In vitro assessment of the antibacterial effect of watery date extracts (Mozafati, Zahedi and Piaroum) on *Staphylococcus aureus* and *Escherichia coli*

Mina Seifzadeh (✉ m_seifzadeh_ld@yahoo.com)

University of Isfahan <https://orcid.org/0000-0001-5277-5834>

Mohammed Rabbani

University of Isfahan

Rasoul Shafiei

University of Isfahan

Research article

Keywords: Bacteria growth curve, CFDA, Date extract, *Escherichia coli* O157: H7, *Staphylococcus aureus*

Posted Date: May 22nd, 2019

DOI: <https://doi.org/10.21203/rs.2.9751/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The present research aimed at investigating the antibacterial effects of date extracts (Mozafati, Zahedi and Piaroum) on the stationary and exponential growth phases of *Staphylococcus aureus* and *Escherichia coli* O157: H7 in vitro. The methods used in this study included flow cytometry and culture. Carboxyl fluorescein diacetate was utilized to determine the effects of extracts and estimate bacterial survivability. Under the impacts of Mozafati, Zahedi and Piaroum extracts, the bacterial elimination percentages recorded in the log phase of *S. aureus* were 30.90, 86.35 and 97.18% respectively. However, the effect of exposure of *E. coli* to date extracts in log phase resulted in the destruction of 8.76, 24.69 and 38.97% of these bacteria respectively. The effect of the extracts on the log phase of *S. aureus* showed a significant increase compared to that of *E. coli* ($p < 0.05$). The extracts had the maximum impacts on the stationary phase of both *S. aureus* and *E. coli* within 3h exposure time. Mozafati and Piaroum extracts yielded the highest bacterial elimination effect within 3h while Zahedi extract had the maximum effect at zero time on stationary phase of *E. coli*. The effect of these extracts on the stationary and logarithmic phases of growth of *S. aureus* and *E. coli* is bacteriostatic. The result of flow cytometry showed an increase as compared with that of culture. The antibacterial effect of Zahedi extract on *S. aureus* and *E. coli* proved to be meaningfully higher than those of other extracts. In order to destroy the examined bacteria in both stationary and logarithmic phase, the use of Zahedi, Piaroum and Mozafati extracts is recommended respectively.

Background

Food borne diseases have been on the rise in recent years, causing major public health problems around the world. The pathogenic bacteria are the main cause of food spoilage that creates serious illnesses in humans. Since food borne pathogens such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* O157: H7 (*E. coli*) bacteria are commonly found in various food stuffs, it is highly important to identify them. The current methods used for identifying food borne pathogens are both difficult and time consuming. Thus a number of quick diagnostic methods of food pathogens have been devised that are required in any investigation related to food borne disease.

In general these quick identification methods are efficient, sensitive and exclusive that saves in time and energy (Woan-Fei Law, 2012). The necessity to develop quick identification methods in early prevention and treatment of food borne illnesses as well as the upsurge of interest in applying quick and automated techniques in food microbiology during recent decades gave rise to the use of fluorogenic substrates along with fluorescence measurement techniques including flow cytometry for quick diagnosis of bacteria (Shapiro, 2003). Given the time required for carrying out the routine tests which can, at times, lead to delayed entry of products into the market, a strong relationship between the results of flow cytometry and shelf life of products might provide a real picture of their quality, allowing their early supply in the market. Meanwhile, the use of flow cytometry enables direct assessment of bacterial growth so as to maintain a controlled microbial biomass during production process. In addition, considering the time needed to obtain the results and confirm the links between flow cytometry and conventional

counting method in the industry, it may provide a suitable predictive means of controlling the quality of product and production process. The advantages of flow cytometry include high precision, no need for enrichment, qualitative and quantitative deliverability, live/dead cell distinction and identification of uncultivable living organisms. In addition, it is possible, via flow cytometry to make distinctions between microbial cells against other bacteria or nonbacterial particles (Endo et al., 2002). So far, flow cytometry has made it feasible to identify, *S. aureus*, *E. coli* (Tanaka et al., 2000) and *Lactococcus lactis* (Bunthof et al., 1999) bacteria present in natural water bodies (Yamaguchi and Nasu, 1997).

