. 63 (inhibition zone 1.8). The other strains (13, 12, 46, 53, and 9) at the same concentration (800 µl) and strains (46 and 63) at concentration of 600 µl were insignificantly increased.

Obtained results for the antimicrobial effect of thyme, peppermint, marjoram, and black seed oils on *E. coli* O157:H7 were presented

in table 9. It could be noted that, the antimicrobial effect for inhibition zone value was increased by increasing the concentration of above mentioned oils. The highest inhibition zone value (2.1 mm) recorded for strain (12) with the concentration of 300µl of peppermint oil, while the lowest one (0.1 mm) was recorded for many strains with all mentioned oils and some concentrations.

Regarding to thyme oil, the highest inhibition zone (0.5 mm) was recorded for strain (10) with the concentration of 300µl. It could be noted that the control strain was affected only by the concentration of 300 µl. Thyme oil had no effect on all the examined strains at concentration of 100 µl. Comparing the control strain with other strains. While the concentration of 150 µl of the same oil had only affected three strains i.e. (46, 10, and D1), two of them (46 and 10) with insignificant increase, while one strain (D1) with a significant increase).

Regarding to peppermint oil, the highest inhibition zone (2.1 mm) was recorded for strain (12) with the concentration of 300 µl. The lowest one (0.7) was recorded for strain (13) at concentration of 150 µl while, as well as for strain 9 at concentrations of 150 and 200 µl. The concentration of 100 µl had no effect on all tested strains. In addition the control strain was not affected by any concentrations except with the concentrations of 250 and 300 µl. Strain (71) was not affected by any concentrations of peppermint oil while, strain (D1) was affected only by the concentration of 300 µl with insignificant decrease. In this respect the strain (57) was only affected by the concentrations of 250 and 300 µl with a significant increase in inhibition zone value. Comparing the control strain with other strains, it noted that, the inhibition zones at concentrations ranged from 150 to 300 µl were significantly increased.

Regarding to marjoram oil, the concentrations of (100, 150, 200, and 250 µl) had no effect on seven strains (control, 46, 53, 57, 9, 10, and Ks). In addition, comparing the value of inhibition zones of above mentioned strains with the control strain, it was found that there is no increment in of inhibition zones value except on strain 57 (insignificant increase) at concentration of 300 µl. The inhibition zones of all other examined strains were insignificantly increased except one strain (D1) with the concentrations of 100, 150, and 300 µl.

Regarding to black seed oil, the highest inhibition zone (0.9 mm) was recorded for two strains (12 and 10) with the concentration of 300 µl while, the lowest one (0.1) was recorded for six strains, one strain (D1) with the concentration of 150 µl, two strains (control and 10) with the concentration of 250 µl, and three strains (13, 57, and Ks) with the concentration 300 µl. It is worth to note that black seed oil mostly had no effect on all strains at concentrations of 100, 150, 200, and 250 µl. Strain No.12 showed significant increase (inhibition zone 0.5). On the other hand, strain D1 showed significant increase at concentration of 200 µl, while showed insignificant increase at concentration of 250 µl. Moreover, concentration of 300 µl showed insignificantly increase on strains 46, 53, 63, 9, and 10, while other strains except strain D1 showed insignificant decrease.

The antimicrobial effect of cinnamon oil on *E. coli* O157:H7 with different concentrations (250, 300, 400, 600, 800 µl) were presented in table 10. Regarding to cinnamon oil, it could be noted that, the antimicrobial effect for inhibition zone value was increased by increasing the concentration of cinnamon oil. The highest inhibition zone value (0.9 mm) was recorded

for strain 57 with the concentration of 800µl of cinnamon oil, while the lowest one (0.1mm) was recorded for many strains (control, 71, 53, 63, 10, Ks, and D1) at different concentrations. The concentration of 250µl had no effect on all tested stains including the control strain.In addition the concentrations of 250, 300, 400, and 600 had no effect on six strains (control, 13, 12, 10, Ks, and D1). Comparing the control inhibition zones value with other strains, it is noted that the concentration of 300µl had effect only on two strains (46 and 9) with a significant increase. Also the concentrations of 400 and 600µl had effect on six strains (71, 46, 53, 57, 63, and 9) with a significant increase. While the inhibition zones of six strains (13, 12, 46, 53, 57, and 63) at the concentration of 800µl were significantly increased.

Antimicrobial activity of plant oils against *S.aureus*

By studying the antimicrobial activity of the tested 14 plant oils against the isolated *S.aureus* strains (13, 33, and 40), it found that basil, sage, caraway, fennel, and dill oils have no effect against isolated *S.aureus* strains. While black seed, thyme, marjoram, anise, and cinnamon oils had only effective at some strains and concentrations (Fig. 3). Black seed oil had effect on two strains (13 and 33) at the concentration of 200 µl. Thyme oil had effect on all tested strains at concentration of 250 µl. Marjoram oil had effect on all tested strains at concentration of 300 µl.

