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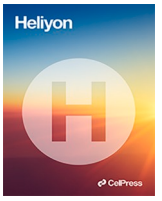

Research article

Peppermint essential oil enhances the vase life of *Dendrobium* orchids

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ABSTRACT

To extend the vase life of cut flowers, there is now a trend of using plant essential oils in place of synthetic chemicals, as they are fully biodegradable, more eco-friendly, and safer. The objective of this study was to examine the possible application and postharvest quality effects of three plant essential oils namely, ginger (*Zingiber officinale* Roscoe), peppermint (*Mentha piperita* L.), and citronella (*Cymbopogon nardus* Rendle), as natural vase solution for cut *Dendrobium* flowers. Peppermint essential oil showed promise as a holding solution for extending the vase life of *Dendrobium* orchids. To confirm vase life extension, emulsions containing peppermint essential oil at concentrations of 50 and 100 μg mL^{-1} combined with 4 % glucose to formulate holding solutions applied to *Dendrobium* orchids. Vase life, some biochemical changes, electrolyte leakage, total microbial count in the holding solution, and physical condition via scanning electron microscopy (SEM) were evaluated over a period of 25 days. The three major compounds in peppermint essential oil were identified as menthol (33.24 %), 1-menthone (18.91 %) and menthofuran (14.85 %). The essential oil was applied in emulsion form as a holding solution. Treatment with 4 % glucose and either 50 or 100 μg mL^{-1} peppermint essential oil prolonged the vase life of *Dendrobium* orchids to up to 28 days. Scanning electron microscopy on Day 7 showed that the xylem vessels of treated orchids remained clear, suggesting reduced microbial plugging at the stalk end. Similarly, on Day 20, a reduced microbial cell count was observed for treated orchids (<1 log CFU mL^{-1}) in comparison with controls (7.20 ± 0.04 log CFU mL^{-1}). Finally, the essential oil improved flower quality by helping preserve petal membrane stability and petal anthocyanin content. Our results suggest the application of peppermint essential oil as a novel alternative to chemicals used in holding solutions for extending the vase life of *Dendrobium* orchids.

1. Introduction

Orchids belong to one of the largest families of flowering plants, Orchidaceae, which contains 880 genera and 26,000 species and is represented in the native floras of almost all parts of the globe. Many orchids are noted for their rare beauty and fine scent. The orchid genus *Dendrobium* contains more than 1000 species and is one of the most commonly encountered genera in the cut flower trade.

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Dendrobium flowers are available in a wide variety of colors, including purple, pink, fuchsia, white, yellow, green, and bicolored, with purples and whites being the most popular commercially [1]. In Thailand, *Dendrobium* orchids are generally considered the top commercial orchids, and the Thai orchid in particular is a product symbol used by many Thai companies; with its high-value, showy, and elegant flowers, the Thai orchid is considered to symbolize a creative economy. The main postharvest quality problems of orchid flowers are wilting, shedding of flower parts, fading, and short vase life. The vase life of cut flowers is especially key to their perceived value and thus is directly related to customer satisfaction. In this way, vase life is also closely associated with repurchasing probability [2]. Therefore, avenues to increase the degree of certainty that a cut flower will last a minimum length of time (that is, the concept of a vase life guarantee) are highly needed not only for sustainability but also for the expansion of the horticultural industry [3].

Various commercial vase solutions are commonly used to extend flower postharvest performance; these solutions usually contain sugars, germicides, acids, and sometimes plant growth regulators [4]. Cut flower vase life can be greatly extended by using exogenous soluble sugars, such as sucrose, glucose, or fructose, in floral preservatives. This can act as a supplement to the flower's food reserves [5]. The main source of energy for cut flowers is the addition of sugars, which prolongs their vase life and improves their water balance while increasing their fresh weight retention [6,7]. Some vase solutions also contain inhibitors of ethylene action or ethylene synthesis, with silver compounds being popular choices. Silver-containing pulsing and holding solutions can indeed considerably extend vase life; however, silver compounds are toxic, and silver thiosulfate in particular requires careful handling and disposal [8,9]. As alternatives to silver compounds, essential oils can also be very effective, even at quite low concentrations; a number of studies have reported that some essential oils considerably prolong vase life [10–13]. Preservative solutions containing essential oils have been found to be very effective in inhibiting the growth of microorganisms and thus delaying or preventing the occlusion of xylem vessels [14]. Cut flower senescence is also associated with pronounced increases in reactive oxygen species (ROS) and activities related to antioxidant systems. Overproduction of ROS causes oxidative damage to cellular proteins, nucleic acids and membrane lipids, leading to membrane deterioration followed by accelerated postharvest senescence [15–18]. Essential oils possess significant scavenging power against free radicals and their ability to mitigate oxidative damage is beneficial in retaining the quality of flowers, delaying the aging process, and prolonging the vase life of cut flowers [14]. So far, numerous chemicals have been used for increasing vase life of orchid cut flower [19–21]. Essential oils extracted from aromatic and medicinal plants are thus attracting significant research interest for their antimicrobial and antioxidant properties. Exogenous plant essential oil at appropriate concentrations has been applied previously in order to delay senescence in cut flower of lisianthus (*Eustoma grandiflorum* Mariachi 'blue') [22], rose (*Rosa hybrida*) [23], and chrysanthemum (*Chrysanthemum morifolium* Ramant 'Arctic Queen White') [24], but in these studies the essential oils were used as a holding vase solution for cut *Dendrobium* orchid.

We hypothesized that plant essential oils would have postharvest quality enhancement of cut *Dendrobium* flowers. Therefore, the objective of this study was to examine the possible application and postharvest quality effects of plant essential oils, as natural vase solution for cut *Dendrobium* flowers. For this purpose, three plant essential oils, namely, ginger (*Zingiber officinale* Roscoe), peppermint (*Mentha piperita* L.), and citronella (*Cymbopogon nardus* Rendle), were screened, and optimal doses were determined for extending the vase life of *Dendrobium* orchids. Subsequently, the effects of glucose in combination with the optimum concentration of the best-performing essential oil, peppermint, were investigated for use in enhancing the postharvest quality of cut *Dendrobium* flowers, evaluated by monitoring water uptake, biochemical activity, microbial count, and physical condition by scanning electron microscopy (SEM).

2. Material and methods

2.1. Plant essential oils and chemical materials

Ginger, peppermint, and citronella essential oils were purchased from the Thai-China Flavors and Fragrances Industry Co., Ltd. (Bangkok, Thailand) and were each provided with a HACCP certificate (hazard analysis critical control point). All chemicals used in this study were of analytical grade (Merck, Darmstadt, Germany), and the water used was distilled.

2.2. Formulation of plant essential oil emulsions

Oil-in-water emulsions were prepared from ginger, peppermint, and citronella essential oils and the anionic surfactant sodium dodecylbenzene sulfonate in ratios of 1:4, 1:1.5, 1:1, 1:0.5, and 1:0.25 (w/w). Each mixture was then titrated dropwise into a conical flask containing a calculated amount of distilled water sufficient to make a 10000 μg mL^{-1} essential oil emulsion. The emulsions were stored at room temperature for 30 days to assess their intrinsic stability and checked periodically for sedimentation by visual observation. Only stable and milky-white emulsions were selected for measurement of the average oil droplet size and ζ-potential. The average oil droplet size which is the difference between the maximum and minimum diameters of dispersed phase droplets, determined by means of light scattering with a NanoPlus Zeta/Nano Particle Analyzer (Particulate Systems, USA) at ~28 °C. All droplet size measurements were taken at least three times, and the average size was reported. The ζ-potential of the oil-in-water emulsion was also measured with the NanoPlus Zeta/Nano Particle Analyzer. The electrophoretic mobility of oil droplets, also known as ζ-potential, can be used to predict the stability of emulsions [25]. The electrophoretic mobility values of colloidal particles were automatically calculated from the ζ-potential using the Smoluchowski equation [26]; [Eq. (1)]:

$$\zeta - \text{ potential (mV)} = 4\pi\eta\mu/\varepsilon \quad (1)$$

where η is the viscosity of the medium (in mPa s), μ is the electrophoretic mobility (in $cm^2s^{-1}V^{-1}$) = v × L/V (v = speed of the particle in cm s^{-1}, L = distance between electrodes in cm, V = voltage) and ε is the dielectric constant of the medium. Each sample was measured at least three times and measurements are reported as ζ -potential in mV.

