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Full Length Research Paper

Effect of *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata* leaf extracts on postharvest performance of cut Mokara red orchid flowers

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The effect and optimum concentration of *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata* single leaf extract (SLE) and double combination leaf extracts (DCLE) on postharvest performance of cut Mokara Red orchid flowers was studied. The 15 mg/L DCLE of *P. guajava* and *A. paniculata* containing floral preservative solutions had the stable pH (initial pH 3.0) compared to the pH of SLE floral preservative solutions, resulting in extended vase life of the cut flowers. Moreover, flowers in the above preservative solutionstreated flowers retained better petal colour than the colour in the other treated flowers. Thus, 15 mg/L DCLE-Pg+Ap was used in subsequent experiments to evaluate the long vase life of cut flowers based on higher rates of preservative solutions uptake. The all-preservative solutions contained two groups of bacteriaand three fungi. The 15 mg/L DCLE-Pg+Ap had lower bacterial populations compared to the 15 mg/L DCLE-Jc+Ap, except in fungal growth. Therefore, DCLE-Pg+Ap had the potential as a natural preservative solutions to extend the 3 days more vase life of orchid flowers compared to the control containing 8-HQC (8-hydroxyquinoline citrate). Future research needs to examine which microbes are responsible for xylem blockage and their effects on the postharvest performance of cut flowers.

Key words: Double combination leaf extracts, floral preservative solutions, microorganism, petal colour, single leaf extract, vase life.

INTRODUCTION

The Mokara Red orchid flower is a tropical, exotic flower with several freckled and broad starfish-shaped florets on the stem. It is commonly used as a cut flower. It is a trigeneric produced from the hybridization of *Arachnis flos-aeris*, *Ascocentrum ampullaceum* and *Vanda peduncularis* (Yam and Thame, 1999) was referred to as Mokara Chark Kuan Orange by C.Y. Mok in Singapore in 1969 as Mokara. However, its vase life is often shortened due to the presence of bacteria and fungi in the floral preservative solutions. These microorganisms and their chemical by-products plug the stem ends and restrict floral preservative solutions uptake, which in turn decreases the longevity of flowers (Dineshbabu et al., 2002). This cause xylem blockage and prevents water uptake which resulting in floret discoloration, as evidenced by fading from deep red to pink, wilting, abscission and bud drop.

Synthetic germicides currently being added intothe floral

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preservative solutions to prevent the growth of microorganisms in the solution. Presently, sodium hypochlorite (NaOCl) and aluminium sulphate [$Al_2(SO_4)_3$] solutions are used as pre-treatments of cut flowers to improve vase life. A rapid pulse treatment with silver nitrate ($AgNO_3$) helps to alleviate postharvest problems in gerbera cultivars, thus, extending flower vase life (Javad et al., 2011). Silver nitrate reduced bacterial contamination in the vase solutions and thus retarded the xylem blockage. The use of sugar + 8-hydroxyquinoline citrate (8-HQC) as a cut preservative solutions to delay flower senescence, enhances post-harvest quality and prolongs the vase life up to 18 days in cut snapdragon flowers (Asrar, 2012). Similarly, Ichimura and Hisamatsu (1999) found that 200 mg/L sucrose plus 200 mg/L 8-hydroxyquinoline sulphate (8-HQS) solutions prolonged vase life by 4.5 days in cut roses (*Rosa hybrida* L. cv. Sonia). However, germicides containing $AgNO_3$ or acetate can pollute the environment as well as cause adverse effects on human health (Damunupola and Joyce, 2006; Knee, 2000).

The extracts of several plant species contain antimicrobial compound that has the potential to be used as a natural germicide in floral preservative solutions of vase cut flowers. *Jatropha curcas* leaf extract has antifungal activity as it contains phorbol esters and antimicrobial compounds (Okoh et al., 2009; Rahman et al., 2014). Similarly, *Psidium guajava* leaf extract showed antimicrobial activity due to its polyphenolic compounds such as guaijaverin, quercetin and avicularin (Suhaila et al., 2009) and for antimicrobial compounds such as squalene, phytol, bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-,[1R-(1Rα,4Z,9S)] and azulene (Rahman et al., 2014). Leaf extract of *Andrographis paniculata* contains two lactones, andrographolide and kalmeghin; and antimicrobial compounds hexadecenoic acid methyl ester, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)-, and 9,12-octadecadienoic acid methyl ester which have antifungal and antibacterial properties (Rahman et al., 2014; Suhaila et al., 2009; Singha et al., 2003).

A combination of plant extracts can produce synergistic effects that manipulate the microbial activity and prolong the vase life of cut flowers. The synergistic effect arises between two or more elements, or substances that produce an effect greater than the sum of their individual effects (Barry, 1976). Evaluation of preservative solutions with double combination of leaf extracts (DCLE) to enhance post-harvest quality and prolong the vase life of cut flowers has not been carried out by any previous researchers. However, use of plant extracts with a preservative or antibiotic has been reported by Ofokansi et al. (2013) where the plant extract of *Phyllanthus muellerianus,* with ciprofloxacin was found to be beneficial against the bacterial pathogen *Staphylococcus aureus* due to potent antibacterial properties.

Antagonism is the opposite of synergism, the effect produced by the contrasting actions of two (or more) chemical groups. It is a condition where the combined effect of two or more compounds is less effective than the individual effects. Antagonism occurs when the antimicrobial activity of one compound is reduced in the presence of a second agent. Pushpa and Chaminda (2011) reported that combinations of *Avicennia marina* plant extracts derived using petroleum ether, chloroform, ethyl acetate, ethanol and water were unable to exhibit synergistic activity against the tested bacterial species, *Staphylococcus* and *Proteus*. All the combinations exhibited antagonistic effects. Thus, combinations of plant extracts can produce synergistic and/or antagonistic effects against microbes in preservative solutions of cut flowers.

The extension of vase life is an important factor of quality for cut flowers. However, vase life reduction and cut flower deterioration occur due to bacterial growth and proliferation in vase solutions (Jowkar, 2006; van Doorn et al., 1991). Numerous bacteria have been known for vascular occlusion of cut flowers, but the mechanism by which bacteria cause the occlusion remained unknown (Ratnayake et al., 2012). Flower vase life is associated with increased concentration of microbes in the preservative solutions. Antimicrobial compounds that have been used in preservative solutions to extend vase life by reducing microbial contamination in cut flowers are: (i) chlorine and bromine compounds, such as NaOCl and (ii) hydroxyquinoline (HQ) compounds, such as 8-HQC (knee, 2000; van Doorn et al., 1990); (iii) silver compounds, such as $AgNO_3$ and (iv) a range of miscellaneous compounds, including $Al_2(SO4)_3$ (Put et al., 1992; Ruting, 1991). Each of these potential biocides (Faragher et al., 2002) may other functions besides antimicrobial activity.

Plant extracts have potential as natural preservatives to control microbes in preservative solutions of cut flowers. Such natural preservative could be used as an alternative to synthetic chemical preservatives to control microorganisms, as well as to extend longevity and maintain quality of cut flowers. Hence, this study was conducted to determine the optimal concentrations of *P. guajava* and *A. paniculata* leaf extracts as effective natural germicides in preservative solutions for longevity of vase life of cut orchid flower.

MATERIALS AND METHODS

Experiment 1. Determination of Optimum Concentration of Single Leaf Extract to Extend Vase Life of Cut Mokara Red Orchid Flower Plant Materials

Leaves of *J. curcas*, *P. guajava* and *A. paniculata* were collected from the University Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor. According to

Rahman et al. (2014) insect- and disease-free leaves of *J. curcas* and *P. guajava* from nodes seven to eight below the youngest leaves were collected at random from 25 plants. While, 17 weeks old *A. paniculata* plants were harvested at 10 cm above the soil surface and the leaves from 50 plants were used for the extraction.

Mokara Red orchid flowers were harvested from a commercial farm in Banting, Selangor, Malaysia (Figure 1). According to Rahman et al. (2014),75% opened florets and satisfactorily rigid and strong stems to hold the florets and buds, was cut at 45-50 cm in length, among 7 to 8 a.m.. The flowers were transported within 1 h after harvest to the Postharvest Laboratory, Department of Crop Science, UPM, for further treatment.

Leaf Extraction

The leaves were extracted according to the method of Rahman et al. (2011) with some modifications. The samples were washed with distilled water and air dried at ambient temperature (25±2 °C) to remove the excess water from the leaf surface. Then the samples were oven-dried (Memmert, ULM 500, Germany) at 45 °C until constant moisture of 14% was obtained. The dried samples were crushed by hand, and then ground separately in a grinding mill (POLYMIX System MFC 13 CZ, the Netherlands). Twenty-gram crushed sample was placed into a 500 mL Erlenmeyer flask. *J. curcas* and *A. paniculata* crushed samples were soaked in 120 mL methanol solvent, while the *P. guajava* sample was soaked in 120 mL ethanol. Then, each flask was sealed with a piece of an aluminium foil to prevent exposure to light and oxygen, followed by wrapping with parafilm to prevent the solutions evaporation. The suspended solutions were allowed to stand for 7 days and then filtered (filter paper 90 mm, Toyo Roshi Kaisha, Ltd., Japan) and evaporated under vacuum using a rotary evaporator (Model CA-1310 Eyela, Tokyo Rikakikal Co., Ltd., Japan) to remove the solvent.

Treatment of Flowers

The basal stem of each flower stalk was re-cut (5 cm) under de-ionized water to avoid stem-end air emboli. Each flowering stem was placed in a 50 mL glass centrifuge tube (DURAN® centrifuge tubes, SCHOTT North America, Inc. NY 10523 USA) containing 40 mL preservative solutions (Table 1). The treatments of preservative solutions were: 1) Control containing 125 mg/L 8-HQC, 2) Single leaf extract (SLE) of *J. curcas*, *P. guajava* or *A. paniculata* at 5, 10, 15 and 20 mg/L, and to each treatment was added 2% sucrose (Suc) and 3% citric acid (CA). The initial pH of preservative solutions were adjusted to pH ~ 3.0 by addition of CA. The stem-end of each flower stalk was held 1 cm from the base of the centrifuge tube, while the flower stalk was held straight with the support of a cotton wool plugged at the brim of the tube. This procedure also prevented evaporation of the preservative solutions. The flowers were kept at ambient temperature (25 ± 2 °C) under continuous white fluorescent light at 1.2 klux throughout the vase life. The preservative solutions were not renewed until the end of the vase life. Vase life was terminated when the florets wilted and dropped, followed by wilting and abscission of petals, or when 30% of the general appearance of flowers were no longer attractive. The final pH and microbial population of preservative solutions were evaluated. Postharvest characteristics such as flower fresh weight and vase life, bud opening, floret dropping and petal colour were evaluated until the end of the vase life.

Experiment 2. Determining the Effects of Double Combination Leaf Extracts to Extend Vase Life of Cut Mokara Red Orchid Flowers

Treatment of Flowers

In this experiment the flowers were evaluated in the same manner as described in experiment 1. The experimental treatments are as shown in Table 2.