Cultivation of date trees goes back to some 4000 Before Christ (BC). Date constitutes one of the most significant species within date palm family which is also the biggest and most ancient tree ever planted by human kind. Date embrace some 200 genera along with 2500 species. There are about 400 species of dates growing in Iran (Ashraf and Hamidi Esfahani, 2011). The country is the second major producer of dates with 14% of total world date production. By-products from dates have, until now, included juice, jam, jelly, and liquid sugar. The fermented products are alcohol, syrup, Tarooneh, wine, organic acids, soft drinks made through fermentation and single cell proteins. Nevertheless, there has, so far, been no extracts made from dates (El Hadrami and Al-Khayri, 2012).

Whilst enjoying common nutritional value and valuable qualities, the various kinds of dates such as Mozafati (soft), Piaroum and Zahedi (semi dry) have also certain biogenic characteristics that distinct them from one another. Although, dates have been consumed as staple food by millions of people throughout centuries, it had not caught the attention of health specialists as a health food and thus, their important antimicrobial properties were remained unnoticed or not fully grasped. Considering that dates are recognized mainly as a phenolic compound and the antibacterial properties of phenolic compounds have already been confirmed in a number of researches (Pereira et al., 2009; Rauha, 2000; Fernandez-Panchon et al., 2008) they can therefore, be regarded as a dietary, functional and natural compound with antibacterial effects. The antimicrobial properties of dates have also been assessed through various means including culture and minimum inhibitory concentrations (MIC) methods (Saleh and Otaibi, 2013). However, up to now there has not been any attempt to confirm the antibacterial effects of date extracts on *S. aureus* and *E. coli* bacteria through flow cytometry technique.

The present research aimed at investigating the bactericidal effects of date extracts (Mozafati, Zahedi and piaroum dates) on the stationary phase and logarithmic (exponential) growth phase of *S. aureus* and *E. coli* O157: H7 in vitro.

Methods

Flow cytometry and culture methods were used to assess the effects of date extracts (Mozafati, Zahedi and piaroum) on the stationary phase and exponential (log) growth phase of *S. aureus* and *E. coli*.

Features of flow cytometry

The samples were examined using Partec PASS, Flow Max software, FL1 channel, Argon laser beam, -20, 100, ultraviolet lamp 100w, diode laser, green fluorescence 488nm from Argon laser with a wavelength of 515-530nm.

Date watery extraction

In order to carry out the watery extraction, the dates (100 g) were first immersed in distilled water (200 ml) for 72 hours in the dark at the refrigeration temperature. Next, the solution was mixed with a mixer and filtered using filter paper No. 1. The suspension was centrifuged using a refrigerated centrifuge at 3000 rpm for 15 minutes. The supernatant was used as extract and was pasteurized at 65°C for 30 minutes. The extracts were refrigerated storage until use (Mehdi pour et al., 2017).

Dye preparation from carboxyl fluorescein diacetate

Carboxyl fluorescein diacetate (CFDA) was used as a bio indicator for live bacteria. To prepare the dye, 0.05g of the dye powder was added to DMSO 1mm.

Preparing positive/negative control for flow cytometry analysis

In order to obtain positive control, 1mm from each bacterial suspension of *S. aureus* and *E. coli* produced in exponential (log) phase was exposed 2h to 1mm of isopropanol. After incubation period, the samples were centrifuged 2800rpm for 15min followed by removal of supernatant. The bacteria pellet was rinsed two times with phosphate buffered saline solution and later dissolved in equal volume of the original phosphate saline solution. Next, 1µl of *Staphylococcus* suspension was mixed with 10µl of dye and 1µl *E. Coli* suspension was added to 20µl of dye. The mix was put to rest in ambient temperature for 15min.

To obtain negative control, 1ml from each suspension of live *S. aureus* and *E. coli* bacteria in the log phase were dyed with 10 and 20µl CFDA respectively. In addition, 1 ml from each solution of live and dead bacteria was mixed with CFDA (Sigma 8166 C). The mix was analyzed using flow cytometry.

The analysis of stationary phase *S. aureus* and *E. coli* bacteria through flow cytometry technique

In order to make synthetic assessment of these bacteria, the stationary phase of growth was used after 6-9h and 6-10h of culture for *S. aureus* and *E. coli* respectively. A 6% date extract was obtained of all the three date species (Mozafati, Zahedi and Piaroum). Then, 2ml suspension of stationary phased bacteria

and 2ml of the examined watery extracts were separately exposed. The solutions were next incubated at 37°C, 120rpm for 48h.