2.3. Preliminary screening of essential oils for extending the vase life of Dendrobium orchids

Cut inflorescences of *Dendrobium* Sonia 'Ear Sakul' were purchased from a commercial grower in Nakhon Pathom, Thailand. The orchid material was placed in the collection of the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, as KMITL-ORC-Den-001. This specimen was morphologically recognized by comparison with the Botanical Garden Organization's primary herbarium database, related literature [27], and confirmation by an orchidologist (Dr. Tassanai Punjansing, Udontani, Thailand). The orchid research was conducted in accordance with appropriate institutional, national, and international rules and regulations. Cut inflorescences were packed dry and transported 150 km at 25 °C to the laboratory at King Mongkut's Institute of Technology Ladkrabang, Bangkok, where they arrived within 2 h of harvest. Export-grade inflorescences, with 4–6 open flowers and 5–10 flower buds, were selected for freshness and uniformity. Stalks of individual inflorescences were recut in air, leaving 12 cm from the lowest open flower. The original 10000 μg mL^{-1} stocks of ginger, peppermint, and citronella essential oil emulsions were used to prepare working solutions of 25, 50, and 100 μg mL^{-1} with the addition of 4 % glucose. Ten treatments were applied to cut *Dendrobium* orchids: (1) distilled water as a control; (2) 4 % glucose + 25 μg mL^{-1} ginger essential oil; (3) 4 % glucose + 50 μg mL^{-1} ginger essential oil; (4) 4 % glucose + 100 μg mL^{-1} ginger essential oil; (5) 4 % glucose + 25 μg mL^{-1} peppermint essential oil; (6) 4 % glucose + 50 μg mL^{-1} peppermint essential oil; (7) 4 % glucose + 100 μg mL^{-1} peppermint essential oil; (8) 4 % glucose + 25 μg mL^{-1} citronella essential oil; (9) 4 % glucose + 50 μg mL^{-1} citronella essential oil; and (10) 4 % glucose + 100 μg mL^{-1} citronella essential oil. Each treatment included ten cut orchid stalks. The inflorescences were individually held in plastic tubes with 30 mL holding solution in the observation room at 30 ± 2 °C, a relative humidity of 60–70 %, and daily illumination of 12 h by fluorescent lamps with a light intensity of approximately 15 μmol m^{-2} s^{-1}. A completely randomized study design was used. In the preliminary screening, vase life, bud opening, and abscission of florets were assessed and used for the selection of adequate holding solutions.

2.4. Chemical composition of peppermint essential oil

Gas chromatography/mass spectrometry (GC/MS) analysis of peppermint essential oil was performed on an Agilent Technologies 6890 GC equipped with an Agilent Technologies 5973 Inert MS (Agilent Technologies, Palo Alto, CA, USA) and coupled to a Finnigan MAT quadruple ion trap detector (ITD) (Thermo Finnegan LLC, Waltham, MA, USA). Separation was conducted on a capillary column (HP-5 column; 30 m length × 0.25 mm diameter and 0.25 μm film thickness). The injection volume was 0.2 μL at a ratio of 1:50 for the identification of volatile compounds. Helium was used as the carrier gas at a pressure of 32.41 kPa. The column temperature was programmed as follows: initial temperature of 100 °C for 3 min, then increasing at a rate of 3 °C/min to 180 °C, and then further increasing at 20 °C/min to 280 °C, which was maintained isothermally for 3 min. The injection port and the detector were maintained at 260 °C. The identification of the compounds from peppermint essential oil was performed according to their retention indices (RI), calculated by injecting a series of linear hydrocarbon standards of C_8–C_{20} n-alkanes (Sigma-Aldrich, St. Louis, Missouri, USA) with the same conditions as for gas chromatography. Individual constituents were distinguished via comparison of their mass spectra (molecular mass and fragmentation pattern) with those of the internal reference mass spectra library (National Institute of Standards and Technology, NIST, 2014). The relative amount of individual components of the total oil was expressed as a percentage peak area relative to total peak area.

2.5. Main study of cut flower quality, biochemical changes, microbial count, and scanning electron microscopic observation

To confirm vase life extension, emulsions containing peppermint essential oil at concentrations of 50 and 100 μg mL^{-1} were combined with 4 % glucose to formulate holding solutions applied to *Dendrobium* orchids, and vase life, change in fresh weight, cumulative uptake, bud opening, flower abscission and wilting, some biochemical changes, electrolyte leakage, total microbial count in the holding solution, and physical condition via scanning electron microscopy (SEM) were evaluated over a period of 25 days. Following preliminary screening, cut inflorescences of *Dendrobium* Sonia 'Ear Sakul' were selected and prepared as described above. The inflorescences were individually placed in plastic tubes containing 30 mL of holding solution. The following three vase solutions were used: (1) distilled water as a control; (2) 4 % glucose + 50 μg mL^{-1} peppermint essential oil (treatment 4G + 50PEO); and (3) 4 % glucose + 100 μg mL^{-1} peppermint essential oil (treatment 4G + 100PEO).

2.5.1. Vase life evaluation

The vase life of cut flowers was evaluated daily. Cut inflorescences were considered to have reached the end of their vase life when 50 % of open florets showed signs of wilting or abscission.

2.5.2. Change in fresh weight

Fresh weights of cut flowers were measured every three days. The following formula was used to determine relative weight [Eq. (2)]:

$$\text{Relative fresh weight } (\%) = (W_t / W_0) \times 100 \quad (2)$$

where W_t represents the fresh weight on the day of observation and W_0 represents the initial fresh weight.

2.5.3. Cumulative uptake

The cumulative uptake of the holding solution was estimated every three days by measuring the remaining holding solution and determining the total loss of holding solution.

2.5.4. Symptoms of bud opening, flower abscission, and wilting

Bud opening, wilting, and flower abscission of both flower buds and open florets were observed for each inflorescence and expressed as a proportion of the value on Day 0.

2.5.5. Determination of electrolyte leakage

Solute leakage measurements were performed on ten petal discs (diameter 0.7 cm, 70–80 mg fresh weight) of floret buds and open flowers cut with a cork borer. Each set of discs was transferred to plastic Petri dishes containing 10 mL of deionized water and incubated at room temperature (25 °C) for 3 h [28]. Subsequently, the electrical conductivity of the solution was determined using a conductivity meter (Consort, C830, Belgium). Total conductivity was expressed as μS cm^{-1} g^{-1}.

2.5.6. Determination of malondialdehyde content

To quantify malondialdehyde (MDA) [29] from floret buds and open flowers, petal samples (0.5 g) were homogenized with 3 mL of 20 % (w/v) trichloroacetic acid (TCA) and centrifuged at 10000×*g* for 20 min. A subsample of 1 mL of the supernatant was mixed with 4 mL of 0.5 % (w/v) thiobarbaturic acid in 20 % (w/v) TCA. The mixture was heated in a water bath at 95 °C for 30 min and then cooled and centrifuged at 10000×*g* at 4 °C for 15 min. The specific absorbance of the thiobarbaturic acid-MDA complex was measured at 532 nm, and the nonspecific absorbance was measured at 600 nm. MDA contents were expressed as nmol g^{-1} on the basis of fresh weight. The following formula was used for MDA calculation [Eq. (3)]:

$$\text{MDA concentration (mmol mL}^{-1}) = [(A_{532}\text{-}A_{600})/155000] \times 10^6 \quad (3)$$

where 155000 is the molar extinction coefficient for MDA.

