

Research Article

Positive effects of different key lime (*Citrus aurantifolia* Linn.) peel extracts on the vase life and quality of lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) cut flowers

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Abstract

Lisianthus flowers are an important cut flower in the international markets. This study was conducted to evaluate effects of peel extract of key lime (*Citrus aurantifolia*) (as extracted by three different solvents including deionized water, ethanol and methanol) on the vase life and quality of cut lisianthus flowers. The experiments were conducted in a completely randomized design with 10 treatments containing 0, 15, 25 and 50 ppm peel extracts of *C. aurantifolia* in three solvents consisted of deionized water, methanol and ethanol with three replicates. The results showed that effect of *C. aurantifolia* peel extracts on the vase life of lisianthus cut flowers significantly varied according to the extraction methods. The water extracted key lime peels significantly increased the vase life (13.66 days) and resulted in the highest fresh weight of the flowers, petal water content. Whereas ethanolic extracts reduced the vase life and water content of the inflorescence. The best quality of flowers during vase life period was obtained from deionized water extracts at 25 and 50 ppm concentrations. Results of our investigation indicated that method of extraction plays an important role in effectiveness of extract. Deionized water was the best one for extraction of active compounds from *C. aurantifolia*. No limitations were founded.

Keywords: Deionized extracts, Ethanol extracts, Methanol extracts, Quality, Vase solution

Introduction

Eustoma grandiflorum (Raf.) Shinn. cut flower is considered as a short vase life flower. Although there are few studies about postharvest physiology of lisianthus, this ornamental flower is reported as a relatively sensitive to the ethylene and this hormone has a great role in the senescence of this cut flower (Ichimura and Korenaga, 1998).

Extract and essential oils are among the plant derived natural compounds that showed to have antimicrobial properties against different pathogens and have been used successfully to control the fungal and bacterial contamination in different horticultural products and increased the postharvest life of different vegetables, fruits and flowers (Bahadorani *et al.*, 2017).

The key lime (*Citrus aurantifolia*), known as Mexican lime, is an important member of Rutaceae family which is widely grown in the tropical and subtropical regions of the world (Costa *et al.*, 2014). The peel extracts of different *Citrus* species are among the plant-based compounds with high biological activities that showed antibacterial effects against a

wide spectrum of bacteria and are well known for their anticancer, immunostimulation, and antigen toxic effects (Diab, 2016). Apigenin, rutin, quercetin, kaempferol and nobiletin were reported as the most abundant flavonoids components of *C. aurantifolia* peel extract conferring high antioxidant activity to this fruit (Loizzo *et al.*, 2012).

Previous report indicated that inclusion of lemon extract successfully prolonged the vase life of cut flowers and strongly inhibited certain pathogens in the vase solution (Prabuseenivasan *et al.*, 2006). According to Mehraj *et al.* (2013) inclusion of sucrose and lemon extracts in the preservative solutions improved the vase life of cut *Chrysanthemum* cv. White Snowball. In addition, previous study has shown that hexane extract of key lime fruit peels successfully inhibited the activity of different strains of *M. tuberculosis* (Sandoval-Montemayor *et al.*, 2012). Citral, 4-hexen-3-one, palmitic acid, linoleic acid and oleic acid were reported as the main constituents of key lime extracts that are active against different strains of *M. tuberculosis* (Sandoval-Montemayor *et al.*, 2012; Diab, 2016).

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According to Loizzo *et al.* (2012) methanol extracts of the peel and leaves of *C. aurantifolia* demonstrated the strongest radical scavenging activity. Results of recent study revealed that deionized water extracted fruit peels of *C. aurantifolia* have high antibacterial activity against different strains of *Helicobacter pylori* and urease activity of these bacteria (Lee *et al.*, 2018). In addition, it is reported that gram-positive bacteria are more sensitive to essential oils of key lime than yeasts and gram-negative ones (Costa *et al.*, 2014).

The fruit peel of key lime constitutes about 30–40% of fruit weigh and is usually regarded as the waste part of the fruit and large quantity of these tissues are produced and wasted annually. Although that extract of key lime tissues possess antimicrobial effects against different microorganisms, however, their effectiveness as postharvest preservative materials were not analyzed. In addition, previous reports indicated that extraction solvents significantly affect the efficiency of plant-derived extracts. Therefore, in the present study, the effect of different extraction solvents and peel extracts of *C. aurantifolia* were investigated on the vase life and quality of lisianthus cut flowers.

Material and methods

Plant materials: The cut inflorescences of lisianthus (*Eustoma grandiflorum*) cv. 'Mriachii Grand White' were obtained from a standard hydroponic greenhouse in Isfahan (32° 39' 8.86" N and 51° 40' 28.63" E), Iran in spring 2019. The plants were grown under standard greenhouse conditions. The flowers were harvested at half-open stage at 7 am and immediately were transferred to the postharvest laboratory of Ardakan University. To avoid air embolism, 5–10 cm of the stalk base were recut under water and flower stalk reached to the height of 40 cm. The prepared cut inflorescence were randomly placed in the glass vases containing 300 ml of the test solutions supplemented with 3% sucrose. Each treatment consisted of three replicates (bottles), and each bottle contained one cut flower stem (one spike). The samples were maintained at the temperature of 22 °C ± 2, relative humidity of 60% ± 10 and 12 μmolm⁻² s⁻¹ light intensity from white florescent lamps with 12 h of daily photoperiod.