For flow cytometric analysis, 1ml of *S. aureus* and *E. coli* suspension was exposed to the examined date extracts and the bacterial culture pellet was dissolved again in phosphate buffered saline following centrifugal procedures and removal of supernatant. Samplings for flow cytometric analysis were carried out at 0, 3, 15, 24 and 48h.

Flow cytometric analysis of *S. aureus* and *E. coli* in log phase

In order to culture log phase *S. aureus* and *Escherichia coli*, OD of 1.45 and 1.42 were applied. The extract preparation stages, the exposure and flow cytometry were similar to those of in stationary phase.

Counting *S. aureus* and *E. coli* in the stationary and log phases of growth via culture method

Preparing samples for the intended bacterial culture in the stationary and log phase of growth was conducted similar to flow cytometry. The culture of *S. aureus* was done in Mannitol salt agar medium and *E. coli* was cultured in Sorbitol MacConkey agar.

Statistical analysis

The results of microbial tests were analyzed by SPSS Software. Samples were compared to later stages of themselves by Two Way Variance Analysis (culture and flow cytometry). The effect of extracts on the number of *S. aureus* and *E. coli* bacteria in the log phase were compared by t-test. The results obtained from flow cytometry were analyzed by Flowing-Software.

Results

Flow cytometry calibration for *S. aureus* and *E. coli* were presented in figures 1 and 2.

Bacterial counts related to *S. aureus* and *Escherichia coli* affected by exposure to date extracts (Mozafati and Piaroum) showed to be minimal at 3h. However, it increased at other sampling times. Due to being exposed to Zahedi date extract, *S. aureus* and *E. coli* showed the least counts at 3h and zero h sampling times respectively whereas their number tended to rise at other sampling times (Figures 3 – 12).

As figures (3 – 6) show, at zero time, the number of *S. aureus* exposed to the stress induced by date extracts (Mozafati, Piaroum and Zahedi) were 49.62, 55.08 and 72.79%, respectively. The effect of Zahedi extract was meaningfully greater than other extracts on reducing the number of *S. aureus*. Compared to

other date extracts, the effect of Mozafati on these bacteria showed a significant decrease. The number of live *S. aureus* cells exposed to the stress induced by date extracts (Mozafati, piaroum and Zahedi) at 3h was significantly lower in comparison to that of zero time. From this point on, the increased exposure duration was mostly associated with an increase in the number of live bacterial cells. These extracts had the highest impact on stationary phase of *S. aureus* at 3h. Based on the results obtained, the effects of extracts from these date species on stationary phase of the bacteria (*S. aureus*) proved bacteriostatic. The results of the present study also showed that the inhibitory effect of date extracts (Mozafati, Piaroum and Zahedi) on *S. aureus* was more significant than that of *Escherichia coli* in stationary phase. The effect of date extracts (Mozafati, piaroum and Zahedi) on the stationary phase of *S. aureus* is bacteriostatic.

At zero time, the bacterial (*Escherichia coli*) growth inhibition rate induced by exposure to date extracts (Mozafati, piaroum and Zahedi) included 10.79, 64.55 and 72.51%. These figures showed to be 97.03, 92.66 and 6.57% at 3h. The number of dead bacterial cells began to decline along with the increase of exposure time of Mozafati and piaroum extracts from 3 h to 15, 24 and 48 and the number of live cells increased. The number of dead bacterial cells decreased with an increase of exposure duration to Zahedi extract from zero to 3h. However, no significant difference was observed upon exposure time increase to Zahedi extract from 3h to 15h but the figures turned out to be significant as the duration of exposure increased from 15h to 24 and 48h. Considering the results obtained, Zahedi extract seems to be more important in eliminating *E. coli* at stationary phase. The effect of date extracts (Mozafati, piaroum and Zahedi) on the stationary phase of *E. coli* is bacteriostatic (Figures 6 – 9).

Based on figures 10, 11 and 12, under the impact of Mozafati, piaroum and Zahedi extracts, the recorded bacterial elimination percentages of *S. aureus* in the log phase were 30.90, 86.35 and 97.18% respectively. The figures related to log- passed *E. coli* cells however, were 8.76, 24.69 and 38.97% respectively. The effect of the examined extracts on bacterial elimination of *S. aureus* in the log phase was a significant increase in comparison to those of *E. coli*. However, compared with other date extracts, Zahedi showed a meaningfully higher inhibitory effect on the log phase of *S. aureus* and *E. coli* cells whereas the effect of Mozafati extract showed a meaningful decrease on the log phase of *S. aureus* and *E. coli* cells. *E. coli* cells showed less sensitivity to Mozafati, Zahedi and piaroum extracts in the log phase of growth. The effects of all the date extracts on the log phase of *S. aureus* were more significant than those of in the stationary phase. However, the effects of these date extracts on *E. coli* cells in the stationary phase showed a significant increase compared to those of in the log phase. So far, there has been no report on the effects of date extracts on the log- phased *S. aureus* and *E. coli* cells. And the present research is the first attempt in this area.