Anise oil had effect only on strain No.13 at concentrations of 25, 50, 75, and 100 µl. Cinnamon oil had effect on strain No.33 at concentrations of 400, 600, and 800 µl.The most effective plant oils (clove, rosemary, peppermint, and lemon) which had antibacterial activity on isolated *S.aureus* strains are shown in table 10.

Obtained results for the effect of clove, rosemary, peppermint, and lemon plant oils on *S. aureus* which isolated from different fresh juices with different concentrations were presented in table 11. It is worth to mention that the antimicrobial effect presented in inhibition zone value was increased by increasing the concentration of above mentioned oils. The highest inhibition zone value (4 mm) recorded for strains (13 and 40) with the concentration of 100µl of clove oil, while the lowest one (0.2 mm) recorded for strain (13) with the concentration of 100µl and 200µl of lemon oil. It is worth to mention that, the clove oil recorded the highest values of inhibition zones compared with other oils. In addition rosemary oil had no antimicrobial effect on strain No. 33 with all concentrations. Also peppermint oil had no antimicrobial effect on strains No.40 with all concentrations (50 – 150 µl).

Similarly to the present study that showed an antibacterial activity of clove, lemon, cinnamon, and thyme plant oils in influencing *E. coli* O157:H7 and *S. aureus*, Alina *et al.* (2011) reported that the essential oil with the widest spectrum of activity was found to be oregano oil followed by white thyme oil, clove bud oil, cinnamon oil, garlic oil, onion oil, and basil oil, in that order. White thyme essential oil presented a higher activity against *E. coli* O157:H7; while oregano essential oil was the most efficient against gram-positive bacteria (*Bacillus cereus* ATCC 11778 and *S. aureus* ATCC 25923). In contrast, in the present study basil oil had no antibacterial activity in influencing *E. coli* O157:H7 and *S. aureus*. Rita *et al.* (2012) found that Combination of 1 CMI of clove with1 CMI of cinnamon leaves with 1CMI of vanillin had a bactericidal effect, reducing the population two log cycles.

Table.1 Oligonucleotide primers used in the study

primer	Target gene	Primer sequence (5`-3`)	Fragment	Reference
O157-4	hlyA	GTA GGG AAG CGA ACA GAG	361	Wang et. al., 1997
O157-3	hlyA	AAG CTC CGT GTG CCT GAA		
Stx1-F	stx1	ACA CTG GAT GAT CTC AGT GG	614	Manna, 2006
Stx-R	STXI	CTG AAT CCC CCT CCA TTA TG		
Stx2-F	Stx2	CCA TGA CAA CGG ACA GCA GTT	779	Manna, 2006
Stx2-R	Stx2	CCT GTC AAC TGA GCA CTT TG		

Table.2 Cycling conditions used for three sets of primers

No.	Step	hly A (*Escherichia coli* O157:H7)	stx1 and stx2 (STEC)
1	Initial denaturation	94^0C/5min.	94^0C/5min.
2	Final denaturation	94^0C/1min.	94^0C/1min.
3	Annealing	52^0C/1min.	60^0C/1min.
4	Initial extension	74^0C/2min.	72^0C/2min.
5	Final extension	74^0C/10min.	72^0C/10min.

Table.3 Microbial load of some Egyptian juices particularly presence of *E. coli* 157:H7 in summer

Type of Juices	Physiochemical characteristics			Bacterial counts (CFU) Log/ml					Log cell/ml MPN Index		Presence-absence of *E. coli* O157:H7
	Temperature	pH	Sugars %	T.C	F	Y	Staph	S.F	Total coliforms	Fecal coliforms	
Sugarcane	33.6	4.14	15.5	5.49	5.53	8.37	4.68	3.3	3.54	3.16	+
Strawberry	33.7	3.77	19	5.37	3.64	3.35	3.25	3	1.76	0	+
Orange	32.4	4.7	9	5.23	4.68	4.52	3.77	2.6	3.16	2.6	+
Guava	34.2	4.7	16.3	6.16	4.88	5.03	4.11	2.1	4.65	3.38	+
Banana	33.6	5.12	14.5	5.21	4.15	3.9	3.37	3	2.81	0.6	+
Cocktail	33.7	4.95	16.5	7.29	6.58	4.81	4.01	4.2	4.85	1.6	+
Tamarind	33.3	2.81	8.9	6.56	4.41	3.79	2.63	3.3	0	0	+
Carrot	34.3	4.67	4.5	5.78	3.78	5.12	3.81	2.7	4.89	3.03	+
Sobia	34.7	5.42	16.6	5.29	5.43	3.9	4.9	3.9	4.76	3.1	+
Mango	33.7	4.61	17.4	6.44	4.25	3.9	3.66	2.6	3.78	2.4	+

Each value represents the mean of three replicates.