Parameter Evaluation for Experiment 1 and 2

Determining Flower Vase Life

Vase life was terminated when 30% of the general appearance of flowers was no longer attractive due to unopened bud, wilting and drying, petal discoloration and wilting, floret epinasty and drop, and stem yellowing.

Determination of pH

The pH of the preservative solutions was determined with a pH meter (Model GLP-21; CRISON; Barcelona). The pH meter was calibrated with buffers at pH 4.0 and 7.0 before being used. The preservative solutions were taken in a clean, dry 250 mL beaker. The glass electrode was submerged in the sample and the pH meter was set aside to stabilize. Then the pH reading was recorded. The pH was measured on the initial day of the experiment after the cut flowers were placed into the preservative solutions and at the end of vase life.

Determining Preservative Solutions Uptake

The preservative solutions uptake were determined by the difference between consecutive weighings of the centrifuge tube plus solution (without the flower). Evaporative water loss from the surface of the preservative solutions were negligible as the cotton wool was plugged at the brim of the tube. Measurements of the preservative solutions uptake and flower fresh weight were taken on alternate days using a balance (Model GF-

Figure 1. Mokara Chark Kuan 'Red' orchid flower.

Table 1. Composition of floral preservative solution in the control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC) and single leaf extract (SLE) treatments [Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment	Composition of floral preservative solution
8-HQC	8-hydroxyquinoline citrate (125 mg/L)
	Jatropha curcas (mg/L)
SLE-Jc	5
	10
	15
	20
	Psidium guajava (mg/L)
SLE-Pg	5
	10
	15
	20
	Andrographis paniculata (mg/L)
SLE-Ap	5
	10
	15
	20

To each floral preservative solution treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solution at pH 3.0.

300; Top Loader Balance; U.S.A) until the end of vase life.

Determining Flower Fresh Weight

The fresh weight of cut flowers was measured every alternate day until the end of vase life. The weight of each inflorescence was determined using a balance.

Determination of Flower Petal Colour

The petal colour of the florets were measured on alternate days until the end of vase life. Measurements were carried out using a chromameter (Model CR-300, Minolta Crop., Tokyo, Japan) calibrated using a white tile and coupled with a Minolta Chroma C (VO.27) software. Calibration values were L* = 97.95, a* = -0.07, b* = 1.66 using the Illuminate C (CIE, 1967). The measurements were made under a constant light source with a fluorescent bulb at room temperature. The petal colour determination was expressed in chromaticity values of L*, C* and hº. The L* coordinate indicated the lightness of colour with values ranging from 0 = black to 100 = white. C*, which refers to the intensity of colour, was computed from values of a* and b* i.e. $C^* = (a^{*2} + b^{*2})^{1/2}$ which represented the hypotenuse of a right triangle

Table 2. Composition of floral preservative solution in the control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combinations of leaf extracts (DCLE) and triple combination of leaf extracts (TCLE) [Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment	Composition of floral preservative solution		
8-HQC	8-hydroxyquinoline citrate (125 mg/L)		
DCLE-Jc+Pg	*Jatropha curcas* (mg/L)	*Psidium guajava* (mg/L)	
	5	5	
	10	10	
	15	15	
	20	20	
DCLE-Jc+Ap	*Jatropha curcas* (mg/L)	*Andrographis paniculata* (mg/L)	
	5	5	
	10	10	
	15	15	
	20	20	
DCLE-Pg+Ap	*Psidium guajava* (mg/L)	*Andrographis paniculata* (mg/L)	
	5	5	
	10	10	
	15	15	
	20	20	
TCLE-Jc+Pg+Ap	*Jatropha curcas* (mg/L)	*Psidium guajava* (mg/L)	*Andrographis paniculata* (mg/L)
	5	5	5
	10	10	10
	15	15	15
	20	20	20

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0.

with values ranging from 0 = least intense to 60 = most intense. The hº, referred to as colour, was the angle of the tangent^{-1} b*/a* where 0º = red, purple, 90º = yellow, 180º = bluish-green and 270º = blue. Three fully opened florets were measured at the middle part in order to calculate the mean.

Determination of Bud Opening

Bud opening was determined visually on alternate days. The bud was considered to have opened if the tip of the petals had left an opening as each bud turns into a floret.

Determination of Floret Drop

Floret drop was determined visually. The floret drop of each inflorescence was determined relatively as a proportion of flower drop on day 0.

Experiment 3. Effects of Selected Double Combinations of Leaf Extracts on Microbial Populations in Preservative Solutions of Cut Mokara Red Orchid Flowers

Treatment of Flowers

Flowers were treated with preservative solutions selected from Experiment-2. The selection was based on the longest vase life (3 days) of the treated flowers. Treatments applied in the experiment were selected from preservative solutions: i) Control (125 mg/L 8-HQC), and ii) DCLE of *J. curcas*+*A. paniculata* (Jc+Ap) and *P. guajava*+*A. paniculata* (Pg+Ap) at 15 mg/L each with each treatment (i, ii) added with 2% Suc + 3% CA (Table 3). Samples of the preservative solutions were taken for microscopic observations of microbial populations after the end of the vase life of the cut flowers.

Parameter Evaluation

Determining Microbial Populations in Preservative Solutions

The microbial populations and microscopic observation of preservative solutions were determined according to the methods of Tongpoothorn et al. (2012) and Rahman et al. (2011) with some modifications. First, 1 mL of preservative solutions were added into a universal bottle (100 mL) containing 9 mL of distilled water as the stock solution. Then, 1 mL of the diluted preservative solutions were added to the 9 mL of distilled water. Each step resulted in a further 8-fold and 6-fold change from the previous diluted preservative solutions for bacterial count and fungal growth determination, respectively. The samples

Table 3. Composition of floral preservative solution in the control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC) and selected double combination of leaf extracts (DCLE) [Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment	Composition of floral preservative solutions	
8-HQC	8-hydroxyquinoline citrate (125 mg/L)	
	Jatropha curcas (mg/L)	*Psidium guajava* (mg/L)
DCLE-Jc+Ap	5	5
	10	10
	15	15
	20	20
	Psidium guajava(mg/L)	*Andrographis paniculata* (mg/L)
DCLE-Pg+Ap	5	5
	10	10
	15	15
	20	20

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0.

were mixed and homogenized in a vortex mixer (Model SA-8; STUART; Switzerland), at a speed of 2500 rpm for 2 min. The nutrient agar (NA) was prepared according to the manufacturer's instruction as labelled on the bottle. Five hundred mL de-ionized water and 11.5 g of NA were placed in a 1 L bottle. The bottle was heated and its content was stirred to dissolve the agar. The solutions were boiled for 1 min, then autoclaved at 121 °C for 15 min and allowed to cool. The agar was poured into sterile Petri-dishes (20 ml). The petri-dishes were covered and left overnight. Then 100 µl of 8-fold final diluted preservative solutions were plated onto the NA in petri-dishes. The inoculated plates were incubated for 48 h at 30 °C. The petri-dishes were turned upside down and cooled in a refrigerator. Potato dextrose agar (PDA) was prepared according to standard instructions provided by Dickinson and Company (Sparks, MD 21152 USA). The 20 g PDA was added to 500 mL distilled water and sterilized at 121 °C and 15 psi for 15 min in an autoclave. A 25 mL pre autoclaved PDA was poured into 90 mm diameter pre sterilized petri-dishes. The PDA was allowed to solidify at room temperature. Then 100 µl of the 6-fold final diluted preservative solutions were plated onto the PDA in the petri-dishes. The inoculated plates were incubated for 7 days at 32 °C. Microorganisms were counted by the standard plate counting method (by counting the number of colonies formed after incubation) to get the number of colony forming units per mL (cfu/mL) (Jowkar, 2006).

Microscopic Observations of Preservative Solutions

The bacterial colonies from the pure culture were obtained and stained with gram stain. The pure culture of bacterial colonies were smeared on a glass slide and stained with crystal violet (primary stain). After a minute of exposure to the staining solutions, the slide was washed with water and the smear was treated with gram's iodine for 1 min. Gram's iodine served as a mordant. The slide was washed again with water and then acetone was used as a decolourisation agent. The process of decolourisation was done quickly so as not to exceed 30 seconds to get a thin smear. Finally, the smear was immediately washed in water and treated with 2-3 drops of counter stain safranin. The smear was washed again before drying. A drop of immersion oil was placed on top of the smear before gently affixing a glass cover slip over the slide. Then the slide was placed in the holder and the bacteria were observed under the 100X lens light microscope (Model E-800; Nikon Eclipse; Tokyo, Japan). The bacterial isolates were differentiated according to their typical morphological and biochemical characteristics (Schaad et al., 2001).

In the case of fungus, a drop of lactophenol cotton blue stain was placed in the centre of a clean glass slide. A small portion (2 mm from the outer boundary) of the fungus colony was scratched and transferred using a teasing needle. Finally, a cover slip was placed on top of the sample slide and gentle pressure was applied for even spreading. Then the slide was placed into the holder and the fungus was observed under the light microscope. The fungal isolates were identified according to Steinkellner and Langer (2004) and Siddiquee et al. (2009).

Experimental Design and Statistical Analysis

The experiments were conducted using the completely randomized design (CRD). Each treatment contained five replications with one flower stem in each replication. All the collected data were subjected to an analysis of variance (ANOVA) to define the differences between treatments. The treatment means were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$ using

SAS software version 9.1. Regression analysis was carried out to determine the relationship between the rate of preservative solutions uptake and days in preservative solutions.

RESULTS AND DISCUSSION

Optimum Concentration of SLE and DCLE to Extend Vase Life of Cut Mokara Red Orchid Flowers

Vase life

The vase life of the cut flowers in all the 15 mg SLE/L treatments were significantly ($p \leq 0.05$) shorter (26-35%) compared to vase life of cut flowers in the 8-HQC treated preservative solutions (Table 4). The vase life of the flowers in the 15 mg SLE/L treated preservative solutions were about 2-3 days shorter, whereas the vase life of flowers in the 8-HQC treated preservative solutions were 9 days. Similarly, the vase life of cut flowers in 5, 10 and 20 mg SLE/L treatments, were also significantly shorter by 3-5 days compared to vase life of cut flowers in the 8-HQC treatment (Table 4).