Experiment design and preparation of *Citrus aurantifolia* peel extract: The experiments were conducted in a completely randomized design with 10 treatments containing 0, 15, 25 and 50 ppm peel extracts of *C. aurantifolia* in three solvents consisted of deionized water, methanol and ethanol with three replicates. The fresh fruits of *Citrus aurantifolia* were obtained from a commercial orchard. The fruit peel was washed by tap water and sterilized by 2% sodium hypochlorite for 5 min. fruit peels were rinsed three times with distilled water. The peels were dried under shade and then were crushed in an electric blender to a fine powder.

Plant materials (20 g) were prepared by soaking in solvents, separately, in autoclaved deionized water,

methanol and ethanol (100 mL of each one) for 24 h in the room condition and then were shook by the routine shake flask method at 28 °C using a rotary shaker at 110 rpm for 24 h. The plant extract was filtered through two layers of Whatman No. 2 and then was centrifuged (at 5000 g for 10 min, at room temperature). The supernatant of plant extract was vacuumed with rotary evaporator at 25 °C. The final plant extraction yields were: 12 g (deionized water), 8.5 g (methanol) and 7.56 g (ethanol). This solvent free residues were suspended in distilled water at a final concentration of 0, 15, 25 and 50 ppm to ready the plant extract standard batches, which, after filter sterilization (filter 0.22 mm pore size), were kept at 4 °C until use. The extraction process was conducted at the laboratory of Agriculture and Natural Resources Faculty of Ardakan University, Iran. Four concentrations (0, 15, 25 and 50 ppm) of each extract were used in the preservative solution (Gholamnezhad, 2018).

Vase life: The most recently opened flowers were tagged at the beginning of the experiment to allow us to determine the number of buds that opened during the postharvest period. Flowers were examined daily for stem discoloration or pedicle bending, and the number of opened and wilted flowers was noted. The end of inflorescence's vase life was considered when 50% of opened flowers showed wilting symptom (Mutui *et al.*, 2006). The harvesting day was considered as day zero.

Counting bacteria: At the end of vase life period, 1 cm of the lower part of stems were cut. The samples were washed three times with sterile deionized water. They were ground and then dilution was made with a 0.9% normal salt solution. The number of bacteria grown in liquid extracts was calculated using the nutrient agar (NA) culture media as described by Jowkar (2006). Briefly, after 100-fold serial dilutions, samples of lisianthus were plated on nutrient agar and incubated for 48 h at 25 °C and the number of bacteria was calculated by the standard plate counting method to generate the number of Colony Forming Units per ml (CFU/ml).

Relative water content (RWC) and petal water content (PW%): Relative water content (RWC) of the leaves was determined at end of vase life period. For RWC evaluation, one gram of leaves was weighed and was considered as fresh weight (FW). Then, the leaves were placed between two layers of completely wet filter paper inside a petri dish and were sealed with parafilm tape. The petri dish was placed in the dark at room temperature for 24 h. After 24 h, the leaves were weighed and this was considered as turgid weight (TW). The leaves then were dried at 70 °C in an oven for 24 h. Then, the samples were reweighed and recorded as dry weight (DW). The RWC was determined based on Barrs and Weatherley (1962):

$$\text{RWC}\% = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

To estimate petal water content (PW%), 1g of the petal from each sample was taken and measured as FW, then were dried at 70 °C for 24 h and their weight were

recorded as DW. PW% was then determined with the following formula (Kalate Jari *et al.*, 2008):

$$\text{PW\%} = \frac{\text{FW}-\text{DW}}{\text{DW}} \times 100$$

Electrolyte leakage: At end of vase life period, electrolyte leakage was computed using the electrical conductivity of leaf discs at 40 °C and 100 °C according to the below formula (Ezhilmathi *et al.*, 2007):

$$\text{Electrolyte leakage} = \left[1 - \left(\frac{C_1}{C_2} \right) \right] \times 100$$

Where C1 and C2 represent the electrical conductivity of leaf discs at 40 °C and 100 °C, respectively.

Enzyme activity assay: Antioxidant enzyme activities of petal harvested samples were measured at the end of vase life. For measuring the activity of peroxidase, 2 mL of reaction mixture and 5 mM guaiacol were mixed and an adequate amount of 25 mM phosphate buffer (pH 7) was added until the final volume of 2 mL was reached. The zero of the spectrophotometer (CECIL 9500, England) was calibrated at the wavelength of 470 nm by the above mixture and then 30 % hydrogen peroxide (H₂O₂) with a volume of 5 µl was added to this mixture. The changes in the light absorptions were quickly measured during 1 minute at a 10-second time interval. Light absorption changes per minute per milligram of protein (ΔOD/min/mg protein) were used to express the enzyme activity amounts (Reuveni *et al.*, 1995).