2.5.7. Determination of anthocyanin content

The total anthocyanin content in petals from floret buds and open flowers was analyzed by the pH differential method [30]. Fresh petal samples (5 g) were extracted in a solution containing 95 % ethanol and 1 % HCl at a ratio of 98:2 by incubation in darkness at 4 °C for 24 h. KCl buffer (pH 1) and sodium acetate buffer (pH 4) were then added to the petal extract, and after 15 min, the absorption was read at 520 nm and 700 mm. The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents per 100 g of sample, determined using the following equation [Eq. (4)]:

$$\text{Anthocyanin pigment } (\text{mg mL}^{-1}) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (4)$$

where A = (A_{520}-A_{700}) pH 1.0 - (A_{520}-A_{700}) pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor; 10^3 = factor for conversion from g to mg; 1 = pathlength in cm; and ε = molar extinction coefficient (26900 L mol^{-1} cm^{-1}).

2.5.8. Total microbial count in holding solution

To determine the number of microorganisms in each vase, a 1 mL aliquot was taken on Days 0, 5, 10, 15, and 20. These samples were serially diluted in 0.85 % (w/v) sterile normal saline (NaCl) to produce a series of six dilutions. Aliquots (1 mL) of each dilution were spread on plate count agar and incubated at 37 °C for 48 h to allow microorganism growth, and the microbial colonies were enumerated [31,32]. All microbial counts were conducted on triplicate subsamples. Colony counting was carried out by calculating the following formula: number of microbial cells per milliliter = [number of colony × dilution factor]/volume of sample taken, and all counted data were converted to values of $\log_{10}$ colony forming units per milliliter (log CFU mL^{-1}) before statistical analysis.

2.5.9. SEM examination

SEM was conducted to examine blockage of stem xylem on Day 7. Two sections (0.5 cm in length) were taken from the base of the orchid stalk. These stalk ends were dried in a dryer apparatus, and the fragments were positioned on stubs prior to tungsten coating in a sputter coater. After subsequently coating with a thin layer of gold, the specimens were examined under a scanning electron microscope (Hitachi SU8020) using the method described by Kim and Lee [33], and photographs were taken.

2.6. Statistical analyses

The research was established according to a complete randomized design (CRD). Vase life evaluation, cumulative uptake, bud opening, and flower abscission and wilting were measured from ten replicate inflorescences. In the test on electrolyte leakage, MDA content, and anthocyanin content, three replications containing 3 inflorescence each were used in each treatment. The data were

subjected to one-way analysis of variance using the Statistical Analysis System (SAS) statistical software, and differences among treatments were evaluated by Tukey's test ($p \leq 0.05$).

3. Results

3.1. Preparation of essential oil emulsion and effectiveness of floral preservation

Formulating oil-in-water emulsions necessitate the use of surfactant. Plant essential oils were mixed with sodium dodecylbenzene sulfonate at different ratios and evaluated for stability, and the best proportion was then used to prepare emulsions. Essential oil/emulsifier ratios of 1:1.5 for ginger essential oil and 1:0.5 for peppermint and citronella essential oils maintained good stability with no occurrence of aggregation and hence were used in preparing emulsions (Table 1). The average droplet diameter and ζ-potential of the emulsions are presented in Table 1. Overall, the average droplet sizes were in the microscale range, with values between 352.69 and 482.8 nm. All exhibited strong ζ-potential, with ginger, peppermint, and citronella essential oil emulsions having values of −80.28, −98.75 and −90.37 mV, respectively.

The results from the screening of ginger, peppermint and citronella essential oil emulsions for use as a holding solution for *Dendrobium* orchids are presented in Table 2 and Supplementary Table S1. *Dendrobium* orchids placed in 4G + 100PEO showed the greatest longevity, with vase life significantly prolonged by more than six days compared to the control treatment. Ginger essential oil showed moderate potential to extend vase life, while citronella essential oil had no significant effect. Peppermint essential oil resulted in maximum bud opening, followed by citronella essential oil, the control, and ginger oil. Additionally, treatment with peppermint essential oil resulted in decreased abscission of flower buds and open florets (Table 2). Therefore, peppermint essential oil shows promise as a holding solution for extending the vase life of *Dendrobium* orchids.

3.2. Chemical composition of peppermint essential oil

The chemical composition of all the oil samples was mainly determined using the GC/MS technique. Following preliminary screening, the composition of peppermint essential oil was previously identified (Fig. 1), and the constituents are listed in Table 3. Monoterpenes are the predominant components of peppermint essential oil (96.02 %), followed by sesquiterpenes (0.90 %). The major constituents were menthol (33.24 %), 1-menthone (18.91 %), menthofuran (14.85 %), limonene (8.84 %), α-pinene (4.43 %), β-pinene (4.24 %) and menthyl acetate (4.13 %); minor constituents were isopulegone (1.63 %), isopulegol (1.55 %), piperitone (1.22 %), α-terpineol (0.96 %), β-caryophyllene (0.90), 3-octanol (0.81 %), neomenthol (0.64 %) and sabinene (0.56 %).

3.3. Vase life evaluation, fresh weight, and vase solution uptake

The environmental impact of chemical preservatives like silver compounds has led to an increase in the use of natural floral preservation. Cut orchids in solutions containing 4G + 50PEO or 4G + 100PEO lasted 6–7 days longer than the control flowers in distilled water, as evidenced by the data presented in Figs. 2 and 3. Orchids stored in distilled water (control) and distilled water plus emulsifier demonstrated mean vase lives of only 21.3 and 19.8 days, respectively (Fig. 2A). Solutions containing distilled water plus the emulsifier sodium alkylbenzene sulfonate had approximately the same effect as distilled water on vase life, so the emulsifier is unlikely to be involved in postharvest preservation of *Dendrobium* orchids.

Table 1
Average particle size, zeta potential, and evaluation of sediment for essential oil emulsions stored at room temperature for 30 days prepared by different formulas.

Plant	Mass ratio of prepared emulsion concentration (w/w)		Sediment	Droplet size (nm)	Zeta potential (mV)
	Oil	Emulsifier			
Ginger	1	4	+	ND	ND
	1	1.5	+	352.69 ± 0.67	−80.28 ± 0.31
	1	1	+	ND	ND
	1	0.5	–	ND	ND
	1	0.25	+	ND	ND
Peppermint	1	4	+	ND	ND
	1	1.5	+	ND	ND
	1	1	+	ND	ND
	1	0.5	–	383.43 ± 1.44	−98.75 ± 0.32
	1	0.25	+	ND	ND
Citronella	1	4	+	ND	ND
	1	1.5	+	ND	ND
	1	1	+	ND	ND
	1	0.5	–	482.81 ± 0.80	−90.37 ± 0.35
	1	0.25	+	ND	ND

Symbols of + and − represent the appearance and no appearance of sediment or creaming.
ND stands for Not Detected.

Table 2
Effect of vase solutions containing glucose and three plant essential oils on vase life, bud opening, and abscission of florets of cut *Dendrobium* orchids during vase time.

Treatment	Vase life (days)	Bud opening (%)	Abscission of floret buds (%)	Abscission of open florets (%)
Distilled water	19.8 ± 1.0d	61.5 ± 3.1bcd	49.2 ± 3.1b	66.6 ± 3.9a
4 % Glucose + 25 μg mL^{-1} ginger oil	22.4 ± 1.1bcd	52.2 ± 1.3d	64.8 ± 2.8 ab	43.5 ± 4.8BCE
4 % Glucose + 50 μg mL^{-1} ginger oil	25.2 ± 0.5 ab	56.2 ± 1.3cd	67.8 ± 5.7a	34.3 ± 1.3c
4 % Glucose + 100 μg mL^{-1} ginger oil	24.8 ± 1.0abcd	51.4 ± 0.6d	55.9 ± 0.7 ab	40.9 ± 3.6BCE
4 % Glucose + 25 μg mL^{-1} peppermint oil	24.5 ± 1.1abcd	64.5 ± 3.1BCE	31.5 ± 3.5c	38.3 ± 3.3BCE
4 % Glucose + 50 μg mL^{-1} peppermint oil	27.2 ± 0.5 ab	72.4 ± 3.0 ab	22.6 ± 0.7c	32.7 ± 0.7c
4 % Glucose + 100 μg mL^{-1} peppermint oil	28.6 ± 0.4a	83.0 ± 2.1a	29.2 ± 3.5c	36.7 ± 3.3BCE
4 % Glucose + 25 μg mL^{-1} citronella oil	21.2 ± 1.8d	66.5 ± 2.6BCE	51.6 ± 0.3 ab	45.3 ± 1.3BCE
4 % Glucose + 50 μg mL^{-1} citronella oil	22.6 ± 1.0bcd	63.±2.6BCE	49.9 ± 6.5b	49.4 ± 4.0b
4 % Glucose + 100 μg mL^{-1} citronella oil	22.7 ± 0.4abc	65.1 ± 3.6BCE	54.7 ± 4.2 ab	45.6 ± 1.9BCE

Data are means ± SE ($n = 10$). Different lower-case letters indicate significantly different values according to Tukey's studentized range test ($p < 0.05$).