It is well known that bacteria, which grow in preservative solutions injure cut flowers through their development. Xylem and their consequent blockage at cut ends, prevents the rate of preservative solutions uptake, resulting in shorter vase life (Kazemi et al., 2010). The 8-HQC, either in preservative solutions or in a pulse treatment, would restrict the microbial growth and subsequent vascular blockage, and promote water uptake, and thus prolong vase life (Chand et al., 2012). This outcome is in agreement with previous research that reported increased vase life of cut flowers when placed in solutions of germicide (Saini et al., 1994). The present study showed longer vase life with 8-HQC, a germicide which was used as the control. The *J. curcas*, *P. guajava* and *A. paniculata* leaf extracts have been described to possess antifungal and/or antimicrobial activities, but when used singly the leaf infusions were not efficient in controlling microbes in the preservative solutions (Okoh et al., 2009; Rahman et al., 2014; Suhaila et al., 2009).The data reported here provides evidence supporting that 8-HQC had a positive effect in prolonging vase life of cut Moraka Red orchid flowers and reduced vascular blockage in the cut flowers (Table 4). When compared with all concentrations, the beneficial essence of each of the 15 mg SLE/L could be due partly to its bactericidal effect. A double combination of the leaf extracts (DCLE) and triple combination of leaf extracts (TCLE) enhanced their potency. These findings were indicated in the results of the subsequent experiment evaluating the strength of DCLE and TCLE treatments. Sucrose alone tends to encourage microbial growth (Pun and Ichimura, 2003).

In the 10 and 15 mg/L DCLE-Pg+Ap of treatments, vase life of cut flowers were significantly ($p \leq 0.05$) longer compared to vase life of cut flowers in the control (Table 5). A 2-3 days longer vase life was observed in flowers treated with 15 mg/L DCLE-Pg+Ap preservative solutions, whereas the vase life of the control was about 9 days. However vase life of cut flowers in all the 5 and 20 mg/L of DCLE-Jc+Pg, DCLE-Jc+Ap and TCLE treatments were 3-5 days shorter compared to the vase life of cut flowers in the control. Similarly, the vase lives of flowers in the TCLE treatments were significantly shorter by 5-6 days compared to flowers in the DCLE-Pg+Ap. The reasons for the shorter vase life of the DCLE-Jc+Pg and DCLE-Jc+Ap treated flowers could be attributed to the presence of microbes in the preservative solutions. Metabolites produced by certain bacteria also decreased vase life and water conductivity in cut carnations (Rahman et al., 2012). The above results could be due to antimicrobial properties of *P. guajava* and *A. paniculata* (Kumar et al., 2012; Suhaila et al., 2009) which reduced stem plugging resulting in longer vase life.

pH Measurement

The final pH value of the cut flowers' preservative solutions containing all the 15 mg SLE/L was significantly ($p \leq 0.05$) higher (42-57%) compared to the final pH of preservative solutions of cut flowers in the 8-HQC (Table 4). Moreover, in all the preservative solutions containing 5, 10 and 20 mg SLE/L treatments, the final pH values were significantly higher (63-114%) compared to the final pH in preservative solutions of cut flowers in the 8-HQC (Table 4). This control treatment maintained a pH that was low enough to prevent bacterial growth for an extended period. An increase in pH of solutions decreased its positive effects on vase life. Lowering the pH of the 8-HQC solution, using CA, also gave a longer vase life than the SLE. However, the vase life was not as long as that was found in treatments with SEL+2%SUC+3%CA. Possibly, the SLE-Jc preservative solutions containing *J. curcas* had antifungal properties (Saetae and Suntornsuk, 2010) and cannot control all the microbes in the preservative solutions. Apparently, SLE-Pg had antibacterial properties (Mittal et al., 2010), which could slightly control the pH of the preservative solutions. The SLE-Ap had antimicrobial properties (Wei et al., 2011), which produced an antagonistic effect with the preservative solutions, resulting in higher pH values. The final pH value of the preservative solutions containing 15 mg/L DCLE-Pg+Ap treatment was significantly ($p \leq 0.05$) lower (12%) compared to the final pH of the 8-HQC preservative solutions with cut flowers (Table 5). The final pH values in all 5, 10 and 20 mg DCLE/L treatments were significantly higher (33-140%) compared to the final pH in preservative solutions of cut flowers in the 8-HQC (Table 5). Moreover, the final pH values, in the different concentrations of TCLE treatments, were significantly

Table 4. The effect of floral preservative solutions containing control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extracts (SLE) on cut Mokara Red orchid flower vase life and final pH [Leaf extracts comprised *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment		Vase life (day)	pH (final)
8-HQC	8-hydroxyquinoline citrate	8.61 a[z]	4.04 g
	Jatropha curcas (mg/L)		
SLE-Jc	5	4.18 e	8.03 b
	10	5.07 d	7.47 c
	15	5.63 cd	6.35 e
	20	3.59 f	8.64 a
	Psidium guajava (mg/L)		
SLE-Pg	5	4.43 e	7.04 d
	10	5.35 d	6.60 e
	15	6.38 b	5.73 f
	20	3.98 ef	7.60 c
	Andrographis paniculata (mg/L)		
SLE-Ap	5	4.30 e	7.32 cd
	10	5.14 d	6.65 e
	15	6.02 bc	5.84 f
	20	3.91 ef	7.70 bc

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0. [z]Means, followed by the same letters within each column, are not significantly different by DMTR ($p \leq 0.05$). n=5.

higher (99-150%) compared to the control (Table 5). Moreover, the TCLE treatments at all concentrations showed similarly higher (127-185%) final pH values compared to the final pH in preservative solutions of cut flowers in the 5, 10 and 15 mg/L DCLE (Table 5). The results indicate that the 15 mg/L DCLE treatments were more effective in maintaining the pH values and thus prolonging vase life of cut flowers. It may be due to synergistic effects of each of the leaf extracts. Preservative solutions with low pH (3.0) increased solution uptake by gladiolus (*Gladiolus* L.) flowers, thus resulting in prolonged vase life, compared to solutions with a high pH (Khan et al., 2009). The cut rose flower stems in acidic solutions with a low pH of ~ 3.0 maintained fresh weight, improved water uptake, and prolonged vase life (Regan and Dole, 2010).

Floral Preservative Solutions Uptake and Fresh Weight

The preservative solutions used significantly ($p \leq 0.05$) affected the rate of preservative solutions uptake in cut flowers. The rate of preservative solutions uptake were measured daily for 10 days. The preservative solutions in the control contained 8-HQC, while the SLE contained *J. curcas*, *P. guajava* and *A. paniculata*, each at a concentration of 5, 10, 15 and 20 mg/L. Flowers in the preservative solutions containing 8-HQC showed a significant linear relationship (R^2=0.87) between the rate of preservative solutions uptake and days flowers were left in the preservative solutions. There was a reduction of 78% in the rate of preservative solutions uptake by day 10 compared to day 2 (Figure 2). Similar linear relationships were observed between the rates of preservative solutions uptake and time in preservative solutions for flowers in the 5, 10 and 15 mg/L SLE-Jc. There were reductions of 60-78% in the rates of solution uptake by day 10 compared to day 2 (Figure 2A). However, there was a significant, negative and quadratic relationship (R^2=0.84) between the rates of preservative solutions uptake and time for flowers in preservative solutions in the 20 mg/L SLE-Jc. The preservative solutions containing SLE-Pg showed significant linear relationships between the rates of preservative solutions uptake at 5 (R^2=0. 84), 10 (R^2=0. 86) and 15 (R^2=0. 88) mg/L and days of flowers in the preservative solutions, with a reduction of 57-77% in the rate of preservative solutions uptake by day 10 compared to day 2 (Figure 2B). There was a significant, negative and quadratic relationship between the rate of preservative solutions uptake and time in preservative solutions for the flowers in the *P. guajava* at 20 mg/L SLE preservative solutions.

Table 5. The effect of floral preservative solutions containing control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) and triple combination leaf extracts (TCLE) on vase life and final pH [The DCLE combination contains two leaf extracts and TCLE combination contains three leaf extracts; Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment				Vase life (day)	PH (final)
8-HQC	8-hydroxyquinoline citrate			8.61 d[z]	4.04 h
	Jatropha curcas (mg/L)	*Psidium guajava* (mg/L)			
DCLE-Jc+Pg	5	5		4.77 h	8.03 d
	10	10		6.07 f	5.93 f
	15	15		7.22 e	4.13 h
	20	20		4.07 ij	9.69 b
	Jatropha curcas (mg/L)	*Andrographis paniculata* (mg/L)			
DCLE-Jc+Ap	5	5		5.95 f	7.98 d
	10	10		7.25 e	5.85 f
	15	15		8.40 d	4.05 h
	20	20		5.25 g	9.68 b
	Psidium guajava (mg/L)	*Andrographis paniculata* (mg/L)			
DCLE-Pg+Ap	5	5		9.04 c	7.62 e
	10	10		10.34 b	5.39 g
	15	15		11.49 a	3.54 i
	20	20		8.34 d	9.62 b
	Jatropha curcas(mg/L)	*Psidium guajava* (mg/L)	*Andrographis paniculata* (mg/L)		
TCLE-Jc+Pg+Ap	5	5	5	4.45 hi	9.83 b
	10	10	10	5.75 f	8.91 c
	15	15	15	6.90 e	8.02 d
	20	20	20	3.75 j	10.08 a

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0. [z]Means, followed by the same letters within each column, are not significantly different by DMTR ($p \leq 0.05$). n=5.

As for flowers in the preservative solutions containing 10 and 15 mg/L SLE-Ap, the rates of preservative solutions uptake showed similar significant linear relationships and reduction in the SLE-Pg treated preservative solutions by day 10 compared to day 2 (Figure 2C). Conversely, there were significant, negative and quadratic relationships between the rates of preservative solutions uptake and time flowers were in the SLE-Ap preservative solutions at 5 (R^2=0.82) and 20 (R^2=0.83) mg/L (Figure 2C). Flower vase life is affected by water uptake and the cut flowers rate of water uptake decreases with time (van Doorn, 1997). This confirmed that the SLE treated preservative solutions were not effective in maintaining solution uptake and thus reduced the vase life of cut orchid flowers. Here,