Table.4 Microbial load of some Egyptian juices particularly presence of *E. coli* 157:H7 in winter

Type of Juices	Physiochemical characteristics			Bacterial counts (CFU) Log/ml					Log cell/ml MPN Index		Presence-absence of *E. coli* O157:H7
	Temperature	PH	Sugars %	T.C	F	Y	Staph	S.F	Total coliforms	Fecal coliforms	
Sugarcane	25	5.7	14.75	5.9	3.3	4.6	3.4	1.4	3.9	0.59	+
Strawberry	25	3.85	12	4.2	3.1	2.9	0.5	2.6	2.4	0.38	+
Orange	20.3	3.7	8.5	4.8	3.5	3.8	2.4	2.7	1.7	0.22	+
Guava	25.3	4.31	16	4.7	3.4	43	0	3.7	1.4	0.15	+
Banana	21.4	4.96	22.5	5.5	4.3	4.3	0	3.1	2.4	0.38	+
Cocktail	20.3	5.10	18	5.2	3.9	4.5	3.3	2.8	3.1	0.5	+
Tamarind	19.6	2.90	7.5	4.9	2.8	5.4	0	2.6	0	0	-
Carrot	25.2	6.51	5	6.2	4.3	4.4	4.7	2.6	5.1	0.71	+
Sobia	25.4	5.65	17	2.5	3.9	4	5.2	2.7	5.1	0.71	+
Mango	25.5	4.37	19	5.2	3.5	3.5	5.4	3.1	2.3	0.36	-

Each value represents the mean of three replicates.

Table.5 Results of Biochemical characterization of *Staphylococcus aureus* isolated from Egyptian fresh juices

Biochemical test		Result
Gelatin Hydrolysis		+
Catalase		+
Litmus milk		Acid, clot formation
Coagulase		+
Acid from sugars	**Glucose**	+
	Mannitol	+
	Maltose	+
	Lactose	+
	Sorbitol	+
	Sucrose	+

Table.6 Occurrence of *E. coli* O157:H7 in different fresh juices samples

sample	Results by PCR for E. coli O157:H7	stx1	hylA	Both stx1 & hylA
Sugarcane	6	1	0	5
Strawberry	2	1	0	1
Orange	1	0	1	0
Guava	2	1	1	0
Cocktail	1	0	0	1
Tamarind	1	0	1	0
Carrot	1	0	0	1
Sobia	3	2	0	1
Mango	2	0	2	0

Table.7 The antimicrobial effect of clove oil on *E. coli* O157:H7 with different concentrations (20, 25, 50, 75, and 100 µl)

E. coli Strains	Clove oil concentrations				
	20µl	**25µl**	**50µl**	**75µl**	**100µl**
control	1.9	2	2.5	2.5	3.3
71	0.4	0.5	2	3	3.5
13	0.4	0.5	0.6	1	1.5
12	1.4	1.5	1.6	2.5	3
46	0.3	0.5	0.5	1.3	2.4
53	0.4	0.5	0.5	1.5	2
57	0.8	1	2.1	2.5	3.5
63	1.5	2.7	3.3	3.7	4
9	0.2	0.6	1	2.8	2.8
10	0.3	0.5	0.6	2	3.2
Ks	2	2.3	2.4	2.5	2.8
D1	1.6	1.9	2.1	2.8	3.3

Each value represents the mean of three replicates.
Concentrations (A) Strains (B) LSD (A x B 0.001) = 0.9219

Table.8 The antimicrobial effect of lemon oil on *E. coli* O157:H7 with different concentrations (100, 150, 200, 250, 300, 400, 600, and 800 µl)

E. coli Strains	Lemon oil concentrations							
	100µl	**150µl**	**200µl**	**250µl**	**300µl**	**400µl**	**600µl**	**800µl**
control	0.7	0.7	0.7	0.7	0.7	1	1	1.1
71	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.9
13	0.3	0.3	0.3	0.3	0.3	0.3	0.4	1.4
12	0.3	0.3	0.3	0.3	0.3	0.3	1.1	1.4
46	0.3	0.3	0.3	0.3	0.4	0.4	0.4	1.2
53	0.2	0.3	0.5	0.6	0.7	0.8	0.9	1.3
57	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.8
63	0.2	0.3	0.4	0.5	0.6	0.8	1.2	1.8
9	0.1	0.1	0.2	0.2	0.3	0.3	0.6	1.2
10	0.3	0.3	0.3	0.3	0.4	0.4	0.6	1
Ks	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.9
D1	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.8

Each value represents the mean of three replicates.
Concentrations (A) = 0.10825 Strains (B) = 0.13258 LSD (A x B 0.005) = 0.3750