According to figure 12, the effect of extracts on the number of *S. aureus* and *E. coli* cells in the log phase showed significant decrease in Zahedi, piaroum and Mozafati treatments. The effects of all three extracts on the log phase of *S. aureus* were significantly higher than those on *E. coli*. The number of *S. aureus* in treatments experiencing stress induced by Mozafati, Piaroum and Zahedi extracts were CFU/g 1×10^7 , 18×10^6 , 1×10^3 respectively which proved lower than that of in control. The *E. coli* number exposed

to stress caused by date extracts (Mozafati, Piaroum and Zahedi) was lower than control by CFU/g 1×10^3 , 1×10^2 , 1×10^1 respectively.

In each sampling stage for bacterial counting in culture and flow cytometry in both stationary and log phase of bacterial growth, the number of live *S. aureus* and *E. coli* cells specified in flow cytometry method turned out to be higher than that of culture. Nevertheless, the rising or the downward projection of bacterial count in culture showed to be in conformity with that of flow cytometry technique (Figs 3 - 12).

The results of Flowing-Software are showed in figures 1, 2, 3, 4, 5, 7, 8, 9, 10 and 11.

Discussion

Physiological activity of pathogenic bacteria in food, should be investigated to yield useful information pertinent to food hygiene and public health (Tanaka et al., 2008). As Figs 1 and 2 show, in this study dead cells were separated from live ones because enzyme (esterase) is inactive in dead cells. According to results, in most cases the number of bacterial cells at 3h experiencing deterred growth due to exposure to the examined date extracts reached its maximum level. From that point onward, increasing the duration of exposure time resulted in the higher increase of live bacterial cells. There has, so far been no research concerning the impact of date extracts on the synthetic growth phase of *S. aureus* and *E. coli* cells. Synthetic examinations on the growth of *S. aureus* (Figs 3, 4, 5 and 6) and *E. coli* (6, 7, 8 and 9) exposed to watery extracts of Mozafati, Piaroum and Zahedi suggest their relative inhibiting potentials on the growth of *S. aureus* and *E. coli*. The greater increase of bacterial population in certain cases may have its origin in their adaptation to the environment. Despite their antimicrobial effects on *S. aureus* and *E. coli*, these extracts possess certain compounds and nutritional contents required for the growth of bacteria and a gradual compromise takes place between bacteria and the environment provided by date extracts (Figs. 3, 4, 5, 6, 7, 8 and 9). In the present study, flow cytometry was first time used to assess the effect of date extracts on *S. aureus* and *E. coli*, the results of which were in line with the general findings of bacterial culture method.

Based on diagrams 10, 11 and 12, contrary to Mozafati, the extracts of Piaroum and Zahedi were found to be able to inhibit the growth of log-phased *S. aureus* but *E. coli* cells in the log phase were observed to be less affected by exposure to extracts of Mozafati, Piaroum and Zahedi. Up to the present, there has not been any research on the effect of date extracts on the log phase of *S. aureus* and *E. coli* cells. This study is the first in its kind to probe into the issue, though the role of flavonoids in deterring bacterial growth has already been investigated (Rauha, 2000). In yet another study by Seifzadeh and Rabbani (2019), the role of bacterial flora was assessed to be of greater significance than flavonoids in exerting the antibacterial impacts of date extracts. Thus, in addition to flavonoids the sensitivity of *S. aureus* to Piaroum and Zahedi extracts and the relative sensitivity to Mozafati might be attributed to bacterial flora present in the extracts. The bacterial flora in Zahedi extract belonged to *Bacillus subtilis* strain UD1022, in Piaroum to *Lecunostoc mesenteroides* subsp *mesenteroides* and in Mozafati to *Pediococcus parvalus* strain SC8B. All these were probiotic bacteria. In addition, it was found in this study that the examined date

extracts were unable to fully destroy or rupture the cell walls of *S. aureus* and *E. coli* since after sometime *S. aureus* and *E. coli* cells reoccurred in date extracts. This was confirmed by the results of flow cytometry and culture obtained at varying sampling times.