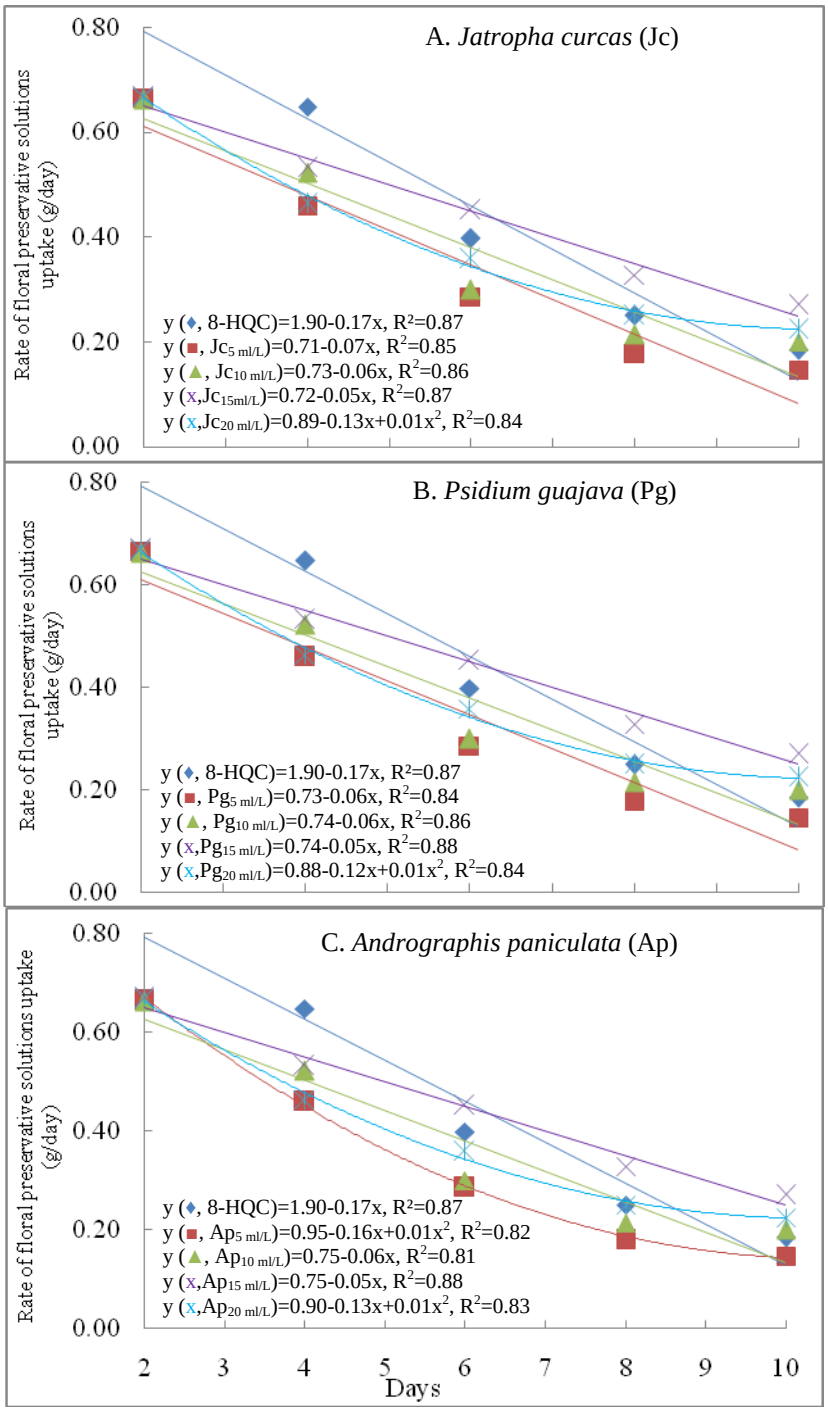

Figure 2. Relationship between rates of floral preservative solutions uptake and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extract (SLE) of *J. curcas* (A), *P. guajava* (B) and *A. paniculata* (C) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flower; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid. A solid line indicates a significant relationship $p \leq 0.05$. n=5].

sucrose is necessary to maintain water balance and delay turgor loss (Sven and Jose, 2004). Moreover, synergistic effect of 8-HQS+2% sucrose in increasing vase life due to suppression of microbial growth (Ketsa et al., 1992), resulted in increased solution uptake. The results of this study is in agreement with the findings of (Rahman et al., 2012) who reported that the leaf extracts of *Psidium guajava* or *Piper betle*, was able to prolong the vase life of carnation

flowers by maintaining the rate of water uptake. Similar results were obtained in a study on carnations which showed that the shorter vase life of flowers were associated with the presence of microbes in the preservative solutions and metabolites produced by certain bacteria reduced the rate of preservative solutions uptake (Thwala et al., 2013). A reduction in the rate of vase solution uptake by *Epidendrum ibaguense* flowers in the final phase of development was associated with senescence and less water of water uptake resulting shorter vase life (Santos et al., 2012).

The treatments of 15 mg/L DCLE-Jc+Ap and DCLE-Pg+Ap had the highest rates of preservative solutions uptake compared to the control (Figure 3). The rates of preservative solutions uptake for the cut flowers were measured daily for 12 days. The preservative solutions contained 8-HQC as the control, and DCLE and TCLE of *J. curcas*, *P. guajava* or *A. paniculata* each at 5, 10, 15 and 20 mg/L. The flowers in the 8-HQC preservative solutions showed a significant linear relationship (R^2=0.87) between the rates of preservative solutions uptake and days in preservative solutions, with a reduction of 82% in the rate of preservative solutions uptake by day 12 compared to day 2 (Figure 3). Similar linear relationships were observed between the rates of preservative solutions uptake and time in preservative solutions for flowers in the 5, 10 and 15 mg/L DCLE-Jc+Pg, with a reduction of 60-80% in the rate of solution uptake by day 12 compared to day 2 (Figure 3A). However, there was a significant, negative quadratic relationship (R^2=0.88) between the rate of preservative solutions uptake and time in preservative solutions for the flowers in the 20 mg/L DCLE-Jc+Pg treatment. The preservative solutions containing DCLE-Jc+Ap showed significant linear relationships between the rates of preservative solutions uptake at 5 (R^2=0.86) and 15 (R^2=0.88) mg/L and days in preservative solutions, with a reduction of 70-84% in the rate of preservative solutions uptake by day 12 compared to day 2 (Figure 3B). There was a significant, negative cubic relationship between the rate of preservative solutions uptake and time in preservative solutions for the flowers in the 10 mg/L DCLE-Jc+Ap preservative solutions, while a quadratic relationship was shown for flowers in the 20 mg/L DCLE-Jc+Ap preservative solutions (Figure 3B). For the cut flowers in the 15 mg/L DCLE-Pg+Ap preservative solutions, the rate of solution uptake showed a similar significant linear relationship as flowers in the DCLE-Jc+Ap treated preservative solutions, with a reduction on day 12 compared to day 2 (Figure 3C). There was a significant negative quadratic relationship between the rate of preservative solutions uptake and time in preservative solutions for flowers in the 20 mg/L DCLE-Pg+Ap (R^2=0.86) treatment, with a reduction of 85% in the rate of solution uptake by day 12 compared to day 2. On the contrary, there were significant negative cubic relationships between the rates of preservative solutions uptake and time in preservative solutions for flowers in the 5 (R^2=0.85) and 10 (R^2=0.86) mg/L DCLE-Pg+Ap treatment, with a reduction of 77-89% in the rate of solution uptake by day 12 compared to day 2 (Figure 3C). For the flowers in preservative solutions containing 5 and 15 mg/L TCLE, the rates of preservative solutions uptake showed similar significant linear relationships and a reduction of 58-64% in the rate of solution uptake by day 12 compared to day 2 (Figure 3D). There was a significant, negative quadratic relationship between the rate of preservative solutions uptake and time in preservative solutions for flowers in the TCLE at 20 mg/L (R^2=0.85), with a reduction of 95% in the rate of solution uptake by day 12 compared to day 2. However, there was a significant negative cubic relationship between the rate of preservative solutions uptake and time in preservative solutions for flowers in the 10 mg/L TCLE (R^2=0.86), with a reduction of 75% in the rate of solution uptake by day 12 compared to day 2 (Figure 3D). Preservative solutions uptake of flowers in the 8-HQC was not significantly ($p \leq 0.05$) different from the DCLE-Jc+Pg treatment, but had different TCLE. The DCLE-Pg+Ap leaf extract was most effective in maintaining the rate of preservative solutions uptake, as it reduced early wilting and prolonged vase life of flowers. The flower vase life was affected by the rates of water uptake. In general, the rate of water uptake of cut flowers decreases with time (van Doorn, 1997). As mentioned earlier, the *P. guajava* and *A. paniculata* leaf extracts showed antimicrobial and antibacterial activity. Moreover, the antimicrobial properties reduced stem vascular tissue blockage and increased preservative solutions uptake of carnation (*Dianthus caryophyllus* cv. White Sim) flowers. Here, sucrose could maintain the water balance and delay turgor loss (Ketsa et al., 1992). The translocation of sucrose tended to accumulate in the flower and increased the osmotic concentration to improve solution absorption.

The fresh weights of the cut flowers were measured daily for 10 days. In 10 and 15 mg/L SLE of *J. curcas*, *P. guajava* and *A. paniculata*, the flower fresh weights increased during the first few days in the vase, except for 5 and 20 mg/L treatments (Figure 4). The fresh weights of the cut flowers were significantly affected by the SLE treated preservative solutions. The fresh weight of cut flower containing 8-HQC showed a significant negative quadratic relationship (R^2=0.87) between the fresh weight and days in preservative solutions, with a reduction of 7% in fresh weight by day 10 compared to day 2 (Figure 4A). A similar linear relationship was observed between fresh weights and time in preservative solutions for flowers in the 15 mg/L SLE-Jc, with a reduction of 2% in fresh weight by day 10 compared to day 2 (Figure 4A). However, there was a significant negative cubic relationship (R^2=0.84) between the fresh weight and time in preservative solutions for the flowers in each of the 5 and 10 mg/L SLE-Jc. There was a significant linear relationship between the fresh weight in the 20 mg/L (R^2=0.88) and days in preservative solutions, with a