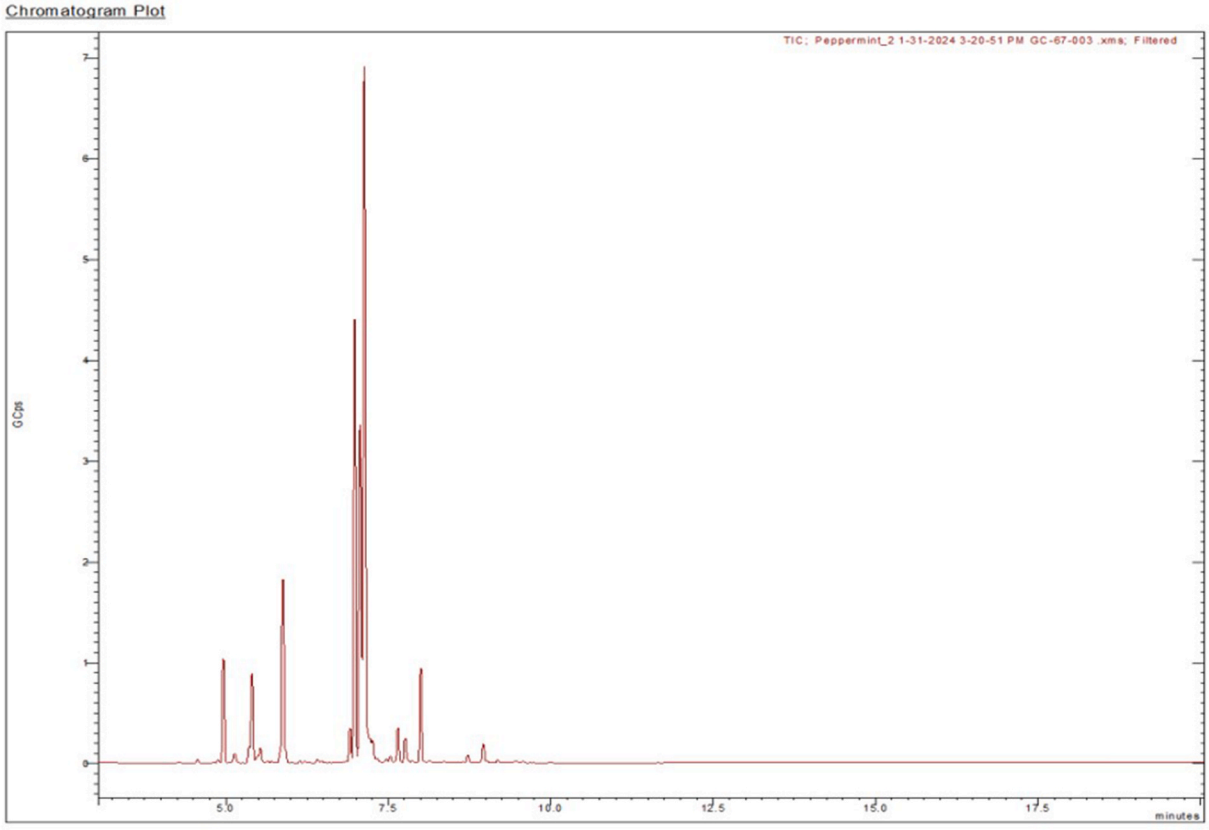

Fig. 1. Gas chromatography/mass spectrometry chromatogram of peppermint essential oil.

Relative inflorescence fresh weight was initially observed to increase (over approximately the first six days) for both peppermint essential oil treatments and the distilled water control. This presumably reflects an initial rehydration by all inflorescences as they took up vase solution to compensate for water lost by transpiration between harvest and the start of the experiment. Thereafter, all inflorescences lost fresh weight steadily (Fig. 2B). On Day 9 of vase time, the relative fresh weight of orchids placed in distilled water was less than the initial weight, while the fresh weight of orchids placed in either peppermint solution did not start declining until Day 12 of vase time.

The cumulative solution uptake by orchid inflorescences placed in water or peppermint essential oil is presented in Fig. 2C. Over the first 12 days, solution uptake was lower in the two essential oil treatments than in the water control, but after Day 12, both essential oil treatments took up water faster than the control. Clearly, peppermint essential oil increased the cumulative uptake of solution over the course of the vase life experiment compared with the control.

Table 3
Constituents of essential oil from peppermint leaf.

Number	Class	Constituent	Formula	RT (min)	RI	% of total oil
1	Monoterpene	α -Pinene	$C_{10}H_{16}$	4.963	936	4.430
2		Sabinene	$C_{10}H_{16}$	5.360	973	0.556
3		β -Pinene	$C_{10}H_{16}$	5.404	977	4.240
4		3-Octanol	$C_8H_{18}O$	5.533	992	0.807
5		Limonene	$C_{10}H_{18}O$	5.878	1028	8.837
6		Isopulegol	$C_{10}H_{18}O$	6.914	1148	1.554
7		l-Menthone	$C_{10}H_{18}O$	6.981	1152	18.912
8		Menthofuran	$C_{10}H_{18}O$	7.067	1164	14.853
9		Menthol	$C_{10}H_{20}O$	7.130	1174	33.243
10		Neomenthol	$C_{10}H_{20}O$	7.227	1185	0.640
11		α -Terpineol	$C_{10}H_{18}O$	7.258	1189	0.961
12		Isopulegone	$C_{10}H_{16}O$	7.651	1240	1.634
13		Piperitone	$C_{10}H_{16}O$	7.766	1255	1.215
14		Menthyl acetate	$C_{12}H_{22}O_2$	7.002	1294	4.134
15	Sesquiterpene	β-Caryophyllene	$C_{15}H_{24}$	8.961	1420	0.904
	Monoterpene					96.016
	Sesquiterpene					0.904
	Total					96.920

RT: Retention time; RI: Retention indices relative to C_8–C_{20} n-alkanes on HP-5MS capillary column; % of total oil: Relative area percentage (peak area relative to the total peak area, %).