For measuring catalase activity, the reaction mixture containing 50 mM potassium phosphate buffer (pH = 7) and 15 mM hydrogen peroxide was utilized. The reaction was initiated with the addition of the 100 µl enzyme extract until the final volume of 3 mL. The changes in the absorption were measured at 240 nm during three minutes on the basis of mM of H₂O₂ per mg of protein (ΔOD/min/mg protein) (Dazy *et al.*, 2008).

The concentration of soluble protein was assayed as described by Bradford (1976) while BSA (bovine serum albumin) was used as the standard protein.

Statistical analysis: Analysis of variance (ANOVA) was performed to compare the fruit peel extract-treated and control flowers as well as to determine whether there were any statistically significant differences among the extraction solvents as well as the fruit peel extract concentrations. Data analysis was performed using SAS software ver. 9.1 and means were compared using Duncan multiple range test (DMRT) in 1% probability level.

Results

Vase life: The vase life of lisianthus cut flower was significantly ($P \leq 0.01$) influenced by the concentration and extraction methods of *C. aurantifolia* peel extract in the preservation solution (Table 1). *C. aurantifolia* peel extracts significantly prolonged the vase life of lisianthus cut flowers. In addition, the effects of different extraction methods as well as their concentrations were significant on the vase life of the lisianthus cut flower. Among different extraction

solvents that were tested for evaluation of *C. aurantifolia* peel extract, deionized water showed to be the most effective one for improving the vase life of lisianthus cut flowers (Table 2, Figure 1). The longest vase life (13.66 days) was attained by application of 25 and 55 ppm of water extracted *C. aurantifolia* peel extract (figure 1b-c), while the lowest vase life (7.83 days) was recorded in the control plants (figure 1a). In addition, lower concentrations (15 and 25 ppm) of ethanolic as well as higher concentration (50 ppm) of methanolic extracts improved the vase life of lisianthus cut flowers (figure 1. h-j).

Bacterial contamination: Bacterial growth was significantly affected by application of different concentrations and kinds of *C. aurantifolia* peel extract ($P \leq 0.01$) (Table 1). The deionized water *C. aurantifolia* peel-extracts showed to be the most efficient treatment for inhibiting bacteria growth in the preservation solution. The lowest number of bacteria (492) was detected in the 50 ppm concentration of deionized water extracts and afterward in 25 ppm concentration (695) (Table 2). All concentrations of methanolic extracts and 25 ppm of ethanolic extracts also reduced the number of bacteria in the solution (Table 2). However, the lowest vase life (6.33 days) and the highest bacteria contamination were recorded in the 50 ppm ethanolic extract (Table 2).

RWC and PW: The RWC and PW of lisianthus cut flowers were also significantly influenced by peel extracts of *C. aurantifolia* ($P \leq 0.01$ and $P \leq 0.05$, respectively) (Table 1). High concentrations of water-extracted *C. aurantifolia* peel resulted in the highest content of RWC (45.28% and 43.33% for 50 and 25 ppm, respectively) (Table 2). The highest concentration of ethanolic extract (50 ppm), which was resulted in the lowest vase life and the highest bacteria numbers, also resulted in the lowest RWC (13.48%) in the cut flowers. However, 25 ppm concentrations of methanolic and ethanolic extracts resulted in the highest (90.00%) and the lowest (73.33%) PW, respectively.

Electrolyte leakage: Among different extraction methods that were tested in this study, water-extracted *C. aurantifolia* peel was the only treatment that significantly reduced the electrolyte leakage in the petals of lisianthus. The lowest electrolyte leakage (25.43%) was recorded in the samples that have been treated by 50 ppm concentration of water extracted peels.

Enzymatic activity: Catalase and peroxidase enzymatic activities were influenced by different concentrations of *C. aurantifolia* peel extracts ($P \leq 0.01$) (Table 1). The highest enzymatic activity for both enzymes was recorded in the control plants (Table 2). The lowest peroxidase activity was obtained from 25 ppm water extracted *C. aurantifolia* peel, while the lowest activity of catalase was recorded in the 25 ppm of water extracted as well as 50 ppm of water and methanolic extracted fruit peel (Table 2).

Table 1. Analysis of variance (ANOVA) of the effect of different concentrations and kinds of *Citrus aurantifolia* peel extract on various traits in *Eustoma grandiflorum* cut flowers.

Source of Variation	Degree of freedom	Mean of square						
		Vase life	Number of bacteria	RWC	PW	Electrolyte leakage	Peroxidase	Catalase
Treatments	9	21.07**	1070695.95**	419.09**	65.18*	1439.93**	0.0004**	0.0004**
Error	20	1.30	3413.96	1.43	42.50	33.52	0.00000004	0.0000002
CV%		13.02	5.38	4.58	7.88	11.96	2.52	4.62

** significant at 1% probability, * significant at 5% probability.

Table 2. Mean comparison of the effect of different concentrations and types of *Citrus aurantifolia* peel extract on various traits of *Eustoma grandiflorum* cut flowers using Duncan multiple range test (DMRT).