Table.9 The antimicrobial effect of (thyme, peppermint, marjoram, and black seed oils) on *E. coli* O157:H7 with different concentrations (100, 150, 200, 250, and 300 µl)

E.oli strains	Concentrations of oils of																			
	Thyme oil					Peppermint oil					marjoram oil					Black seed oil				
	100µl	150µl	200µl	250µl	300µl	100µl	150µl	200µl	250µl	300µl	100µl	150µl	200µl	250µl	300µl	100µl	150µl	200µl	250µl	300µl
control	0	0	0	0	0.1	0	0	0	0.1	0.2	0	0	0	0	0.1	0	0	0	0.1	0.2
71	0	0	0.2	0.2	0.2	0	0	0	0	0	0	0.3	0.3	0.4	0.5	0	0	0	0	0.2
13	0	0	0	0.4	0.4	0	0.7	0.8	1	1.2	0	0.6	1	1	1.3	0	0	0	0	0.1
12	0	0	0.1	0.3	0.3	0	1.3	1.5	1.8	2.1	0.2	0.2	0.4	2	3	0	0	0	0.5	0.9
46	0	0.1	0.1	0.2	0.2	0	0.8	0.8	0.9	1.1	0	0	0	0	0.1	0	0	0	0	0
53	0	0	0.1	0.3	0.3	0	1	1.1	1.3	1.8	0	0	0	0	0.1	0	0	0	0	0.8
57	0	0	0.1	0.2	0.2	0	0	0	1.2	2	0	0	0	0	0.3	0	0	0	0	0.1
63	0	0	0	0.2	0.2	0	0.8	0.8	1	1.3	0.2	0.2	0.3	0.3	0.4	0	0	0	0	0.8
9	0	0	0.1	0.3	0.3	0	0.7	0.7	0.8	1.1	0	0	0	0	0.1	0	0	0	0	0.8
10	0	0.2	0.3	0.3	0.5	0	1.4	1.5	1.6	1.8	0	0	0	0	0.1	0	0	0	0.1	0.9
Ks	0	0	0	0	0.1	0	1.5	1.5	1.6	1.7	0	0	0	0	0.1	0	0	0	0	0.1
D1	0	0.3	0.3	0.3	0.3	0	0	0	0	0.1	0.1	0.1	0.2	0.2	0.2	0	0.1	0.3	0.3	0.3

Each value represents the mean of three replicates.
Oils (A) Concentrations (B) Strains (S) LSD (A x B x S 0.001) = 0.2599

Table.10 The antimicrobial effect of cinnamon oil on *E. coli* O157:H7 with different concentrations (250, 300, 400, 600, 800 µl)

E. coli Strains.	cinnamon oil concentrations				
	250µl	300µl	400µl	600µl	800µl
control	0	0	0	0	0.1
71	0	0	0.1	0.2	0.2
13	0	0	0	0	0.3
12	0	0	0	0	0.3
46	0	0.2	0.3	0.3	0.4
53	0	0	0.1	0.2	0.3
57	0	0	0.2	0.6	0.9
63	0	0	0.1	0.2	0.4
9	0	0.2	0.2	0.2	0.2
10	0	0	0	0	0.1
Ks	0	0	0	0	0.1
D1	0	0	0	0	0.1

Each value represents the mean of three replicates.
Concentrations (A) Strains (B) LSD (A x B 0.001) = 0.1416

Table.11 The effect of clove, rosemary, peppermint, and lemon plant oils on *S. aureus* with

different concentrations

St strains	Concentrations of oils of															
	Clove				Rosemary				Peppermint				Lemon			
	A(25µl)	B(50µl)	C(75µl)	D(100µl)	A(25µl)	B(50µl)	C(75µl)	D(100µl)	A(50µl)	B(75µl)	C(100µl)	D(150µl)	A(100µl)	B(200µl)	C(400µl)	D(600µl)
13	2.7	3	3.5	4	0	0.4	1	1.3	0.6	0.9	1.6	2.5	0.2	0.2	0.3	0.4
33	0.6	0.7	1	2	0	0	0	0	0.8	1	1.2	1.4	0.7	0.8	1.1	1.4
40	2.6	3.2	3.5	4	0	0.2	0.5	0.6	0	0	0	0	0.4	0.7	0.8	1.2

Each value represents the mean of three replicates.
Oils (A) Concentrations (B) Strains (S) LSD (A) = 0.29057 LSD (B) = 0.29057 LSD (S) = 0.25164
LSD (A x S 0.001) = 0.5033

Fig.1 PCR analysis of *E. coli* O157:H7 (Stx1 gene),M = DNA ladder, N = negative control strain, Lanes 1 and 2= PCR products of strains, P = positive control strain