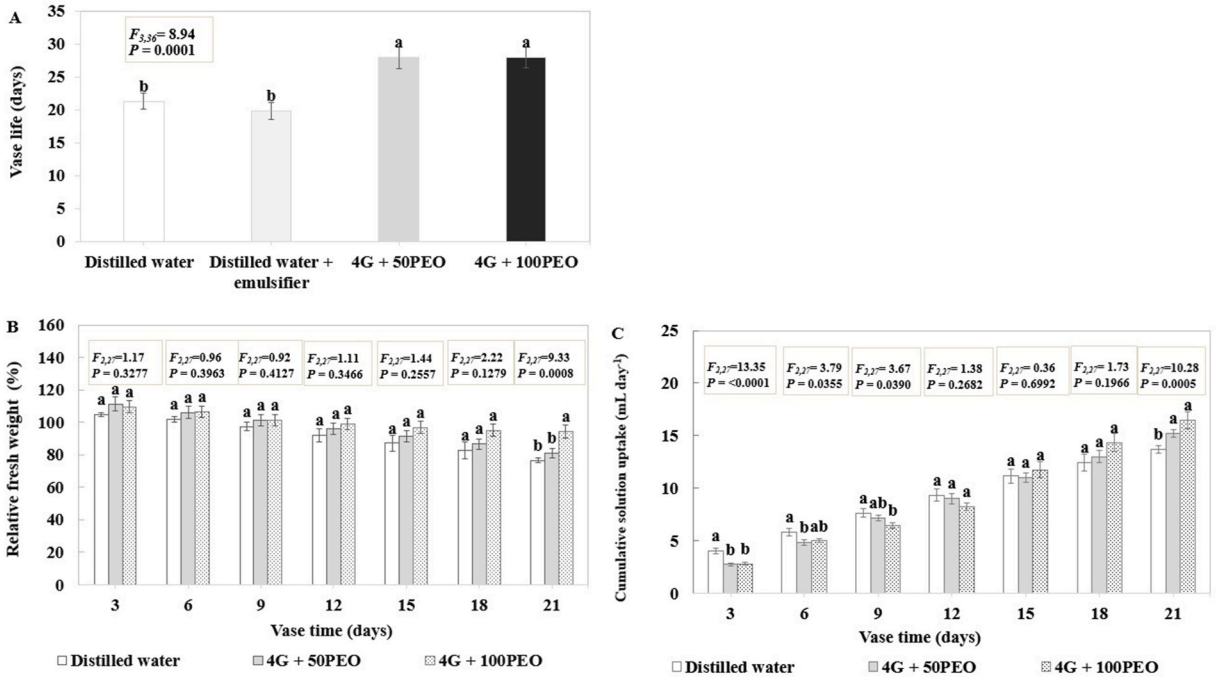

Fig. 2. Effects vase solutions containing glucose and peppermint essential oil on vase life (A), relative fresh weight (B), and cumulative solution uptake (C) of cut *Dendrobium* orchids during vase time. Data are means ± SE (vertical bars), $n = 10$. Different letters above bars indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test. 4G + 50PEO: vase solution containing 4 % glucose +50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose +100 μg mL^{-1} peppermint essential oil; F value: calculated F value of variance analysis; *P*-value >0.05: significant difference; *P*-value <0.05; non-significant.

3.4. Bud opening, flower wilting, and flower abscission

Treatment 4G + 50PEO was more effective in promoting bud opening (53.66 %), followed by treatment 4G + 100PEO (51.37 %) and the control (36.50 %) (Fig. 4A). Regarding wilting of flower buds, none showed signs of wilting within the first three days. In control inflorescences, wilting increased beginning on Day 6 and reached a maximum on Day 21 (19.41 % of all buds). On Day 21, orchids treated with either essential oil solution showed less wilting than the control treatment (Fig. 4B). Regarding abscission of flower buds, none abscised within the first three days. In controls, bud abscission increased rapidly beginning on Day 6 and reached a

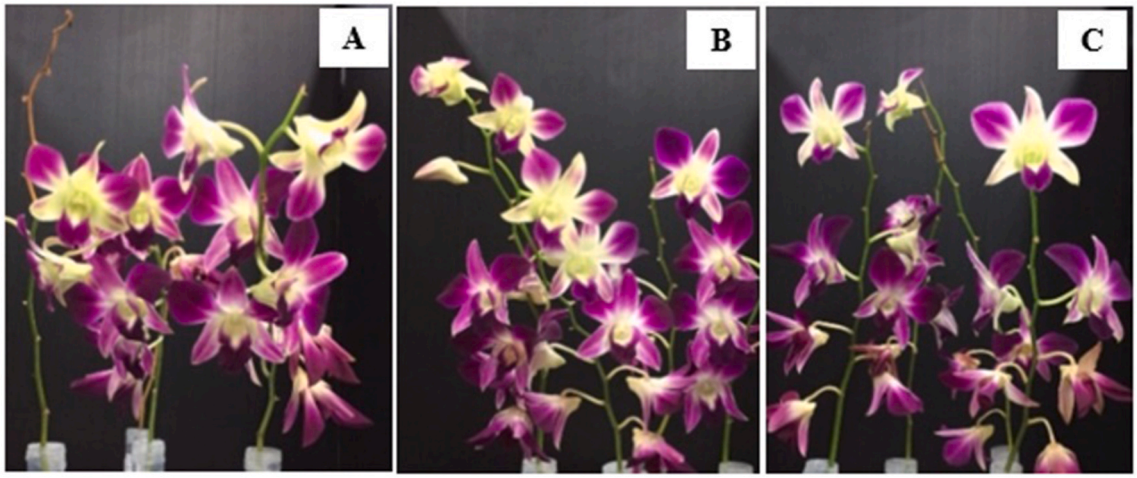

Fig. 3. Photographs represent the vase life of *Dendrobium* orchids kept in distilled water (A), 4 % glucose + 50 μg mL^{-1} peppermint essential oil (B), and 4 % glucose + 100 μg mL^{-1} peppermint essential oil (C) on Day 25.

maximum on Day 21 (51.17 % of buds). From Day 6 to Day 18, both peppermint oil treatments exhibited a relatively positive effect on bud abscission, and abscission on Day 21 was significantly reduced compared with that in the distilled water treatment (Fig. 4D). For open florets, wilting and abscission increased with vase time in all treatments. From Day 3 to Day 18, no differences in wilting and abscission of open florets were observed between control and treated orchids; however, on Day 21, abscission were significantly lower in orchids treated with peppermint essential oil than in controls (Fig. 4C and E).

3.5. Electrical conductivity, MDA content, and anthocyanin content

The elevated electrical conductivity of petal disc leachate indicates electrolyte leakage from the tissues. Here, conductivity increased with vase time for both peppermint essential oil treatments and the control (Fig. 5). Electrical leakage from bud floret petal exhibited a significant decrease in orchid treated with peppermint essential oil. The electrical conductivity in the flower bud petals in samples treated with 4G + 100PEO and 4G + 50PEO decreased by 13.51 and 9.31 %, respectively, on day 6, relative to the control group. Similalrly, electrical leakage from open flower petals exhibited a significant decrease in orchids treated with peppermint essential oil (Fig. 5A). The electrical conductivity in the open flower petals in samples treated with 4G + 100PEO and 4G + 50PEO decreased by 10.50 and 3.43 %, respectively, on day 25, relative to the control group (Fig. 5B).

The level of membrane damage was assessed by MDA content. Assays of MDA content showed that petal MDA accumulation decreased in peppermint essential oil-treated floret buds compared to controls throughout the evaluation period (Fig. 6A). In open flowers, the initial petal MDA value was 49.53 μmol/g, which increased with vase time in both the control and peppermint essential oil treatments (Fig. 6B). Relative to the control, MDA accumulation in open florets was significantly lower with 4G + 100PEO throughout the study period, while the 4G + 50PEO treatment achieved significance by Day 20. On Day 25, the MDA content with 4G + 50PEO was 10 % lower and that with 4G + 100PEO was 18 % lower than that of the control.

Anthocyanins are the common pigments found in most orchids, including *Dendrobium*. In flower buds, the petal anthocyanin content generally decreased during the evaluation period (Fig. 7A). On Days 2 and 4, no difference was observed between the control and treated orchids. On Day 6, the petal anthocyanin content was significantly higher in peppermint-treated flower buds than in controls. Petal of open flowers showed similar patterns of anthocyanin content change (Fig. 7B), with a general decrease as vase time increased. On Day 0, samples showed high anthocyanin content (55.84 mg/100 g (FW)); on Day 25, control flowers (13.08 mg/100 g (FW)) had significantly less anthocyanin than those treated with either essential oil solution (29.18–31.87 mg/100 g (FW)). For both buds and open florets, controls showed a rapid decrease in anthocyanin content throughout the evaluation period.

3.6. Total microbial counts in vase solution

The major causes of vase life reduction in cut flowers are the microbial proliferation in vase solution. After a very short vase time of 1 h (on Day 0), the control vase solution showed abundant growth of microbial cells; this initial value of 4.25 ± 0.05 increased to 7.20 ± 0.04 log CFU mL^{-1} by Day 20. Throughout the evaluation period, the control exhibited significantly more bacterial colonies than either peppermint essential oil treatment. Interestingly, the minimum average microbial counts of the peppermint treatments were <1 log CFU mL^{-1} (Table 4).