Treatments	Extraction solvent	Concentration (ppm)	Vase life (day)	Number of bacteria (Log ₁₀ CFU ml ⁻¹)	RWC (%)	Electrolyte leakage (%)	Peroxidase (ΔOD/min/mg protein)	Catalase (ΔOD/min/mg protein)	PW (%)
Control		0	7.83 ^{bc}	1108.33 ^c	14.76 ^{ef}	33.05 ^{bc}	0.41 ^a	0.04 ^a	80.00 ^{ab}
Peel extract of <i>Citrus aurantifolia</i>	Deionized water	15	7.00 ^{bc}	953.33 ^d	15.98 ^e	53.58 ^b	0.002 ^f	0.003 ^g	86.66 ^a
		25	13.66 ^a	695.00 ^e	43.33 ^a	32.46 ^{cd}	0.001 ^h	0.001 ^h	83.33 ^{ab}
		50	13.66 ^a	492.00 ^f	45.28 ^a	25.43 ^d	0.002 ^g	0.001 ^h	80.00 ^{ab}
	Ethanollic	15	8.00 ^{bc}	1440.00 ^b	16.26 ^e	53.42 ^b	0.004 ^d	0.0034 ^{fg}	83.33 ^{ab}
		25	8.66 ^{bc}	856.67 ^d	30.92 ^c	42.84 ^{cd}	0.004 ^e	0.004 ^{ef}	73.33 ^b
		50	6.33 ^c	2633.33 ^a	13.48 ^f	87.02 ^a	0.007 ^c	0.005 ^e	80.00 ^{ab}
Methanolic	15	7.33 ^{bc}	920.00 ^d	34.48 ^b	37.04 ^c	0.002 ^g	0.021 ^b	86.66 ^a	
	25	7.33 ^{bc}	886.67 ^d	23.59 ^d	85.51 ^a	0.008 ^b	0.016 ^c	90.00 ^a	
	50	8.00 ^{bc}	856.00 ^d	23.72 ^d	33.50 ^{cd}	0.007 ^c	0.010 ^d	83.33 ^{ab}	

Values with the same letters in each column have no significant differences at 1% probability.

Discussion

Application of natural decay-preventing substances, which are more environmentally friendly than the chemical agents, are getting more acceptances in recent years to prolong the postharvest life of cut flowers (Bautista-Banos *et al.*, 2006). According to our results, extracts of *C. aurantifolia* significantly improved the vase life of lisianthus cut flowers. Positive effects of different herbal extracts on the quality and vase life were reported in different cut flowers including gerbera (Bahadorani *et al.*, 2017), gypsophila (Khenizy *et al.*, 2014) and chrysanthemum (Hashemabadi *et al.*, 2016). This positive effect may be arising from the active compounds of herbal extracts, which will confer the antimicrobial properties to the vase solution. It is well documented that accumulation of microorganisms as well as their decay products in the vascular tissues are among the common causes of vessel blockage in the cut flowers (Williamson *et al.*, 2002), which can reduce the water uptake and its transport, and consequently reduces the fresh weight and the vase life of cut flowers. Antimicrobial compounds can improve water conductance in the plants by reducing bacterial growth and preventing xylem vessels occlusions. Lisianthus cut flowers are highly sensitive to the bacteria contamination in the vase solution (De La Riva *et al.*, 2009). In addition, accumulation of bacteria slime in the vase solution can indirectly induce various stresses including water deficiency as well as ethylene production in the cut flowers. Lisianthus is considered

as a highly sensitive plant species to ethylene production (Ichimura and Korenaga, 1998). Therefore, reduction in the bacteria contamination can greatly reduce the likelihood of occurrence of different stresses and improve the vase life of lisianthus cut flowers. The antimicrobial effects of *C. aurantifolia* peel extract has been reported in previous studies and these effects were contributed to its high contents of antioxidant and phenolic compounds such as apigenin, rutin, quercetin, kaempferol and nobiletin (Loizzo *et al.*, 2012; Sandoval-Montemayor *et al.*, 2012; Lee *et al.*, 2018). In this experiment, deionized water extract showed to be the most effective one for improving the vase life of cut lisianthus and was the most efficient treatment for inhibiting bacteria growth, which may be due to the greater effect of water in extracting the active ingredients of key lime peel. However, the lowest vase life and the highest bacteria contamination were recorded in the 50 ppm ethanollic extract. The highest concentration of ethanollic extract showed negative effects. So, the lower concentration of it is recommended.

RWC and PW also significantly affected by addition of key lime extracts in the vase solution. Petals of cut flowers are among the main ornamental features of flowers and their turgidity contribute to the good looking and marketability of the products and have great importance on the consumer acceptance. Petal turgidity largely depends on the water uptake and its maintenance (Vahdati *et al.*, 2012). Proper water balance is an