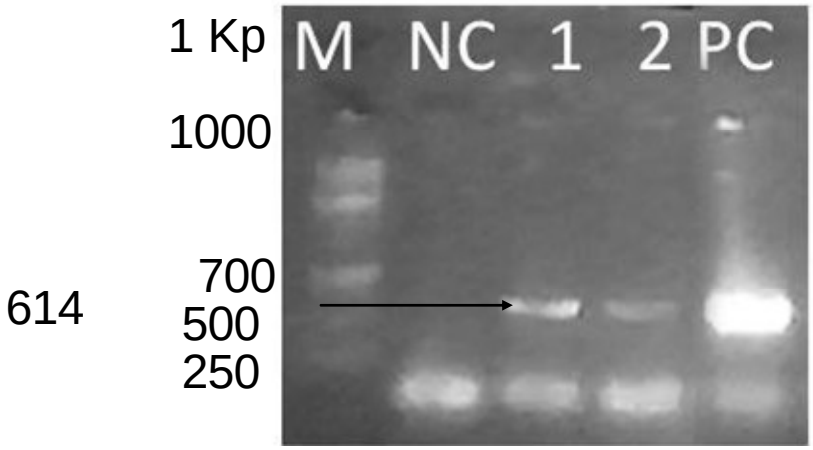


Fig.2 PCR analysis of *E. coli* O157:H7 (hylA gene), M = DNA ladder, P = positive control strain, N = negative control strain, Lanes1, 2, 3, 4, and 5 = PCR products of strains

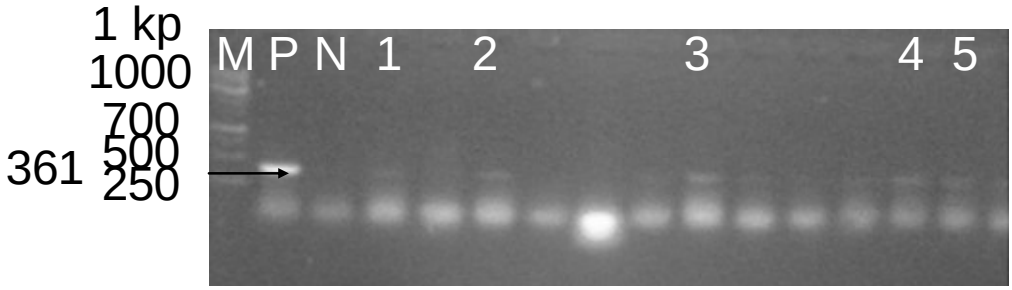
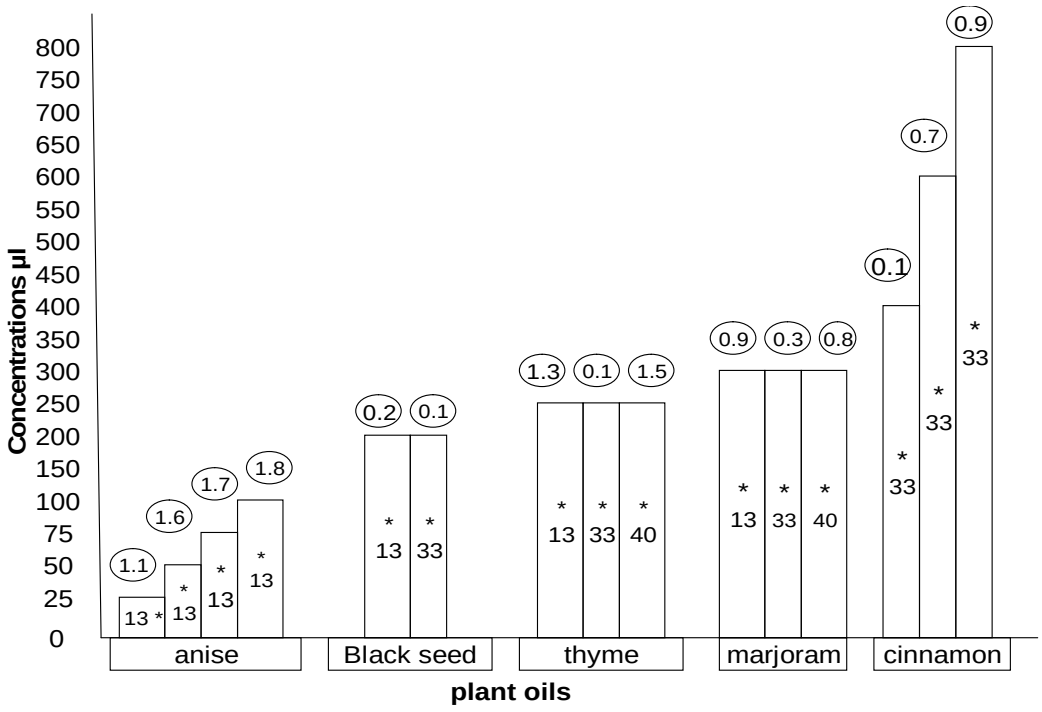