According to diagrams (3 – 12) bacterial counts revealed in flow cytometry showed higher numbers than those of in cell culture. Such a greater number might stem from lack of growth of injured cells at sub lethal level in culture medium. Having esterase enzymes, the affected cells react to dye and are thus specifiable by flow cytometry due to their preserved physiologic functions.

CFDA is applied for specifying the number of active bacterial cells having esterase such as *Klebsiella pneumonia* (Diaper and Edwards, 1994), *Listeria monocytogenes* (Nexmann Jacobsen et al., 1997) as well as counting bacterial number in pure water aimed at use in pharmaceutical industry (Kawai et al., 1999) and bacterial survivability (Porter et al., 1995). In this study CFDA was used to dye *S. aureus* and *E. coli* cells for flow cytometric analysis. CFDA is used here as substrate for the cell esterase. This enzyme is present in the living organism. It is a non- fluorescent substrate, broken up by inner cell enzymes which result in the production of fluorescence. Considering that the cell is of inner esterase activity with healthy membrane, a pile up of broken substrate is formed within the cell, creating fluorescence (Hoefel et al., 2003).

Renggli et al (2013) determined *E. coli* treated with bactericidal antibiotics by flow cytometry. Malmberg (2013) found that flow cytometry worked equally well as in cell culture in bacterial identification and counting of *E. coli* exposed to antibiotic stress in a way that it could substitute quick counting method used in conventional cell culture. Endo (et.al 2002) gave a report on *E. coli* number and the total bacterial counts on the surface of surimi products through flow cytometry and parallel method of cell culture.

Shariati et al. (2010) evaluated antibacterial effects of date pit extract on multidrug-resistant *S. aureus*. Alawi et al. (2017) found that the date extract had inhibitory effects on *S. aureus* and *E. coli*. The antimicrobial property of date against *S. aureus* and *E. coli* was proven by Baliga et al. in 2011. Idris et al. (2017) demonstrated that the date extract had antibacterial effects on *S. aureus* and *E. coli*. These results are in line with the results of the current study.

Ou (et.al 2017) reported a combined culture trial consisting of live/dead *E. coli* cells through both flow cytometry and parallel traditional method. However, in this study, the number of cells specified in flow cytometry showed a rise compared to that of in culture method. This might be accounted for by different steps in preparing the strain and the bacterial sample. Fröhling and Schlüter (et.al 2015) reported the survivability of negative gram *E. coli* exposed to Peracetic acid and ozone via flow cytometry. With a view to evaluate the inactivating impacts of heat, Peracetic acid and ozone on *E. coli* population. Vorgelegt (et.al 2011) acquired the results through flow cytometry and compared them with those of culture. The results were in conformity with findings of the present study.

Rüger (et. al 2012) investigated the use of flow cytometry in survivability estimates of *S. aureus* in combined culture method which were similar to the findings of the present study. Shrestha (et. al 2012)

could identify Methicillin-Resistant *S. aureus* (MRSA) via flow cytometry. These researchers detected live and dead cells using flow cytometry and reported that the results could be considered as equal to those of culture method or dealt with greater sensitivity.

Conclusion

The emergence of microbial strains resistant to antibiotics is one of problems facing pharmaceutical industry. *S. aureus* and *E. coli* constitute the major cause of food poisoning and other serious illnesses. Considering the noticeable effects of date extracts on these bacteria and their potential uses in pharmaceutical and/or food industries and in order to prevent and cure infections, it is recommended to utilize date extracts either separately or in combination with antibiotics.

List Of Abbreviations

CFDA: Carboxyl fluorescein diacetate

BC: Before Christ

S. aureus: Staphylococcus aureus

E. coli: *Escherichia coli* O157: H7

MIC: Minimum inhibitory concentrations

Declarations

Ethics approval and consent to participate

'Not applicable' for that section

Consent for publication

All authors agree with the release of this article

Availability of data and material

The related results are presented in this article. There are no confidential information for this article. The materials needed to conduct this research are easily available.

Competing interests

I have received my research grants from Dr Rabbani (my supervisor). He has received his grants from University of Isfahan.

Funding

There is no grant for publishing this article.

Authors' contributions

The article was written by the first person. Dr Rabbani and Dr. Shafiei are my supervisors. All authors have the same share in this article.