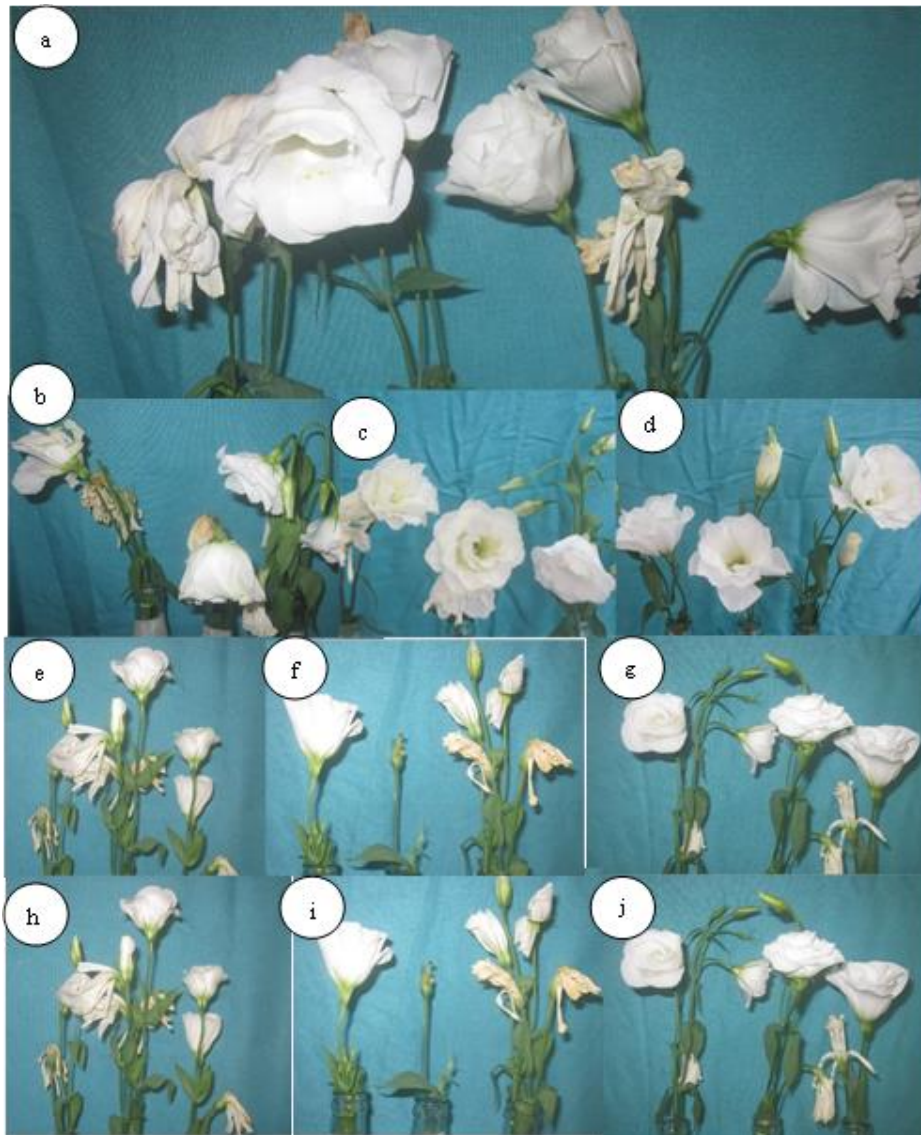


Figure 1. Effect of different vase solutions of peel extract of key lime (*Citrus aurantifolia*) on vase life and quality of cut lisianthus flowers. a. Control b-d. 15, 25 and 50 ppm of deionized *C. aurantifolia* peel extract solution, respectively e-g. 15, 25 and 50 ppm of ethanolic extracts of *C. aurantifolia* peel extract solution, respectively h-j. 15, 25 and 50 ppm of methanolic extracts of *C. aurantifolia* peel solution, respectively at end of vase life.

important factor to influence the vase life of ornamental cut flowers (van Doorn, 2012). The water balance of cut flower, which is determined by the amount of the water uptake and the water losses, is easily impressed by the vascular obstruction. It is reported that imperfect water uptake system is able to disarrange the water balance and subsequently result in the precocious wilting of the petals of cut flowers (Lu *et al.*, 2010). According to our results, the highest RWC and PW were recorded the water-extract treated samples. Suppression of microbial growth in the vase solution can postpone the flower wilting and extend the vase life of many cut flowers. Previous study has shown that citric and ascorbic acid had positive effects on the qualitative characteristics and vase life of different cut flowers including lisianthus (Azizi and Onsjnejad, 2015). *C. aurantifolia* peel extract is a rich source of ascorbic acid that may contribute to the antimicrobial activity of peel extract and result in

the higher vessels conductivity and water uptake. Moreover, the high acidic nature of peel extract will impress the pH of the preservative solutions and subsequently microorganism community and water uptake.

Blockage of water conducting tissues, in particular the xylem vessels, which can be resulted by air emboli, microorganisms accumulation as well as physiological plugs, tyloses and gels, is regarded as the most probable causes of RWC reduction (van Doorn, 2012). Moreover, sedimentation of suberin and tannin in the conducting tissues, which can be resulted from increase in the activity of polyphenol oxidase (PPO) enzyme, are able to reduce the water uptake capacity in cut flowers (van Doorn, 2012). Therefore, intense reduction of RWC (that is observed in the high concentration of ethanolic extract treated cut flowers) might be contributed somewhat to the augmentation of antioxidant systems in