Fig.3 The effect of anise, black seed, thyme, marjoram, and cinnamon plant oils on

S. aureus with different concentrations



*: No. of strain
O: Value (mm) of inhibition zones

All the assayed combination of cinnamon bark with vanillin gave bactericidal effect reducing the population of *E. coli* O157: H7 between one and two log cycles. Rosa *et al.* (2009) also indicated that clove, lemon and cinnamon oils had an antibacterial activity against *E. coli* O157:H7. Suree and Pornpan (2011) reported that essential oils of anise, bastard cardamom, cinnamon, dill, mace, zedoary, prikhom, and bitter ginger were determined for their antimicrobial and antioxidant activities. Of all, cinnamon oil had the highest antibacterial activity. Two oil combinations: i) cinnamon and mace oils and ii) cinnamon and prikhom oils showed a synergistic effect against *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Salmonella Rissen* (0.32–0.38 mg/mL fractional inhibitory concentration index, FICI). Mosqueda-Melgar *et al.* (2008), reported higher reductions of *S. enteritidis* and *E. coli* O157:H7 in strawberry and orange juices containing 0.1% (v/v) of cinnamon bark oil than in apple and pear juices under same conditions.

In conclusion, all tested juices showed the occurrence of high microbial load for tested microbial groups under investigation. That indicates to unsanitary conditions, unhygienic practices during or after production as well as poor quality of used water source. The use of fallen fruit that has been in contact with contaminated soil, water, sewage or manure, and use of contaminated water in washing or processing fruit juices. Essential plant oils can be used effectively to reduce pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices, so we can use plant oils as a food additives which has an antimicrobial effect against microorganisms. In the present investigation clove, lemon, and thyme oils were the most effective

antibacterial activity in influencing *E. coli* O157:H7 in addition of *S. aureus*.While dill, fennel, basil, and anise oils had no effect. The results suggested that these plant oils could be used in juices as an additive supplements which have an antibacterial activity in influencing different *E. coli* O157:H7 and *S. aureus* strains especially clove and lemon oils, because of their acceptable taste and odor in juices and the need of lower concentrations compared with other oils concentrations.

Reference

Abdul-Raouf, U. M., and Ammar M. S. (1996). Isolation and identification of *E. coli O157:H7* from some Egyptian foods. *Egypt. J. Microbiol.*, 31(3): 469–474.

Alina, Dobre, A., Valeria Gagiu., Petru Niculita, 2011. Preliminary studies on the antimicrobial activity of Essential oils against food borne bacteria and Toxigenic fungi. *Fascicle VI – Food Technol.*, 35(2): 16–26.

Al-Zogibia Onizan, G., Moussa, I., Mohamed, Ashgan, M., Hessain, Jakeen, K. El-Jakeed, Saleh A. Kabli, 2015. Molecular and serotyping characterization of shiga toxogenic *Escherichia coli* associated with food collected from Saudi Arabia. *Saudi J. Biol. Sci.*, 22: 438–442.

Andres, S.C., Giannuzzi, L., Zaritzky, N.E. 2004. The effect of temperature on microbial growth in apple cubes packed in film and preserved by use of orange juice. *Int. J. Food Sci. Technol.*, 39(9): 927–933.

Ankur Titarmare, Pranoti Dabholkar, Suchitra Godbole, 2009. Bacteriological analysis of street vended fresh fruit and vegetable juices in Nagpur City, India. *Internet J. Food Safety*, 11: 1–3.

Barnett, J.A., Payne R.W., Yarrow, D. (Eds), 2000. Yeasts: characteristics and identification, 3rd ed., Cambridge University Press, Cambridge. Pp. 23–81.

Bates, R.P., Morris, J.R., Crandall, P.G. 2001. Principles and practices of small- and medium-scale fruit juice processing. *FAO Agricult. Services Bull.*, 146: 59.

Bauer, A.V., Kirby, W.M.M., Sherris, J.C., Truck, M., *Am. J. Clin. Pathol.*, 45: 493–496.

Bello Olorunjuwon, O., Bello Temitope, K., Fashola Muibat, O., Oluwadun Afolabi, 2014. Microbiological quality of some locally-produced fruit juices in Ogun State, South western Nigeria. *E3 J. Microbiol. Res.*, 2(1): 001–008.

Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds), 2005. Bergey's manual of systematic bacteriology, 2nd edn., Vol. 2, Parts A, B and C. Springer-Verlag, New York, NY.

Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., *et al.* 2004. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovat. Food Sci. Emerg. Technol.*, 5(2): 135–149. doi:10.1016/j.ifset.2003.11.005.

Cheng, H.Y., Yang, H.Y., Chou, C.C. 2002. Influence of acid adaptation on the tolerance of *Escherichia coli* O157:H7 to some subsequent stresses. *J. Food Prot.*, 65(2): 260–265.