Acknowledgements

We are sincerely grateful to the experts at Isfahan university and to the Inland Waters Aquaculture Research Center for their unwavering support.

References

- Al-Alawi RA, Al-Mashiqri AH, Al-Nadabi JSM., Al-Shihi BL Baqi Y. Date Palm Tree (*Phoenix dactylifera* L.): Natural Products and Therapeutic Options. *Front Plant Sci.* 2017; doi: 10.3389/fpls.2017.00845. eCollection 2017
- Ashraf Z, Hamidi Esfahani Z. Date and Date Processing: A Review. *J Food Rev Int.* 2011; DOI: 10.1080/87559129.2010.535231
- Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food Res Int.* 2001; <https://doi.org/10.1016/j.foodres.2010.07.004>.
- Bunthof C, van den Braak S, Pieter Breeuwer P, Rombouts FM, Abee T. Fluorescence assessment of *Lactococcus lactis* viability. *Int J Food Microbiol.* 2000; DOI: 10.1016/S0168-1605(00)00170-7
- El Hadrami A, Al-Khayri MJ. Socioeconomic and traditional importance of date palm. *Emir J Food Agric.* 2012; 371-385
- Diaper JP, Edwards C. 1994. The use of fluorogenic esters to detect viable bacteria by flow cytometry. *J Appl Bacteriol.* 1994; doi.org/10.1111/j.1365-2672.1994.tb03067.x
- Endo H, Nakamura J, Ren H, Hayashi T. Flow Cytometry for Rapid Determination of Number of Microbial Cells Grown on Fish. *Food Sci Technol Res.* 2002; 342-346.
- Fernandez-Panchon MS, Villano D, Troncoso AM Garcia-Parrilla MC. Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. *Crit Rev Food Sci Nutr.* 2008; doi: 10.1080/10408390701761845.
- Fröhling A, Schlüter O. Flow cytometric evaluation of physico- chemical impact on Gram-positive and Gram-negative bacteria. *Front Microbiol.* 2015; doi: 10.3389/fmicb.2015.00939. eCollection 2015.

- Hoefel D, Grooby WL, Monis PT, Andrews S, Saint CP. A comparative study of carboxyfluorescein diacetate and carboxyfluorescein diacetate succinimidyl ester as indicators of bacterial activity. J Microbiol Method. 2003; 379 – 388.
- Idris II, Ado A, Adamu H. Evaluation of inhibitory effect of *Phoenix dactylifera* ethanol seeds extract against *Escherichia coli* and *Staphylococcus aureus*. Niger J Chem Res. 2017; 1-7.
- Malmberg C. Evaluation of flow cytometry as replacement for plating in *in vitro* measurements of competitive growth under antibiotic stress. Uppsala: University School of Engineering; 2013.
- Mehdipour F, Shahrokhi N, Esmaeilpour K, Kalantaripour TP, Oloumi H, Basiri M, Asadi-Shekaari M. Aqueous Date Fruit Extract can't Ameliorate β -amyloid induced memory impairments in male rats. J Biol Sci. 2017; doi: 10.1016/j.biopha.2017.02.037.
- Nexmann Jacobsen C, Rasmussen J, Jakobsen M. Viability staining and flow cytometric detection of *Listeria monocytogenes*. J. Microbiol. Methods. 1997; DOI: 10.1016/S0167-7012(96)00960-8.
- Odeh I, Al-Rimawi F, Abbadi J, Obeyat L, Qabbajeh M, Hroub A. Effect of harvesting date and variety of date palm on antioxidant capacity, phenolic and flavonoid content of date palm (*Phoenix Dactylifera*). J Food and Nutr Res. 2014; DOI: 0.12691/jfnr-2-8-11
- Ou F, McGoverin C, Swift S, Vanholsbeeck F. Absolute bacterial cell enumeration using flow cytometry. Department of Physics. Auckland:Univ Auckland; 2017.
- Pereira DM, Valentão P, Pereira J. A, Andrade PB. Phenolics: From Chemistry to Biology. Mol. 2009; doi:10.3390/molecules14062202
- Porter U, Pickup R, Edwards C. Evaluation of flow cytometric methods for the detection and viability assessment of bacteria from soil. Soil Biol Biochem. 1997; doi.org/10.1016/S0038-0717(96)00254-4
- Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 2000; 3-12.
- Renggli S, Keck W, Jenal U, Ritz D. Role of auto fluorescence in flow cytometric analysis of *Escherichia coli* treated with bactericidal antibiotics. J Bacteriol. 2013; DOI: 10.1128/JB.00393-13.
- Saleh FA, Otaibi MM. Antibacterial activity of date palm (*PhoenixDectyliferaL.*) fruit at different ripening sages. J Food Process Technol. 2013; DOI: 10.4172/2157-7110.1000285
- Seifzadeh, M and Rabbani, M. 2019. Identification of probiotic bacteria in Mozafati, Piaroum and Zahedi watery extract. Cell Mol Biol. Accepted.
- Shapiro, H. M. Practical Flow Cytometry, 4th Edition. New Jersey: John Wiley and Sons; 2003.