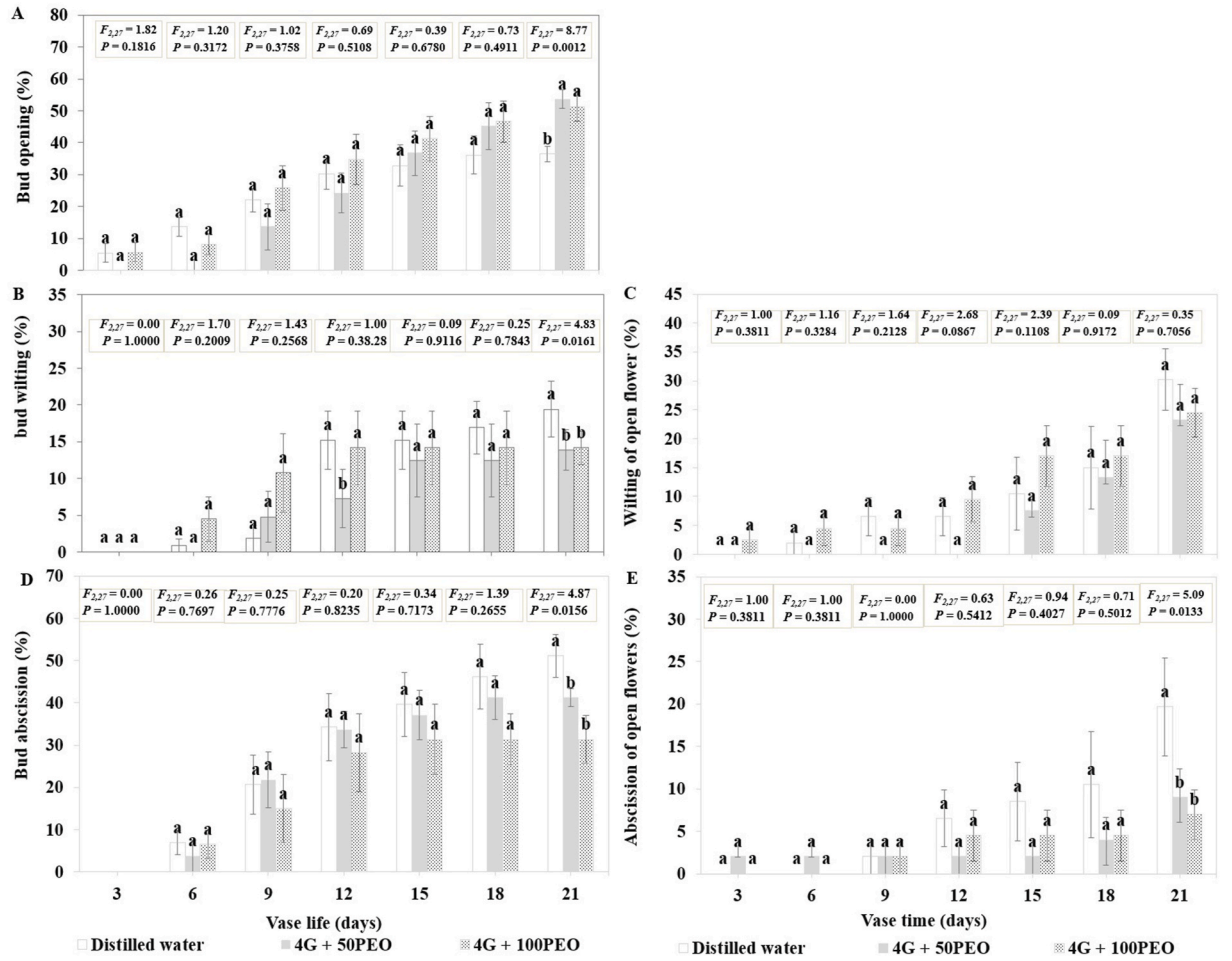

Fig. 4. Effects vase solutions containing glucose and peppermint essential oil on bud opening (A), bud wilting (B), wilting of open florets (C), bud abscission (D), and abscission of open florets (E) of cut *Dendrobium* orchids during vase time. Data are means ± SE (vertical bars), $n = 10$. Different letters above bars indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test. 4G + 50PEO: vase solution containing 4 % glucose +50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose +100 μg mL^{-1} peppermint essential oil; F value: calculated F value of variance analysis; *P*-value >0.05: significant difference; *P*-value <0.05; non-significant.

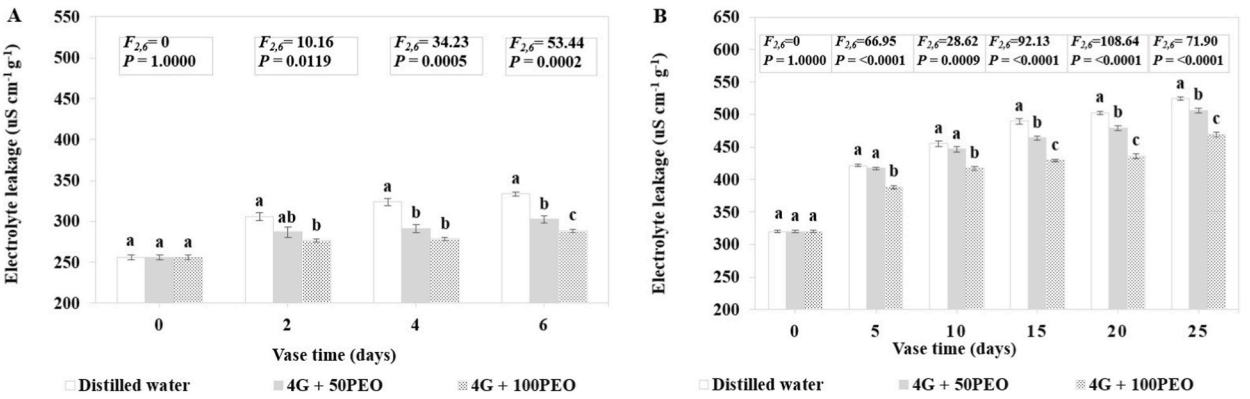

Fig. 5. Effects of vase solutions containing glucose and peppermint essential oil on electrolyte leakage from petals of floret buds (A) and open florets (B) of cut *Dendrobium* orchids during vase time. Data are means ± SE (vertical bars), $n = 3$. Different letters above bars indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test. 4G + 50PEO: vase solution containing 4 % glucose +50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose +100 μg mL^{-1} peppermint essential oil; F value: calculated F value of variance analysis; *P*-value >0.05: significant difference; *P*-value <0.05; non-significant.

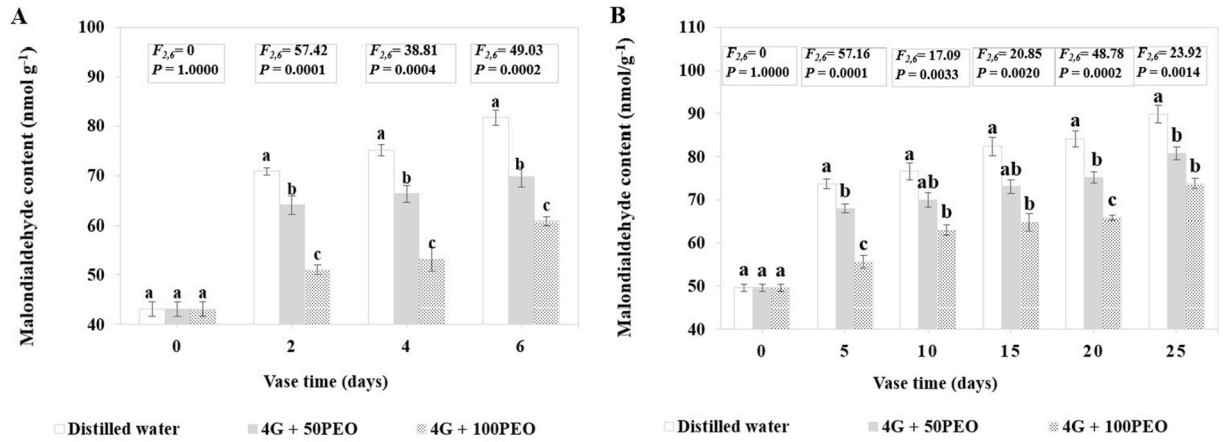

Fig. 6. Effects of vase solutions containing glucose and peppermint essential oil on petal malondialdehyde content in floret buds (A) and open florets (B) of cut *Dendrobium* orchids during vase time. Data are means ± SE (vertical bars), $n = 3$. Different letters above bars indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test. 4G + 50PEO: vase solution containing 4 % glucose +50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose +100 μg mL^{-1} peppermint essential oil; F value: calculated F value of variance analysis; *P*-value >0.05: significant difference; *P*-value <0.05; non-significant.