Clarke, H., Cowan, S.T. 1952. Biochemical methods for bacteriology. *J. Gen. Microbiol.*, 6: 187–197.

Cunnif, P. (Ed.), 1995. Official methods of analysis, AOAC International, 16th edn. AOAC International, Gaithersburg, MD.

Downes, F.P., Ito, K. (Ed.), 2001.

Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C

Doyle, M.P., Schoeni, J.L. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.*, 53: 2394–2396.

Durgesh, Mahale, P., Ranjana, Khade, G., Varsha, Vaidya, K. 2008. Microbiological analysis of street vended fruit juices from Mumbai City, India. *Internet J. Food Safety*, 10: 31–34.

Harrigan, W.F. 1998. Laboratory methods in food microbiology, 3 rd edn., Academic press Limited, UK. 532 Pp.

Hill, W.E., Datta, A.R., Feng, P., Lampel, K.A., Payne, W.L. 1998. FDA Bacteriological analytical manual, 8th edn. Identification of Foodborne Bacterial Pathogens by Gene Probes. AOAC International, Gaithersburg, MD

Holt, J.G., Krieg, N.R. 1994. In: Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R. (Eds.), Enrichment and isolation methods for general and molecular bacteriology. ASM Press, Washington, D.C. Pp. 205.

Horwitz (Ed.), 2007. Official methods of analysis of AOAC international, 18th ed., online. AOAC International, Gaithersburg, Md.

Hyun Jung Kim, Minseon Koo, A-Ram Jeong, Seung-Youb Baek, Joon-Il Cho, Soon-Ho Lee, In-Gyun Hwang, 2014. Occurrence of pathogenic *Escherichia coli* in commercially available fresh vegetable products in Korea. *J. Korean Soc. Appl. Biol. Chem.*, 57(3): 367−37

Jarett, L., Sonnenwirth, A.C. (Eds), 1980. Gradwohl's and parasitic infections, 7th edn. American Public Health Association, Washington, D.C.

Javed Ali, Arshad Hussain, Ziarurahman, Shafqatullah, Ghulam Mohiuddin Paracha, Muhammad Siddique Afridi, Inayat Ur Rahman, Said Hassan, 2015. Microbiological quality evaluation, preservation and shelf life studies of sugar cane juices sold in Peshawar City, Khyber Pakhtunkhwa-Pakistan. *Am. Eur. J. Agric. Environ. Sci.*, 15(4): 485–489.

Javid Ali, Naseem Ullah, Farhat Ali Khan, Saeed Akhtar, Zia-ur-Rahman and Irshad Ahmad, 2013. Comparative microbiological quality evaluation of un-branded and branded juices of street vended sold in retail out let of Peshawar City. *Am. Eur. J. Agric. Environ. Sci.*, 13(8): 1155–1159.

Jordan Madic, 2010. In: Mariapia Viola Magni (Ed.), Methods for detection of shiga-toxin producing *Escherichia coli* (STEC): Detection of bacteria, viruses, parasites and fungi, Chapt. 2. Springer Science + Business Media B.V.

Joy, Lewis E., Patrina, Thompson, BVVBN Rao, Kalavati C., Rajanna, B. 2006. Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. *Internet J. Food Safety*, 8: 35–38.

Kiranmayi, Ch. Bindu, Krishnaiah, N. 2010. Detection of *Escherichia coli* O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique. *Veter. World*, 3(1): 13–16.

Law, D. 2000. Virulence factors of *Escherichia coli* O157 and other Shigatoxin-producing *E. coli*. *J. Appl. Microbiol.*, 88: 729–745

Lewis, J.E., Thompson, P., Rao, B.V.V.B.N., Kalavati, C., Rajanna, B. 2006. Human bacteria in street vended fruit juices: A case study of Visakhapatnam City, India. *Internet J. Food Safety*, 8: 35–38.

Mabrouk, E.E.M. 2001. Search for *E. coli* O*:157*H7 in Egyptian foods and dairy products, PH.D. Thesis, Al-Azhar

University.

MacFaddin, J.F. 2000. Coagulase test. Biochemical tests for identification of medical bacteria, 3rd edn. Lippincott Williams and Wilkins, Philadelpha. Pp. 105–19.

Manna, S.K. 2006. Detection of *Escherichia coli* O157 in foods of animal origin by culture and multiplex polymerase chain reaction. *J. Food Sci. Technol.,* 43(1): 77–79.

Mosqueda-Melgar, J., Raybaudi-Massilia, R.M., Martín-Belloso, O. 2008. Non-thermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. *Innovat. Food Sci. Emerg. Technol.,* 9: 328–40.