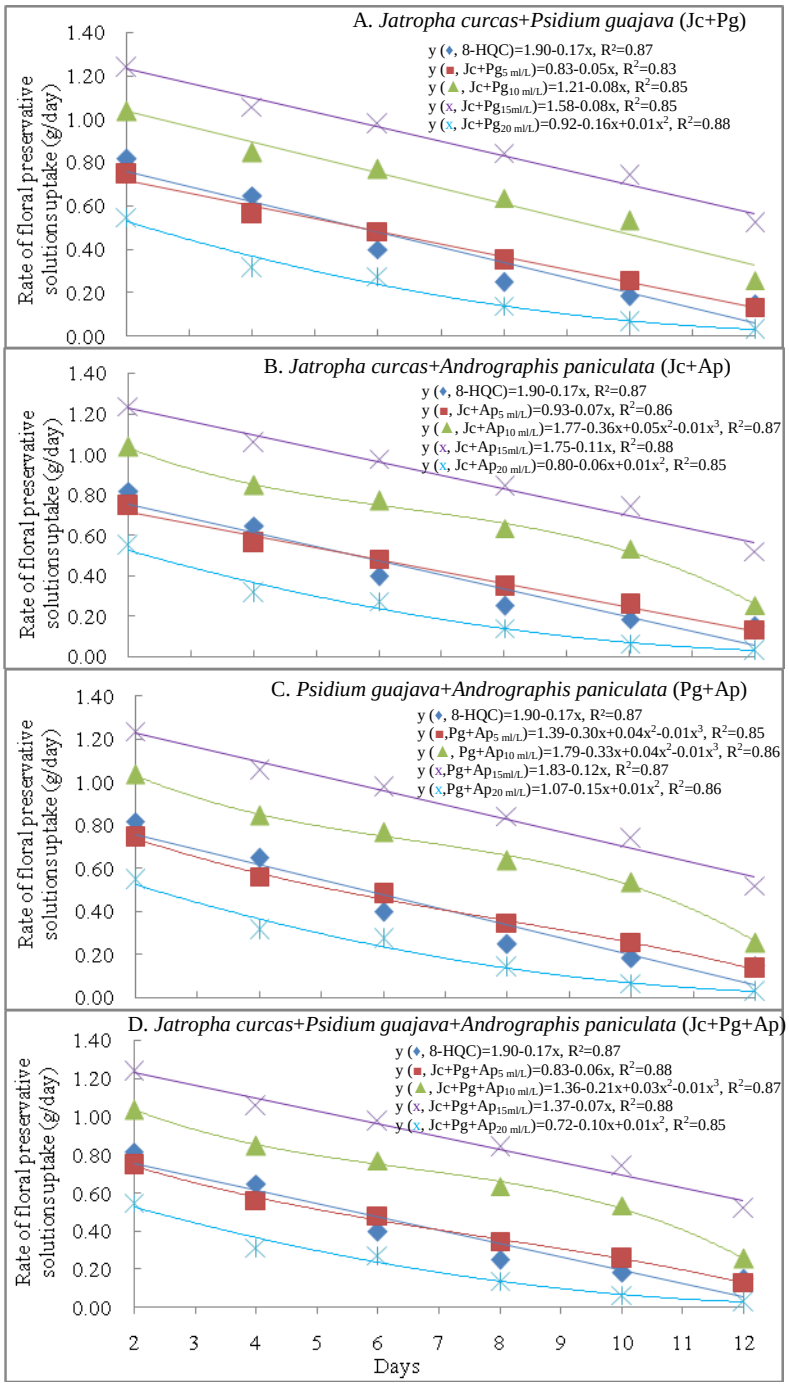

Figure 3. Relationship between rates of floral preservative solutions uptake and days in vase solution containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and double combination leaf extracts (DCLE) of *J. curcas+P. guajava* (A), *J. curcas+A. paniculata* (B) and *P. guajava+A. paniculata* (C) and triple combination leaf extract (TCLE) of *J. curcas+P. guajava+A. paniculata* (D) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flower; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

reduction of 19% in fresh weight on day 10 compared to day 2 (Figure 4A). For the 20 mg/L SLE-Pg (R^2=0.74), the cut flowers showed a significant linear relationship between the fresh weight and days in the preservative solutions, with a reduction of 15% in fresh weight on day 10 compared to day 2 (Figure 4B). There were significant negative quadratic relationships between the fresh weight and time in preservative solutions for the flowers in the 5,

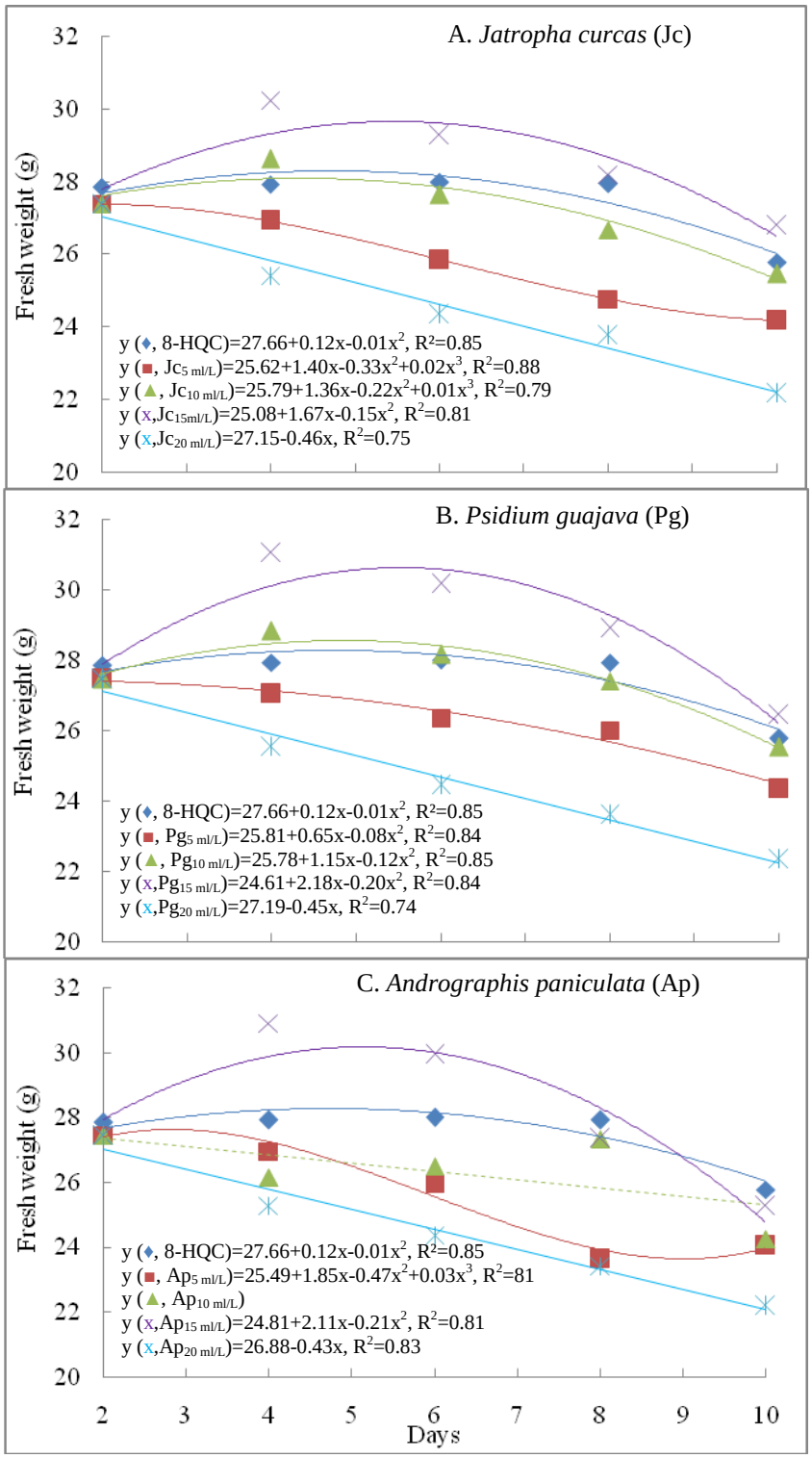

Figure 4. Relationship between fresh weights and days in vase solution containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extract (SLE) of *J. curcas* (A), *P. guajava* (B) and *A. paniculata* (C) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

10 and 15 mg/L SLE-Pg. Flowers in the preservative solutions containing 20 ml/L SLE-Ap, the fresh weight showed a similar significant and linear relationship, with a reduction in flower weight in the SLE-Pg treatment on day 10

compared to day 2 (Figure 4C). Conversely, there was a significant, negative cubic relationship between the fresh weight and time in the 5 mg/L SLE-Ap (R^2=0.81) preservative solutions, whereas there was a significant, negative quadratic relationship between the fresh weight and time in preservative solutions for flowers in the 15 mg/L (SLE-Ap (R^2=0.81). In the present study, the cut flowers treated with preservatives at concentrations 5, 10, 15 and 20 mg/L SLE of *J. curcas*, *P. guajava* and *A. paniculata* had 2-19% lower fresh weights compared to the flowers in the 8-HQC preservative. This suggested that the fresh weights of cut orchid flowers were not affected by the SLE treated preservative solutions in order to reduce solution uptake, resulting in a decrease in vase life. However, solutions containing 8-HQS+sucrose were reported to extend the vase life of *Dendrobium* and cut rose flowers and improve flower quality and fresh weight (Rogers, 1973; Dineshbabu et al., 2002; Elgimabi and Ahmed, 2009).

The fresh weights of the DCLE treated cut flowers were measured daily for 12 days. In all concentrations, flower fresh weights increased during the first few days in the vase (Figure 5). Flowers in preservative solutions containing 8-HQC showed a significant negative quadratic relationship (R^2=0.87) between the fresh weight and days in preservative solutions. There was a reduction of 6% in fresh weight on day 12 compared to day 2 (Figure 5). Similar quadratic relationships were also observed between the fresh weights and time in preservative solutions for flowers in the 5, 15 and 20 mg/L DCLE-Jc+Pg treatments, with a reduction of 7-9% in fresh weights by day 12 compared to day 2 (Figure 5A). However, there was a significant linear relationship between the fresh weight at 10 mg/L (R^2=0.73) DCLE-Jc+Pg treatment and days in preservative solutions, with a reduction of 9% in fresh weight by day 12 compared to day 2 (Figure 5A). The DCLE-Jc+Ap preservative treatment showed a significant linear relationship between the fresh weight at 15 mg/L (R^2=0.78) and days in preservative solutions, with a reduction of 8% in fresh weight by day 12 compared to day 2 (Figure 5B). There was a significant negative quadratic relationship between the fresh weights and time in preservative solutions for the flowers in the 10 and 20 mg/L DCLE-Jc+Ap treatment, with a reduction of 7-9% in fresh weights by day 12 compared to day 2 (Figure 5B). As for flowers in the preservative solutions containing 5 and 20 ml/L DCLE-Pg+Ap treatment, the fresh weights showed similar significant quadratic relationships and reductions in fresh weight by day 12 compared to day 2 (Figure 5C). Moreover, there were significant, negative quadratic relationships between the fresh weights and days in preservative solutions for flowers in the 5 mg/L (R^2=0.86) and 20 mg/L (R^2=0.86) TCLE, with a reduction of 7% in fresh weight by day 12 compared to day 2. However, there was a significant, negative linear relationship between the fresh weight and days in preservative solutions for flowers in the 15 mg/L (R^2=0.87) TCLE, with a reduction of6% in fresh weight by day 12 compared to day 2 (Figure 5D). In the 15 mg/L DCLE-Pg+Ap treatment, the fresh weight of cut flower vase life was maintained until the end of vase life. The application of 8-HQS increased the vase life as well as the fresh weight of the cut flowers (Kumar et al., 2012), as well as DCLE also increased vase life by maintaining flower fresh weights. In *Dendrobium* cut flowers, holding solutions containing 8-HQS+sucrose extended the vase life and improved flower quality, water consumption, fresh weight, and flower freshness, and reduced respiration rate and weight loss (Dineshbabu et al., 2002). The total solution uptake and vase life of rose cut flowers were increased, while flower senescence and bent neck were inhibited when using 8-HQS+sucrose compared to the control (Elgimabi and Ahmed, 2009). Moreover, the synergistic effect of 8-HQS+2% sucrose in increasing vase life was due to suppression of microbial growth (Ketsa et al., 1992), resulting in increased solution uptake. Hence, addition of sucrose to the holding solutions might have led to increased uptake of the holding solutions (Knee, 2000).