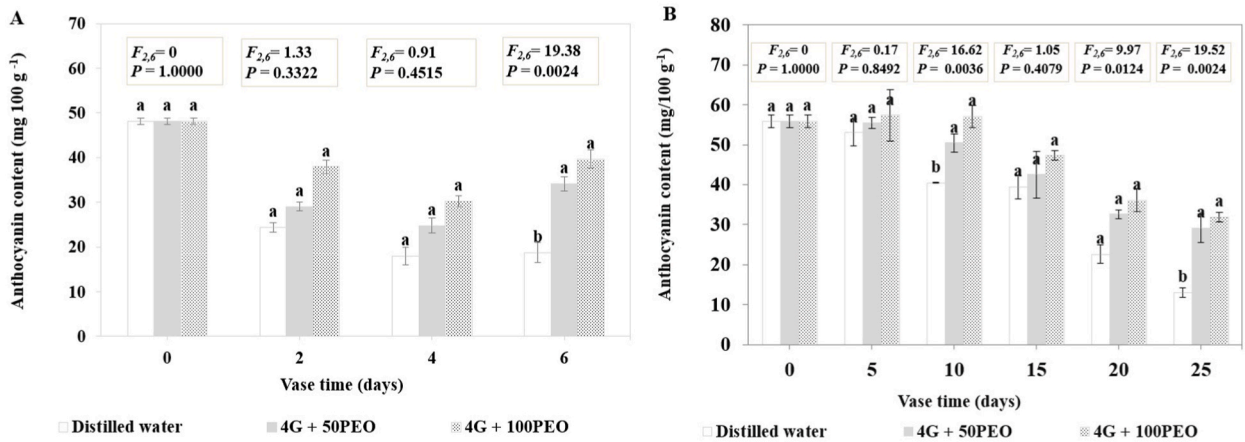

Fig. 7. Effects of vase solutions containing glucose and peppermint essential oil on the petal anthocyanin content in floret buds (A) and open florets (B) of cut *Dendrobium* orchids during vase time. Data are means ± SE (vertical bars), $n = 3$. Different letters above bars indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test. 4G + 50PEO: vase solution containing 4 % glucose +50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose +100 μg mL^{-1} peppermint essential oil; F value: calculated F value of variance analysis; *P*-value >0.05: significant difference; *P*-value <0.05; non-significant.

Table 4
Microbial number in vase solutions of florets of cut Dendrobium orchids held in vase solutions containing glucose and peppermint essential oil during vase time.

Treatment	Microbial count (log CFU mL^{-1})				
	Day 0	Day 5	Day 10	Day 15	Day 20
Distilled water	4.25 ± 0.03	6.74 ± 0.06	6.88 ± 0.01	6.92 ± 0.02	7.20 ± 0.02
4G + 50PEO	<1	<1	<1	<1	<1
4G + 100PEO	<1	<1	<1	<1	<1

CFU: colony forming units per milliliter; 4G + 50PEO: vase solution containing 4 % glucose + 50 μg mL^{-1} peppermint essential oil; 4G + 100PEO: vase solution containing 4 % glucose + 100 μg mL^{-1} peppermint essential oil.

3.7. SEM observation

SEM observation was used to detect xylem occlusion by microorganisms at the base of the cut orchid stem. SEM was performed on the stalk ends of untreated and essential oil-treated *Dendrobium* orchids on Day 7 (Fig. 8). In the control stalk, a majority of vessels were occluded, and a layer of occluding substances surrounded the cut stalk end (Fig. 8A). This occluding material probably comprised bacterial cells and associated plant exudates. Orchids placed in either peppermint treatment showed stem xylem vessels with less blockage and intact stem vasculature (Fig. 8B–C).

4. Discussion

Essential oils have low water solubility, which hinders their incorporation in floral preservative solutions mainly composed of water. Hence, there is a need to formulate an emulsion that can be used as a delivery system for such bioactive compounds and incorporated in preservative solutions. In oil-and-water emulsions, oil droplets are dispersed in an aqueous phase and stabilized with surfactants. Here, oil-in-water emulsions were prepared with three essential oils and the anionic surfactant sodium dodecylbenzene sulfonate; these solutions displayed an average oil droplet size between 352.69 and 482.8 nm and ζ-potential between −80.28 and −98.75 mV (Table 1), indicating emulsion stability. Oil droplet size is dependent on emulsifier type and concentration because the emulsifier adsorbs to the droplet surface to prevent coalescence [34]. An emulsion is usually stable when the ζ-potential is more positive than +30 mV or more negative than −30 mV [35].

The initial screening in this work showed ginger and citronella essential oils to have only a slight effect on the vase life of *Dendrobium* orchids, while peppermint essential oil notably extended vase life. It has been indicated that the efficacy of plant essential oils is dependent on their phytochemical composition and concentration [36] and, more specifically, on antimicrobial and antioxidant activities that reflect the composition and concentration of the oil's bioactive constituents [14,37]. The vase life of cut orchids in solutions containing 4 % glucose in combination with peppermint essential oil was 6–7 days longer than that of control flowers in distilled water (Fig. 2A). Distilled water alone was used as the control because distilled water promotes water uptake in cut flowers and tap water composition changes with location and season [38]. While exogenous application of sucrose supplies a cut flower with a much-needed substrate for respiration and enables flowers harvested at the bud stage to open [39], a straight sugar-water solution