the stressed plants. This observation was further supported by increase in the activities of antioxidant enzymes in the treatments with higher bacteria accumulation (Table 2). Catalase and peroxidase are two main antioxidant enzymes whose activities will increase in the plant tissues when they encountered with an unfavorable condition. Increase in the activity of antioxidant enzymes is one of the most efficient systems used by plants against pathogen and water deficit-induced oxidative stress to reduce the detrimental effects of reactive oxygen species (ROS) and prevent senescence and cell death (Faghieh *et al.*, 2017). Decrease in the activities of antioxidant enzymes can be attributed to the preparation of better conditions that were provided by preservation solution for the cut flowers. According to Saeed *et al.* (2016) increase in the number of bacteria and fungi in the vase solution, can result in the water deficit stress and subsequent increase oxidative stress in the cut flowers and accelerate flora senescence. On the other hand, the stressed plants increase their antioxidant activity to cope with the stressful conditions. Our results are in accordance with the previous results indicated that moderate and high concentrations of geranium extracts on the preservative solution of chrysanthemum cut flowers reduced the activity of antioxidant enzymes such as peroxidase and superoxide dismutase (Hashemabadi *et al.*, 2016).

According to our results, adequate water uptake is an important factor for maintaining the favorable water balance and highly contributes to the postharvest life of cut flowers. These observations are in agreement with the reports of other cut flowers including carnation and gerbera (Liu *et al.*, 2009; Perik *et al.*, 2012). Fresh weight losses, which is usually accompanied by reduction in the shelf life and quality of flower, is one of the most important physiological disorders in postharvest life of cut flowers (Saeed *et al.*, 2016). In addition, high fresh weight of cut flowers is a good indicator of proper plant reservoir materials as well as their water content, two factors that highly contribute to the quality and commercial value of cut flowers (Saeed *et al.*, 2016).

Several reports indicated that application of floral preservatives is able to extend the postharvest life of flowers and adjusting their water balance both through decreasing the water loss or increasing the water uptake (Li *et al.*, 2012; Shan and Zhao, 2015). However, according to our data from vase solution weight, the latter is more probable about the water and methanolic extracts of *C. aurantifolia* peel for maintaining the adequate water balance in the lisianthus cut flowers.

Electrolyte leakage was also significantly reduced in the water-extract treated flowers. Electrolyte leakage as an indicator of membrane integrity is among the main factors that affect the water losses in different plant tissues (Faghieh *et al.*, 2019). Increase in the membrane

permeability and subsequent electrolyte leakage have reported during the senescence period of vase life of other cut flowers (Gul and Tahir, 2013). In addition, it is well documented that membrane breakdown components are highly associated with the senescence signal transduction pathways (Gul and Tahir, 2013).

Water extracted *C. aurantifolia* peel showed the highest antimicrobial effects against the predominant bacterial contamination in the preservative solution. It is reported that different extraction solvents significantly affect the antimicrobial properties of *C. limon*. For instance, methanolic and ethanolic extractions of *C. limon* were highly effective against *Salmonella typhimurium* and *Micrococcus aureus*, respectively. Moreover, previous reports indicated that key lime essential oils showed higher antimicrobial activity against gram-positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermidis* than gram negative ones (Costa *et al.*, 2014). Therefore, it seems that water extracts of *C. aurantifolia* peel were the most effective treatment for controlling the microorganisms that were presented in the preservative solution of lisianthus cut flower.

Conclusion

Collectively, results of present work indicated that inclusion of *C. aurantifolia* peel extract in the preservative solution is highly efficient for extending postharvest life of lisianthus cut flowers. In addition, types of solvent used for extraction of *C. aurantifolia* peel play an important role in the quality and effectiveness of the final extract. Based on our observation, positive effects of key lime peel extract on the vase life of lisianthus cut flowers, could be due to the reduction of pH of vase solution and subsequent antimicrobial properties of such tissues and improvement in the water, ion and carbohydrate uptake. However, supplementary investigations are required to elucidate the mechanism involved in the antibacterial effect of *C. aurantifolia* and its positive role on the extending vase life of lisianthus cut flowers, as well as the causal association between these two. From the results of the present study, it can be concluded that 25 and 50 ppm deionized water extract from fruit peel of *C. aurantifolia* can retain the quality of lisianthus cut flower, improve water uptake and metabolic processes in the inflorescence and subsequently prolong the vase life of this flower. Therefore, 25 and 50 ppm of deionized *C. aurantifolia* peel extract solution is suggested to be used commercially in the cut flower preservative solutions for prolonging the vase-life and postharvest quality of lisianthus cut flowers.

Conflict of interest

The authors have no conflict of interest to report.