Petal Colours

The petal colours (as indicated by h^0, C* and L* colour values) of the cut flowers in the vase solutions containing the 8-HQC and the single leaf extract (SLE) of *J. curcas*, *P. guajava* and *A. paniculata* were measured on alternate days (Figure 6). There was a significant negative linear relationship ($p \leq 0.05$) between h^0 values of petals and days in vase solutions containing the 8HQC (R^2=0.89) and 15 (R^2=0.94) mg/L SLE (Figure 6A). The h^0 colour values of petals in the 8-HQC vase solution showed a slight decrease of 25% over 10 days in the vase solution. However, there were significant, negative cubic relationships between the h^0 colour values of petals and days in vase solutions containing 5 (R^2=0.91), 10 (R^2=0.93) and 20 (R^2=0.89) mg/L SLE-Jc, with a gradual decrease of 53-77% in petal colour at day 10 compared to day 0 (Figure 6A). There were significant negative quadratic relationships between the petal h° values and days in vase solutions for the flowers in the 5, 10 and 20 mg/L SLE-Pg. As for flowers in the vase solution containing 15 mg/L SLE-Pg, the petal h^0 values showed a similar significant linear negative relationship and a gradual decrease in the SLE-G treated vase solution by day 10 compared to day 0 (Figure 6B). Conversely, there were significant negative cubic relationships between the petal colour and days in vase solutions for flowers in the 5 (R^2=0.90), 10 (R^2=0.92) and 20 (R^2=0.87) mg/L SLE-Ap (Figure 6C).

There were significant negative linear relationships ($p \leq 0.05$) between C* values of petals and days in vase solutions containing the 8-HQC and 5, 10, 15 and 20 mg/L SEL (Figure 7). The C* colour values of petals in

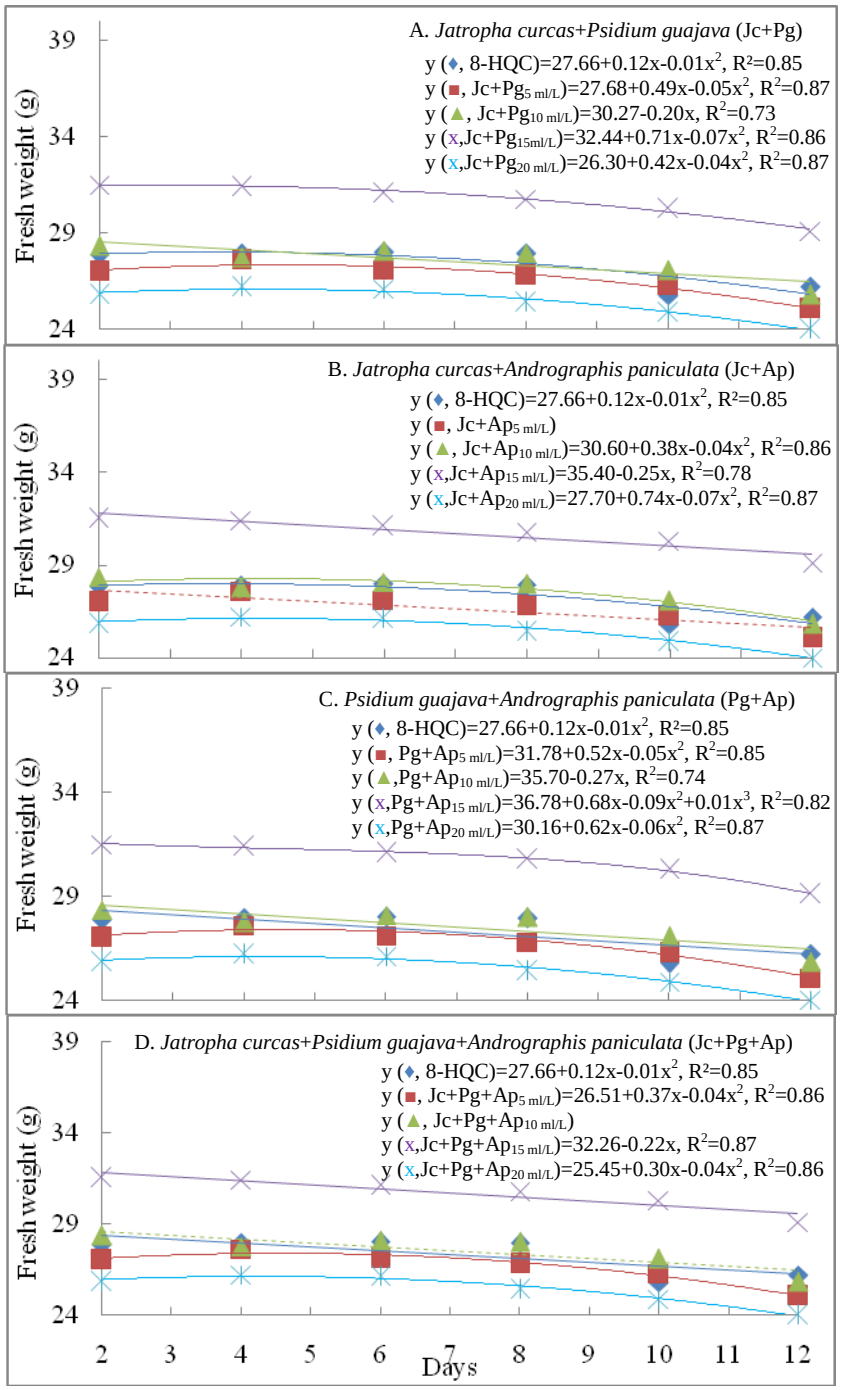

Figure 5. Relationship between fresh weights and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) *J. curcas+P. guajava* (A), *J. curcas+A. paniculata* (B) and *P. guajava+ A. paniculata* (C), and triple combinations of leaf extracts (TCLE) of *J. curcas+P. guajava+ A. paniculata*(D) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

the STD vase solutions showed a decrease of 50% at day 10 (R^2=0.69). Similar linear negative relationships were observed between petal C* values and days in vase solutions in the 5 (R^2=0.92), 10 (R^2=0.91), 15 (R^2=0.88) and 20 (R^2=0.93) mg/L SLE-Jc, with a decrease of 45-52% in C* values by day 10 compared to day 0 (Figure 7A). The SLE-Pg, showed significant, negative linear relationships at 5 (R^2=0.89), 10 (R^2=0.88), 15 (R^2=0.85)

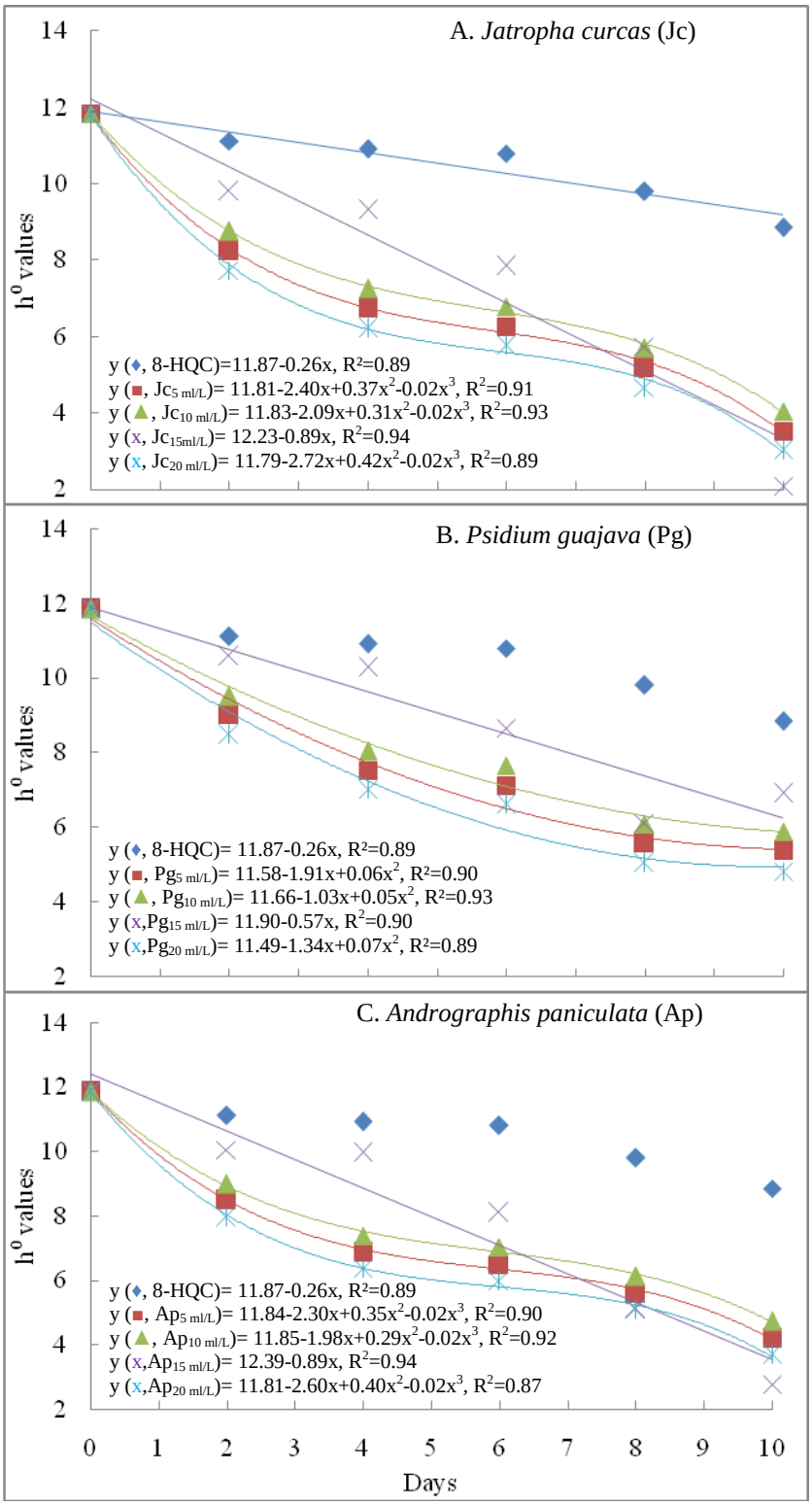

Figure 6. Relationship between h^0 colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), single leaf extracts (SLE) of *J. curcas* (A), *P. guajava* (B) and *A. paniculata* (C) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

and 20 (R^2=0.91) mg/L, with a 45% decrease by day 10 compared to day 0 (Figure 7B). The petals in the 5 (R^2=0.90), 10 (R^2=0.88) 15 (R^2=0.85) and 20 (R^2=0.91) mg/L SLE-Ap vase solutions had a similar linear trend of C* values, with 45 to 46% lower C* values by day 10 compared to day 0 (Figure 7C).

There was a significant negative linear relationship ($p \leq 0.05$) between L* values of petals and days in vase solutions containing 8-HQC, with a reduction of 30% in petal colour by day 10 compared to day 0 (Figure 8). Similar linear negative relationships were observed between petal colour and days in vase solutions for flowers in the 5 (R^2=0.94), 10 (R^2=0.94) and 15 (R^2=0.92) mg/L SLE-Jc, with reductions of 40-46% in petal colour by day 10 compared to day 0 (Figure 8A). However, there was a significant negative cubic relationship between the petal colour and days in vase solutions for the flowers in the 20 (R^2=0.94) mg/L SLE-Jc, with a reduction of 43% in petal colour at day 10 compared to day 0 (Figure 8A). The SLE-Pg treatments showed similar significant negative linear relationships between the petal colour and days in vase solutions for the flowers at 5, 10 and 20 mg/L. As for flowers in the vase solutions containing 15 mg/L SLE-Pg, the petal colour showed a similar significant quadratic negative relationship, with a reduction of 36% in petal colour at day 10 compared to day 0 (Figure 8B). The petals in the SLE-Ap treatments were significant, and showed negative linear relationships between the petal colour and days in vase solutions at 5 (R^2=0.91), 10 (R^2=0.89) and 20 (R^2=0.92) mg/L (Figure 8C). Similarly, there was a significant, negative quadratic relationship and reduction between the petal colour and days in vase solutions for flowers in the 15 (R^2=0.85) mg/L SLE-Ap (Figure 8C).