Moushumi Ghosh, Sarvnarinder Kaur, Abhijit Ganguli, 2007. Inactivation of *Escherichia coli* O157:H7 by cinnamon extracts in carrot-kinnow mandarin blends. *Indian J. Microbiol.*, 47: 377–381

Neelam Arun, Shalini Gupta, Singh, D.P. 2012. Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*,

Nicolas, B., Razack, B.A., Yollande, I., Aly, S., Tidiane, O.C.A., Philippe, N.A., De Souza, C., Sababénédjo, T.A. 2007. Street-vended foods improvement: contamination mechanisms and application of food safety objective strategy: Critical review. *Pak. J. Nutr.*, 6(1): 1–10.

Nostoc Muscorum, 2012. *Chlorella Pyrenoidosa* against some pathogenic bacteria and fungi. *IJPSR*, 3(12): 4866–4875.

Oliveira, A.C.G., Seixas, A.S.S., Sousa, C.P., Souza, C.W.O. 2006. Microbiological evaluation of sugarcane juice sold at street stands and juice handling conditions in São Carlos, São Paulo, Brazil. *Cad. Saúde Pública, Rio de Janeiro,* 22(5): 1111–1114.

Rashed, N., Aftab, U, Md., Azizul, Md. H., Saurab, K.M., Mrityunjoy, A., Majibur, R.M. 2013. Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh. *Int. Food Res. J.*, 20(2): 1011–1015.

Reza Robati, Sakineh Gholami, 2013. Estimation of virulence genes of Shiga toxin producing *Escherichia coli* from juice purchase and vegetables in Tehran/Iran. *Eur. J. Exp. Biol.,* 3(2): 457–462.

Rita María Cava-Roda, Amaury Taboada-Rodríguez, María Teresa Valverde-Franco, Fulgencio Marín-Iniesta, 2012. Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *listeria.monocytogenes* and *E. coli* O157:H7 in milk. *Food Bioprocess Technol.,* 5: 2120–2131.

Rosa M. Raybaudi-Massilia, Jonathan Mosqueda-Melgar, Robert Soliva-Fortuny and Olga Martín-Belloso, 2009. Control of pathogenic and spoilage micro organisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Compr. Rev. Food Sci. Food Safety,* 8(3): 157–180.

Shakir Uddin Ahmed, M., Tania Nasreen., Badrunnessa Feroza, Sahana Parveen, 2009. Microbiological quality of local market vended freshly squeezed fruit juices in Dhaka City, Bangladesh. *Bangladesh J. Sci. Ind. Res.,* 44(4): 421–424.

Silva, F. de A.S.E., Azevedo, C.A.V.de., 2009. Principal components analysis in the software assistat-statistical attendance. In: World Congress on Computers in Agriculture, 7, American Society of Agricultural and Biological Engineers, Reno-NV-USA.

Sivakami, R., Sugumar, R., Benila Smily,

J.M., Sumithra, P. 2013. Antibacterial acitvity of *Anabaena circinalis* and synedra ulna against five bacterial pathogens. Vol. I, Issue: VIII, P3.

Suree Nanasombat, Pornpan Wimuttigosol, 2011. Antimicrobial and antioxidant activity of spice essential oils. *Food Sci. Biotechnol.,* 20(1): 45–53.

Syndney M. Finegold, William J. Martin, 1982. Diagnostic microbiology, Chapt. 3. Mosby Co., St. Louis.

Tasnim, F., Hossain, M.A., Nusrath, S., Hossain, M.K., Lopa, D., Haque, K.M.F. 2010. Quality assessment of industrially processed fruit juices available in Dhaka city, Bangladesh. *Malaysian J. Nutr.,* 16(3): 431–438.

Tournas, V.H., Eugenia Katsoudas, 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *Int. J. Food Microbiol.,* 105(1): 11–17.

Tsige, K., Tsegaye, G., Ketema, B. 2008. Microbiological safety of fruit juices served in cafes/resturants, Jimma town, South west Ethiopia. *Ethiopia J. Health Sci.,* 18(3): 98–100.

Victorian Government Department of Human Services, Food Safety Unit Melbourne, Victoria, 2005. Microbiological survey of freshly squeezed juices from retail businesses across Victoria. Available at: http://www.health.vic.gov.au/foodsafety

Vojdani, J.D., Beuchat, L.R., Tauxe, R.V. 2008. Juice-associated outbreaks of human illness in the United States,1995 through 2005. *J Food Prot.,* 71: 356–364.

Wang, R.F., Cao, W.W., Cerniglia, C.E. 1997. A universal protocol for PCR detection of 13 species of foodborne pathogen in foods. *J. Appl. Microbiol.*, 83: 727–736.

Zadik, P.M., Chapman, P.A., Siddons, C.A. 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J. Med. Microbiol.,* 39: 155–158.

Zaika, L.L. 1998. Spices and herbs: their antimicrobial activity and determination. *J. Food Safety,* Pp. 997–118.