References

- Azizi, S., & Onsinejad, R. (2015). Effect of citric acid on vase life, solution uptake and chlorophyll content of cut lisianthus (*Eustoma grandiflorum* L.) flowers. *American Journal of Agricultural and Biological Science*, 10(11), 433-435.
- Bahadorani, A., Asadi-Gharneh, H. A., & Etemadi, N. (2017). Effects of post-harvest treatments of eucalyptus and lemon extracts on qualitative characteristics of gerbera cut flowers. *Research on Crops*, 18 (3), 472-476. 10.3923/biotech.2006.137.142.
- Barrs, H. D., & Weaterley, P. E. (1962). A re-examination of the relative turgidity techniques for the estimating water deficit in leaves. *Australian Journal of Biological Sciences*, 15, 413-428.
- Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., Ait-Barka, E., Bosquez-Molina, E., & Wilson, C. L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, 25, 108-118. <https://doi.org/10.1016/j.cropro.2005.03.010>.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
- Cho, M. S., Celikel, F. L., & Reid, M. S. (2001). Sucrose enhances the postharvest quality of cut flowers of *Eustoma grandiflorum* (Raf.) Shinn. *Acta Horticulturae*, 543, 305- 315. 10.17660/ActaHortic.2001.543.37.
- Chutichudet, P., Chutichudet, B., & Boontiang, K. (2011). Effect of 1- MCP on vase life and other postharvest qualities of patumma (*Curcuma alismatifolia*) cv. Chiang Mai Pink. *International Journal of Agricultural Research*, 6, 29-39.
- Costa, R., Bisignano, C., Filocamo, A., Grasso, E., Occhiuto, F., & Spadaro, F. (2014) Antimicrobial activity and chemical composition of *Citrus aurantifolia* (Christm.) Swingle essential oil from Italian organic crops. *Journal of Essential Oil Research*, 26(6), 400-408. <https://doi.org/10.1080/10412905.2014.964428>.
- Da Silva, J. A. T. (2003). The cut flower: Postharvest considerations. *Biological Science*, 3, 406-420.
- Dazy, M., Jung, V., Ferard, J. F., & Masfarau, J. F. (2008). Ecological recovery of vegetation on a coke factory soil: Role of plant antioxidant enzymes and possible implication in site restoration. *Chemosphere*. 74, 57-63. <https://doi.org/10.1016/j.chemosphere.2008.09.014>.
- De La Riva, F., Mazuela, P. C., Alvaro, J. E., & Urrestarazu, M. (2009). Treatment with peracetic acid extends the vase life of lisianthus (*Eustoma grandiflorum*) flowers. *Horticultural Science*, 44, 418-420.
- Diab, K. A. (2016). In vitro studies on phytochemical content, antioxidant, anticancer, immunomodulatory, and antigenotoxic activities of lemon, grapefruit, and mandarin citrus peels. *Asian Pacific Organization for Cancer Prevention*, 17(7), 3559-67.
- Ezhilmathi, K., Singh, V. P., Arora, A., & Sairam, R. K. (2007). Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase of *Gladiolus* cut flowers. *Plant Growth Regulation*, 51, 99-108.
- Faghih, S., Ghojadi, C., & Zarei, A. (2017). Response of strawberry plant cv. 'Camarosa' to salicylic acid and methyl jasmonate application under salt stress condition. *Plant Growth Regulation*, 36(3), 651-659. <https://doi.org/10.1007/s00344-017-9666-x>.
- Faghih, S., Zarei, A., & Ghojadi, C. (2019) Positive effects of plant growth regulators on physiology responses of *Fragaria × ananassa* cv. 'Camarosa' under salt stress. *International Journal of Fruit Science*, 19(1), 104-114. <https://doi.org/10.1080/15538362.2018.1462291>.
- Gholamnezhad, J. (2018). Effect of plant extracts on activity of some defense enzymes of apple fruit in interaction with *Botrytis cinerea*. *International Journal of Agriculture*, 18(1), 115-123. [https://doi.org/10.1016/S2095-3119\(18\)62104-5](https://doi.org/10.1016/S2095-3119(18)62104-5).
- Gul, F., & Tahir, I. (2013). An effective protocol for improving vase life and postharvest performance of cut *Narcissus tazetta* flowers. *Journal of the Saudi Society of Agricultural Sciences*, 12, 39-46. <https://doi.org/10.1016/j.jssas.2012.06.001>.
- Hashemabadi, D., Abedini-Aboksari, H., & Sedaghatoor Kaviani, B. (2016). Geranium (*Pelargonium graveolens*) extract and mechanical treatment improve water relation, enzyme activity and longevity of cut chrysanthemum *Dendranthema grandiflorum* (Ramat.) Kitamura flowers. *Acta Scientiarum Polonorum, Hortorum Cultus*, 15(5), 185-203.
- Ichimura, K., & Korenaga, M. (1998). Improvement of vase life and petal color expression in several cultivars of cut *Eustoma* flowers using sucrose with 8-hydroxyquinoline sulfate. *Bull Natl Res Veg Ornament Plants Tea Japan*, 13, 31-39.
- Jowkar, M. M. (2006). Water relations and microbial proliferation in vase solutions of *Narcissus tazetta* L. cv. 'Shahla-e-Shiraz' as affected by biocide compounds. *Journal of Horticultural Science and Biotechnology*, 81, 656-660. <https://doi.org/10.1080/14620316.2006.11512120>.
- Kalate Jari, S., Khalighi, A., Moradi, F., & Fatahi moghadam, M. R. (2008). The effect of calcium chloride and calcium nitrate on quality and vase life of rose flowers cv. Red Giant. *Olum Fonun Iran*, 9(3), 163-179. (In Farsi).
- Khenizy, S. A. M., Abd El-Moneim, A. M., & Abdel-Fattah, G. H. (2014). Effect of natural extracts on vase life of gypsophila cut flowers. *Scientific Journal of Flowers and Ornamental Plants*, 1(1),1-16.