The results confirm that the SLE treated vase solutions were not effective in maintaining better petal colour, and thus reduced the vase life of cut orchid flowers. Moreover, the synergistic effect of 8-HQS+2% sucrose in increasing vase life due to suppression of microbial growth resulted in increased in solution uptake of cut orchid flowers (Ketsa et al., 1992). The result of this study are in agreement with the findings of Rahman et al. (2012) who reported that the colour values of carnation flowers were similarly increased to maintain good colour retention with longer vase life. Chandran et al. (2006) found that vase solutions treated with sugar extended flower vase life by maintaining a pool of dry matter and promoting better respiration of petals. The positive effects of adding sucrose have been documented in many cut flower species, with resulting increase in intensity of petal colour (Boo, 1997; Han, 2003). There were significant negative linear relationships ($p \leq 0.05$) between h^0 values of petals and days in vase solutions containing 8HQC (R^2=0.86) and 15 (R^2=0.93) mg/L DCLE (Figure 9A). The h^0 colour values of petals in the 8-HQC vase solutions showed a decrease of 45% by day 12 compared to day 0 (Figure 9). However, there were significant negative cubic relationships between the h^0 colour values of petals and days in vase solutions containing 5 (R^2=0.91), 10 (R^2=0.92) and 20 (R^2=0.89) mg/L DCLE-Jc+Pg, with a gradual decrease of 53-77% in petal colour at day 10 compared to day 0 (Figure 9A). The petals in the 10 (R^2=0.88), 15 (R^2=0.89) and 20 (R^2=0.83) mg/L DCLE-Jc+Ap, had similar linear trends of h^0 values, with 45-52% reductions by day 10 compared to day 0 (Figure 9B). There was a significant negative quadratic relationship between the h° colour values of petals and days in vase solutions for the flowers in the 5 mg/L DCLE-Jc+Ap treatment (Figure 9B). For the cut flowers in vase solutions containing 15 mg/L DCLE-Pg+Ap, the rate of vase solution uptake showed a similar significant linear relationship and reduction as flowers in the DCLE-Jc+Ap treatment by day 12 compared to day 0 (Figure 9C). As for flowers in the vase solutions containing DCLE-Jc+Ap, the petal h^0 values showed significant negative cubic relationships in the 5 (R^2=0.88), 10 (R^2=0.91) and 20 (R^2=0.91) mg/L DCLE-Pg+Ap treatment, with a reduction of 48-56% in h^0 colour values of petals by day 12 compared to day 0 (Figure 9C). There were significant negative cubic relationships between the petal h^0 values and days in vase solutions for flowers in the 5 (R^2=0.83) and 10 (R^2=0.90) mg/L TCLE, with a reduction of 44-98% in the petal h^0 values by day 12 compared to day 0 (Figure 9D). In flowers in vase solutions containing 5 (R^2=0.83), and 10 (R^2=0.90) mg/L TCLE, the h^0 colour values of petals showed similar significant linear relationships and decrease of 63-77% in the petal h^0 values by day 12 compared to day 0 (Figure 9D). In flowers in vase solutions containing 15 (R^2=0.88) and 20 (R^2=0.90) mg TCLE/L, the h^0 colour values of petals showed similar significant linear relationships and a reduction of 63-77% in the h colour values of petals by day 12 compared to day 0 (Figure 9D).

There was a significant negative linear relationship ($p \leq 0.05$) between C* values of petals and days in vase solution containing 8-HQC (Figure 10). The C* colour values of petals in the 8-HQC vase solutions showed a decrease of 44% at day 10 (R^2=0.80). However, the DCLE-Jc+Pg, showed significant negative cubic relationships were observed at 5 (R^2=0.91), 10 (R^2=0.93), 15 (R^2=0.90) and 20 (R^2=0.91) mg/L, with 50-66% decrease by day 10 compared to day 0 (Figure 10A). The DCLE-Jc+Ap, showed significant negative and linear relationships at 10 (R^2=0.94), 15 (R^2=0.91) and 20 (R^2=0.93) mg/L, with 49-56% decrease by day 10 compared to day 0 (Figure 10B). However, a significant, negative quadratic relationship was observed at 5 mg/L (R^2=0.94). The petals in the DCLE-Pg+Ap showed significant negative cubic relationships at 5 (R^2=0.90), and 10 (R^2=0.88) mg/L while the 15 (R^2=0.85) and 20 (R^2=0.91) mg DCLE/L vase solutions had negative linear trends of C* values, with 32 to 36% reductions by day 10 compared to day 0 (Figure 10C). The TCLE treated vase solutions containing 5 (R^2=0.83), 10 (R^2=0.90), 15

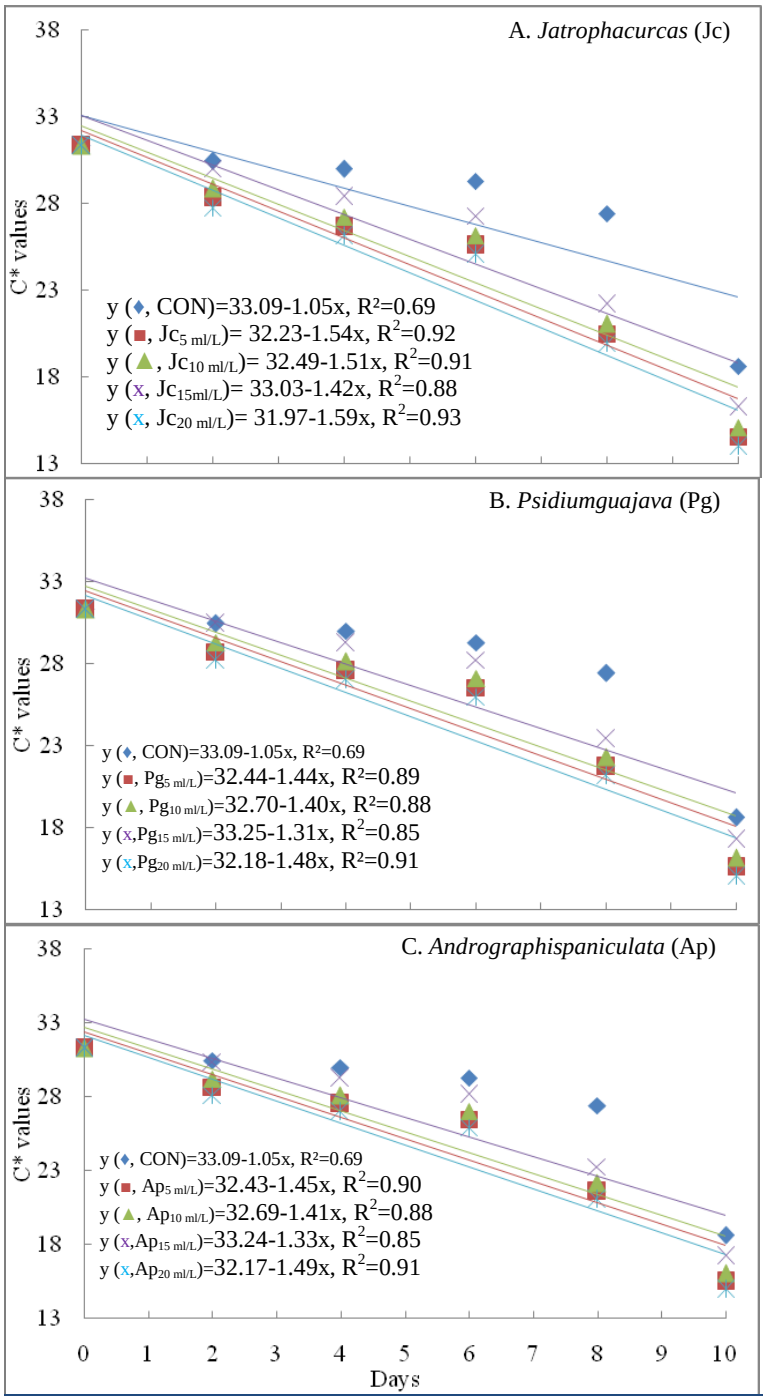

Figure 7. Relationship between C* colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extracts (SLE) of *J. curcas* (A), *P. guajava* (B) and *A. paniculata* (C) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

(R^2=0.88) and 20 (R^2=0.90) mg/L, showed similar significant negative cubic relationships, with a decrease of 53-76% in the petal C* values by day 12 compared to day 0 (Figure 10D).

There was a significant negative linear relationship ((R^2=0.80) between L* values of petals and days in vase decrease of 37% at day 10. The L* values of flower petals in the DCLE-Jc+Pg showed similar significant neg-