Shariati, A., Pordeli, H. R., Khademiyan, A., & Kyaie, E. Evaluation of the antibacterial activity of the extracts of Date Palm (*Phoenix dactylifera* L.) fruite and pits on multi-Resistant *Staphylococcus aureus*. J Food Technol Nutr. 2010; 42-47.

Shrestha NK, Wilson DA, Scalera NM, Oppedahl A, Procop GW. Immuno-flow cytometry for the rapid identification of *Staphylococcus aureus* and the detection of methicillin resistance. Eur J Clin Microbiol Infec Dis. 2012; doi: 10.1007/s10096-011-1514-5.

Tanaka Y, Yamaguchi N, Nasu M. Viability of *Escherichia coli* O157:H7 in natural river water determined by the use of flow cytometry. J Appl Microbiol. 2008; 228-236

Vorgelegt V, Diplom-Ingenieurin V, Fröhling A, Berlin A. A flow cytometric approach to monitor the effects of gentle preservation techniques in the postharvest chain. Berlin: Technical University of Berlin; 2011.

Woan-Fei Law J, Nurul-Syakima AbM, Kok-Gan C, Learn-Han L. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol. 2014 doi: 10.3389/fmicb.2014.00770.

Yamaguchi N, Nasu M. Flow cytometric analysis of bacterial respiratory and enzymatic activity in the natural aquatic environment. Journal of Applied Microbiology. 2003; doi.org/10.1046/j.1365-2672.1997.00165.x

Figures

Figure 1

Flow cytometric calibration for *Escherichia coli* O157: H7 bacteria%

Calibration figures:

Colored alive: Yellow

Colored dead: Green

Without alive: Grey

Without dead: Red

Colored alive and
dead: Dark blue

Figure 2

Flow cytometric calibration for *Staphylococcus aureus* bacteria%

Yellow: zero time

Green: 3 hours

Red: 15 hours

Dark blue: 24 hours

Grey: 48 hours

Figure 3

The effect of Mozafati extract on the stationary phased *S. aureus* by Flowing-software%

Yellow: zero time

Green: 3 hours

Red: 15 hours

Dark blue: 24 hours

Grey: 48 hours

Figure 4

The effect of Piaroum extract on the stationary phased *S. aureus* by Flowing-software%

Figure 5

The effect of Zahedi extract on stationary phased *S. aureus* by Flowing-software%

Figure 6

The effect of Mozafati, Zahedi and Piaroum extracts on stationary phase of *Staphylococcus aureus* and *Escherichia coli* via culture method (logCFU/g)

Figure 7

The effect of Mozafati extract on stationary phased *E. coli* by Flowing-software%

Yellow: zero time
 Green: 3 hours
 Red: 15 hours
 Dark blue: 24 hours
 Grey: 48 hours

Figure 8

The effect of Piaroum extract on stationary phased E.coli by Flowing-software%

Yellow: zero time
 Green: 3 hours
 Red: 15 hours
 Dark blue: 24 hours
 Grey: 48 hours

Figure 9

The effect of Zahedi extract on stationary phased E.coli by Flowing-software%

Yellow: Mozafati
 Green: Piaroum
 Red: Zahedi

Figure 10

The effect of Mozafati, Piaroum and Zahedi extracts on the log phased (after 24h) E. coli bacteria by Flowing-software%

Figure 11

The effect of Mozafati, Piaroum and Zahedi extracts on the log phased (after 24h) *S. aureus* bacteria by Flowing-software%

Figure 12

The effect of Mozafati, Zahedi and Piaroum extracts on the log phase of *Staphylococcus aureus* and *Escherichia coli* via culture method (logCFU/g)