- Lee, S. M., Park, S. Y., Kim, M. J., Cho, E. A., Jun, C. H., Park, C. H., Kim, H. S., Choi, S. K., & Rew, J. S. (2018). Key lime (*Citrus aurantifolia*) inhibits the growth of triple drug resistant *Helicobacter pylori*. *Gut Pathogens*, *10*(1), 16.
- Li, H., Huang, X., Li, J., Liu, J., Joyce, D., & He, S. (2012). Efficacy of nano-silver in alleviating bacteria-related blockage in cut rose cv: movie Star stems. *Postharvest Biology & Technology*, *74*, 36–41. <https://doi.org/10.1016/j.postharvbio.2012.06.013>.
- Liu, J., He, S., Zhang, Z., Cao, J., Lv, P., He, S., Cheng, G., & Joyce, D. C. (2009). Nano-silver pulse treatments inhibit stem-end bacteria on cut gerbera cv: ruikou flowers. *Postharvest Biology and Technology*, *54*, 59–62. <https://doi.org/10.1016/j.postharvbio.2009.05.004>.
- Liu, Y., Heying, E., & Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safty*, *11*(6), 530–45. <https://doi.org/10.1111/j.1541-4337.2012.00201.x>.
- Loizzo, M. R., Tundis, R., Bonesi, M., Menichini, F., De Luca, D., Colica, C., & Menichini, F. (2012). Evaluation of *Citrus aurantifolia* peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. *The Journal of the Science of Food and Agriculture*, *92*, 2960–2967. <https://doi.org/10.1002/jsfa.5708>.
- Lu, P., Cao, J., He, S., Liu, J., Li, H., Cheng, G., Ding, Y., & Joyce, D. C. (2010). Nano-silver pulse treatments improve water relations of cut rose cv: Movie Star flowers. *Postharvest Biology and Technology*, *57*, 196–202. <https://doi.org/10.1016/j.postharvbio.2010.04.003>.
- Mehraj, H., Ona, A. F., Taufique, T., Mutahera, S., & Jamal Uddin, A. F. M. (2013). Vase life quality improvement of white snowball using vase life extending solution. *Bangladesh Research Publication Journal*, *8*(3), 191-194.
- Mir Saeed Ghazi, M. A., Naderi, R., & Kalateh-Jari, S. (2013). Effect of nano titanium, nanosilver and some essential oils on the longevity and quality of cut alstroemeria flower. *Plants and Ecology*, *37*, 85-99.
- Mutui, T. M., Emongor, V. E., & Hutchinson, M. J. (2006). The effects of gibberellin 4+ 7 on the vase life and flower quality of Alstroemeria cut flowers. *Plant Growth Regulation*, *48*, 207-214.
- Perik, R. R. J., Raze, D., Harkema, H., Zhong, Y., & van Doorn, W. G. (2012). Bending in cut *Gerbera jamesonii* flowers relates to adverse water relations and lack of stem sclerenchyma development, not to expansion of the stem central cavity or stem elongation. *Postharvest Biology and Technology*, *74*, 11–18. <https://doi.org/10.1016/j.postharvbio.2012.06.009>.
- Prabuseenivasan, S., Jayakumar, M., & Ignacimuthu, S. (2006). In vitro antibacterial activity of some plant essential oils. *BMC Complementary Medicine and Therapies*, *6*, 39. <https://doi.org/10.1186/1472-6882-6-39>.
- Reuveni, M., Agapov, V., & Reuveni, R. (1995). Induced systematic protection to powdery mildew in cucumber by phosphate and potassium fertilizers: Effect of inoculum concentration and post-inoculation treatment. *Canadian Journal of Plant Pathology*, *17*(3), 245-251. <https://doi.org/10.1080/07060669509500686>.
- Saeed, T., Hassan, I., Abbasi, N. A., & Jilani, G. (2016). Antioxidative activities and qualitative changes in gladiolus cut flowers in response to salicylic acid application. *Scientia Horticulturae*, *210*, 236–241. <https://doi.org/10.1016/j.scienta.2016.07.034>.
- Sandoval-Montemayor, N. E., Garcia, A., Elizondo-Trevino, E., Garza-Gonzalez, E., Alvarez, L., & del Rayo Camacho-Corona, M. (2012). Chemical composition of hexane extract of *Citrus aurantifolia* and anti-*Mycobacterium tuberculosis* activity of some of its constituents. *Molecules*, *17*(9), 11173–84.
- Shan, C., & Zhao, X. (2015). Lanthanum delays the senescence of *Lilium longiflorum* cut flowers by improving antioxidant defense system and water retaining capacity. *Scientia Horticulturae*, *197*, 516–520. <https://doi.org/10.1016/j.scienta.2015.10.012>.
- Van Doorn, W. G. (2012). Water relations of cut flowers: An update. In: Janick, J. (Ed.), Hort Rev John Wiley & Sons, Inc, Hoboken, NJ, USA, pp. 55–106.
- Williamson, V. G., Faragher, J. D., Parsons, S., & Franz, P. (2002). Inhibiting the Postharvest Wound Response in Wildflowers. Rural Industries Research and Development Corporation (RIRDC).