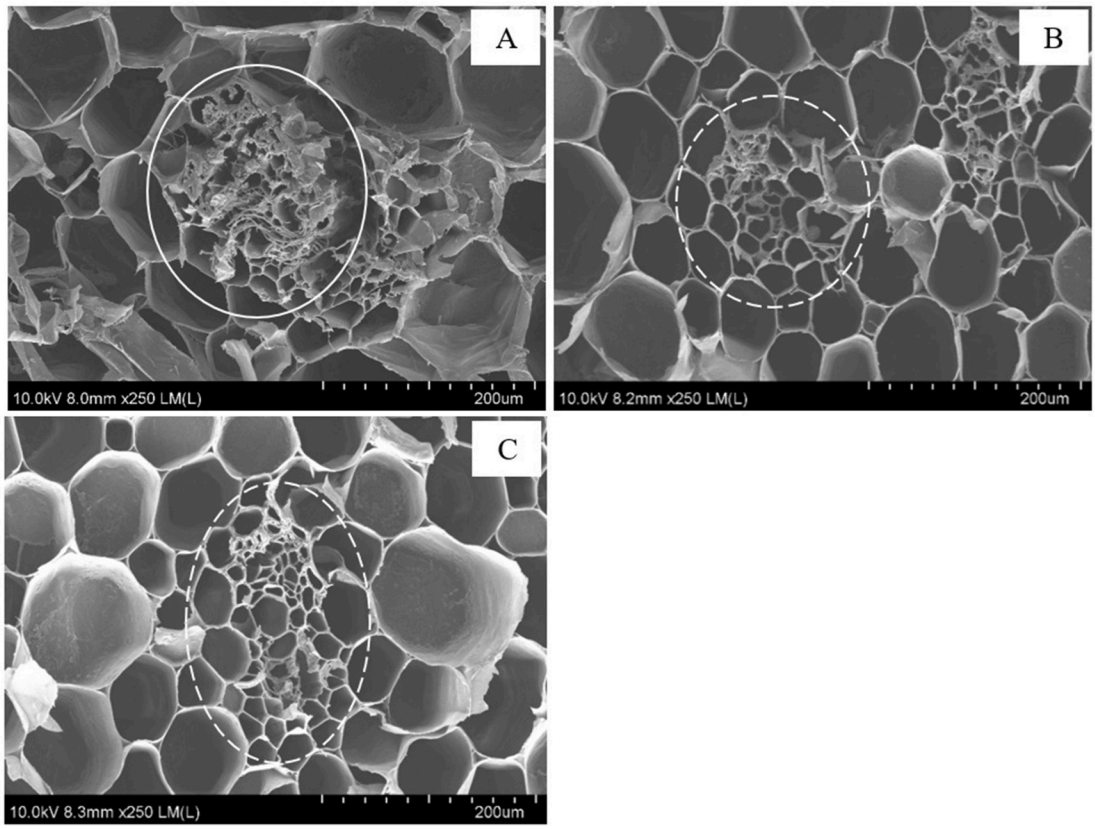

Fig. 8. Scanning electron micrograph showing the cut surfaces of *Dendrobium* orchid flower stem bases after holding for seven days in vase solution containing distilled water (control) (A), 4 % glucose + 50 μg mL^{-1} peppermint essential oil (B), or 4 % glucose + 100 μg mL^{-1} peppermint essential oil (C) during vase time. The solid circle denotes blocked xylem vessels, and the dashed circles denote vessels with less blockage.

promotes bacterial growth and may therefore block the xylem vessels and inhibit uptake of both water and dissolved sugars [40]. Thus, sugar must be combined with an appropriate antimicrobial agent to prevent microbial buildup in the vase solution. In addition to microbial growth, various other factors accelerate the senescence of flowers after harvesting. Cut flower longevity is frequently terminated by petal wilting and flower abscission. A negative water balance develops when water loss via transpiration is not compensated by water absorption from the vase [38]. Finally, water stress may accelerate the increase in ethylene production over time, which significantly promotes abscission in floral buds and induces moderate to high abscission in open flowers [41,42].

In this study, control inflorescences exhibited the highest number of wilted florets (Fig. 4B), which is likely associated with food reserve depletion and the inability to take up water, leading to subsequent color change and loss of cell turgor [43]. Extending the period of positive water balance is a means of preventing early wilting, retaining a high-quality appearance, and increasing vase life [44–46]. Additionally, control buds failed to open (Fig. 4A) because low sugar levels in petals lead to arrest of bud development [47]; conversely, sugar accumulation results in a reduced (i.e., more negative) water potential, which promotes water influx, allows cell expansion, and thus allows the bud to open [48]. Treatment with sugars such as glucose, fructose, and sucrose has been shown to promote flower opening in many cut flowers, including roses [49], spray-type carnations [50] and snapdragons [51]. In addition, as carbohydrates are the primary energy reserves [52], increased carbohydrate levels may promote respiratory activity, thereby improving ATP levels, which in turn facilitate maintenance processes and delay cell death. Thus, bud opening has been related to both petal water and carbohydrate status. From the consumer point of view, adequate flower bud opening is a key quality requirement; that is, incomplete flower bud opening is associated with low perceived value (thus low consumer satisfaction) and is regarded as a primary purchasing barrier [53].

GC–MS analysis of the peppermint essential oil used in this study identified menthol and 1-menthone as the main components (Table 3). This is consistent with prior studies, which have well documented that peppermint essential oil consists predominantly of menthol (36–47 %) and 1-menthone (16–26 %) [54–57]. Variation in the exact percentages may depend on genetic factors, environmental factors, or geographical differences [58]. The positive effect of an essential oil on vase life is due to its biological activities, specifically its effectiveness as an antimicrobial and antioxidant agent. According to the literature [54], Mentha piperita L. essential oil has antimicrobial properties; moreover, menthol and menthone both have the potential to serve as antibacterial and antifungal agents against a wide range of microorganisms. In general, the antimicrobial action of essential oils and their components is based on their hydrophobicity, which enables penetration of the microbial lipid cell wall and membranes, resulting in increased membrane permeability, the leakage of ions and cytoplasmic content, and ultimately cellular breakdown and microbial cell death [59,60]. The antimicrobial activity of peppermint essential oil may thus be correlated with decreased microbial cell counts in the vase solution (Table 4), reduced plugging of the stalk xylem (Fig. 8), and corresponding positive effects on water uptake (Fig. 2C) and maintenance of fresh weight (Fig. 2B). Gradual reduction of water uptake caused by xylem vessel obstruction is a major factor in reduced vase life; thus, increasing vase life involves enhancing the hydraulic conductivity and water relations of cut flowers and thereby avoiding early dehydration of the flower tissues [61,62]. In the present study, control orchid flowers soon lost turgidity and suffered shortened vase life as a result of xylem blockage (Table 3, Fig. 8). In addition to microbial growth, deposition of lignin, suberin, and tannins in the xylem vessel lumens and air embolism also reduce water uptake and vase life [63]. Wound-induced xylem blockage has been noted in some cut flower species (i.e., chrysanthemum, astilbe and bouvardia) [38,64].

Oxidative stress owing to the accumulation of ROS also triggers and aggregates vase life-terminating symptoms. In particular, ROS accumulation is associated with lipid peroxidation and associated membrane damage, electrolyte leakage, and MDA formation [65]. Advancing cut flower capacity to detoxify ROS is thus an alternative means of extending vase life [52], which in general involves slowing the senescence process [61,62]. Over the course of this study, control orchids exhibited decreases in membrane stability (Fig. 5) and anthocyanin content (Fig. 7) and an increase in MDA content (Fig. 6), while in orchids treated with peppermint essential oil, these changes were alleviated, and the MDA content was lower. It can be inferred that treatment of cut orchids with peppermint essential oil reduces ROS activity and increases antioxidant activity. Carbohydrates may act as ROS scavengers, preserving membrane integrity; conversely, carbohydrate starvation elicits ROS formation [46]. Additionally, nonenzymatic antioxidants such as anthocyanin constitute an endogenous defense against ROS in flower tissues, with anthocyanin also contributing to petal color [66]. Many studies on cut flowers have demonstrated that natural antioxidants, including essential oils [67,68], salicylic acid [69], and moringa extract [70], delay senescence and extend vase life by increasing ROS scavenging activity; this also helps the flower retain anthocyanin content. In peppermint essential oil, the most powerful free radical scavenging compounds are menthone and isomenthone [14–16].

5. Conclusion

Our study indicates that 4 % glucose in combination with a peppermint essential oil emulsion at 50 or 100 μg mL^{-1} increases the vase life of *Dendrobium* cut orchids by increasing bud opening and delaying the onset of floret wilting and abscission. In addition, the essential oil improves flower quality by retarding microbial growth in the vase solution, preventing blockage of xylem vessels, and helping retain both membrane stability and high levels of antioxidant content. It is therefore reasonable to propose that essential oil contributes to the regulation of water uptake by preventing an obstruction inside the stem and limiting the number of microorganisms in a vase solution. Furthermore, essential oil may be acting as a ROS scavenger, preserving the membrane integrity and anthocyanin content of the petals for a prolonged time, delaying orchid senescence.

According to the experimental results, combining peppermint essential oil (mostly menthol and menthone) with sucrose can effectively increase the vase life of cut *Dendrobium* orchids. However, more research is needed to compare the efficacy of peppermint essential oil with menthol and menthone, as well as their combination with sucrose, and to explore the possibility of replacing natural active ingredients with oil to introduce the best concentration to increase its longevity. Finally, ethylene is a critical factor in

determining the vase life of climacteric cut flowers, including orchids. Lower ethylene production may have contributed to the vase life enhancement in orchids treated with essential oil emulsions; however, ethylene was not assessed in the current study. The effect of essential oil application on ethylene production in climacteric cut flowers deserves further investigation.

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Data availability statement

Data will be made available on request.

Additional information

Data associated with this study are included in the article/supplementary material referenced in the article. No additional information is available for this paper.

CRediT authorship contribution statement

Montinee Teerarak: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Komkhae Pilasombut:** Writing – review & editing, Methodology. **Chamroon Laosinwattana:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Montinee Teerarak reports financial support was provided by Thailand Science Research and Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31636.

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