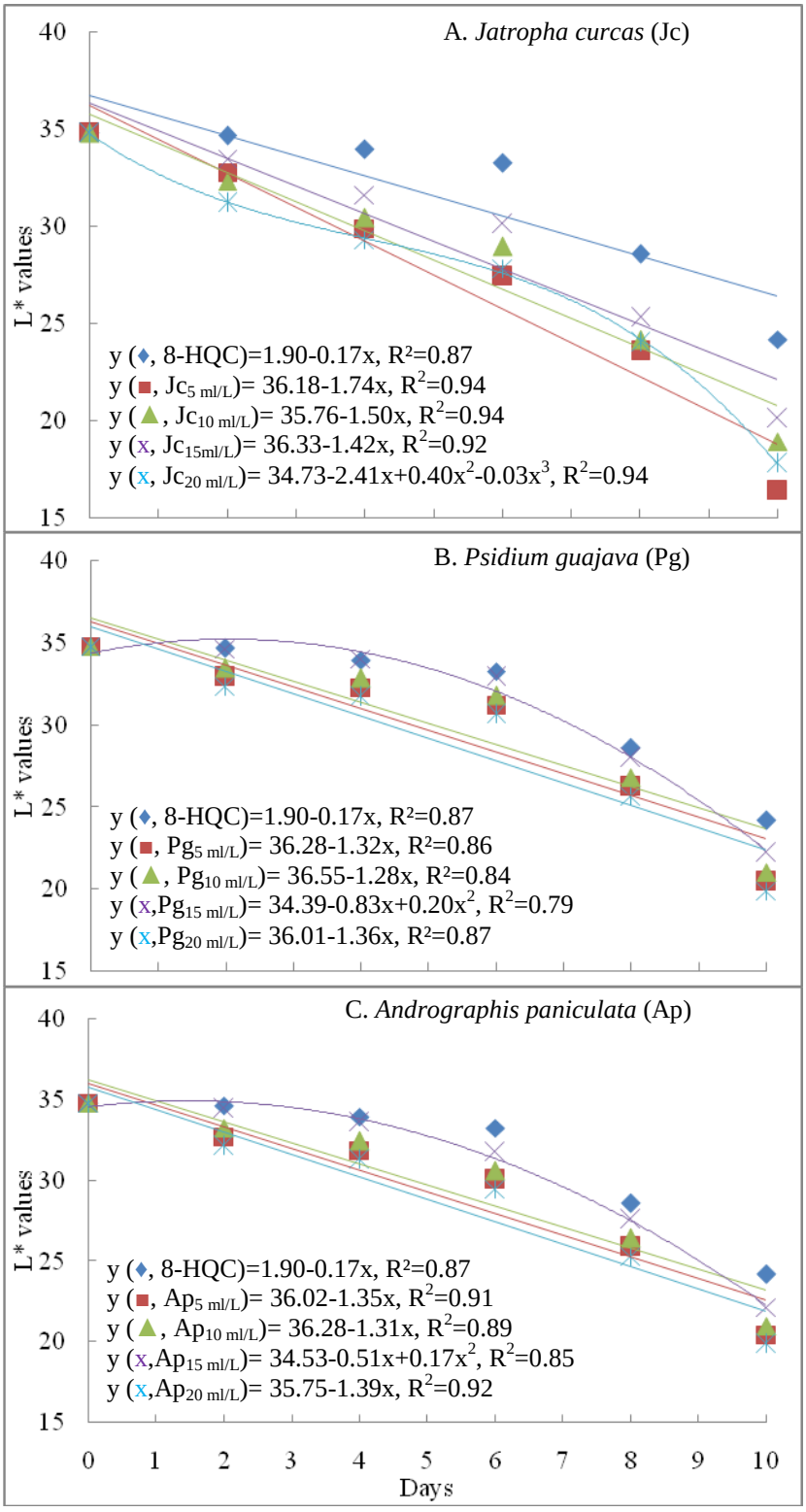

Figure 8. Relationship between L* colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extracts (SLE) of *J. curcas* (A), *P. guajava* (B) and *A. paniculata* (C) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

ative linear relationships ($p \leq 0.05$) when regressed with days in vase solutions containing 5 (R2=0.95), 10 (R^2=0.95) and 15 (R^2=0.94) mg/L (Figure 11A). However, there was a significant negative quadratic relationship

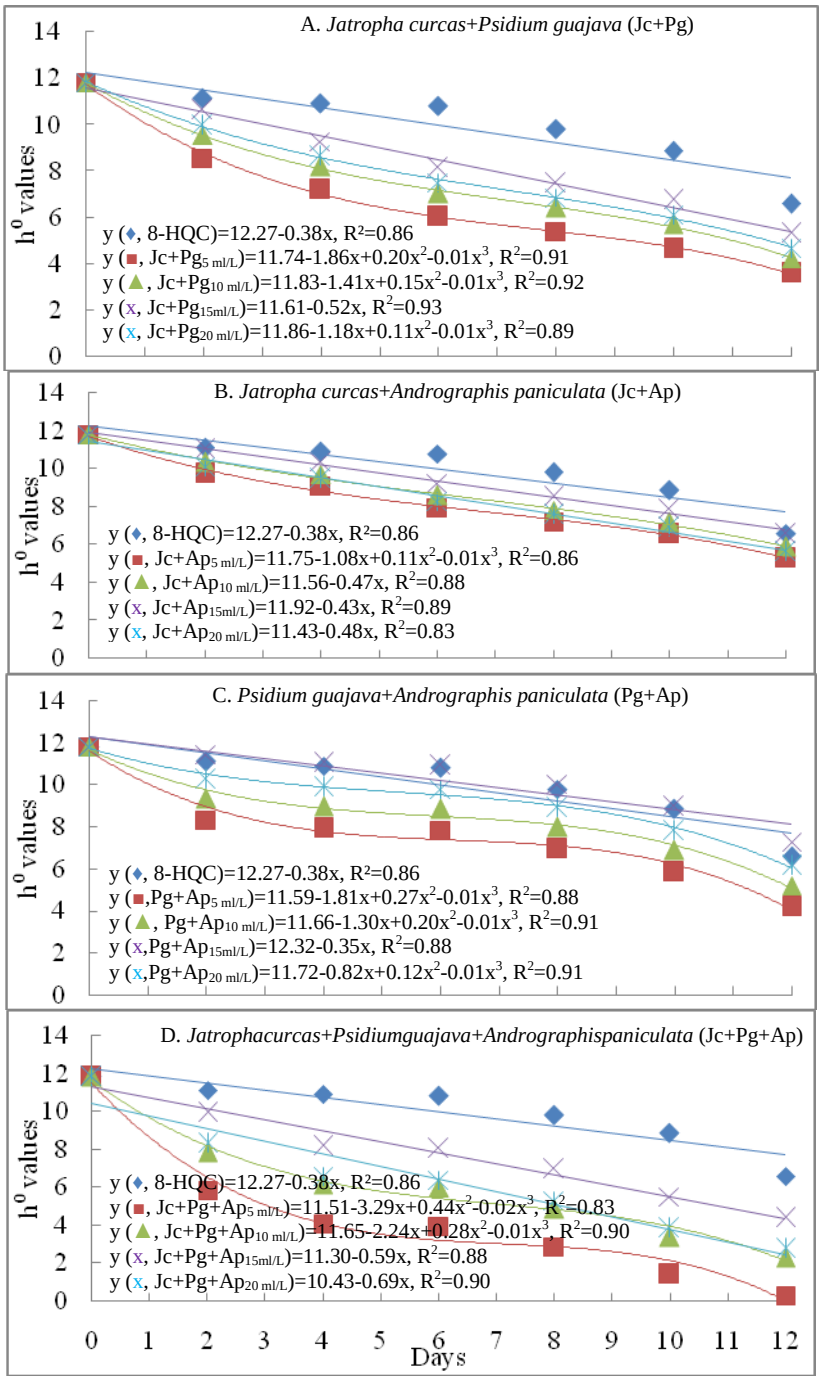

Figure 9. Relationship between h° colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) of *J. curcas+P. guajava* (A), *J. curcas+A. paniculata* (B) and *P. guajava+ A. paniculata* (C), and triple combination leaf extracts (TCLE) of *J. curcas+P. guajava+ A. paniculata*(D) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

(R^2=0.93) between L* values and days in the 20 mg/L DCLE-Jc+Pg vase solution, with a 47% decrease in L* value by day 10 (Figure 11A). The petals in the DCLE-Jc+Ap, showed significant negative linear relationships of L* values at 5 (R^2=0.86) and 15 (R^2=0.85) mg/L, with a 33-42% decrease by day 10 (Figure 11B). However, a significant negative cubic relationship occurred in the 10 mg/L (R^2=0.87) vase solution, while a quadratic relationship

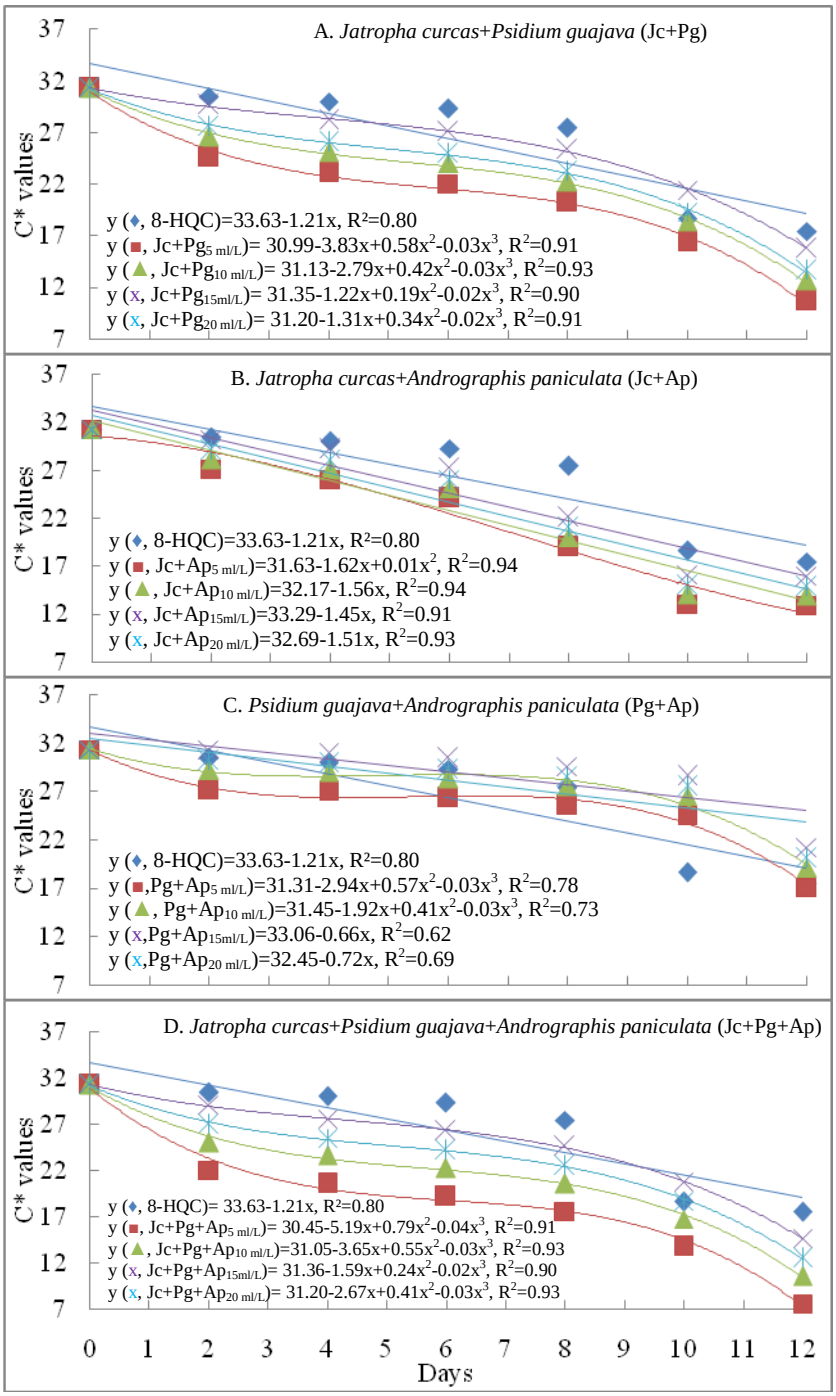

Figure 10. Relationship between C* colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) of *J. curcas+P. guajava* (A), *J. curcas+A. paniculata* (B) and *P. guajava+ A. paniculata* (C), and triple combination leaf extracts (TCLE) of *J. curcas+P. guajava+ A. paniculata*(D) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

was observed at 20 mg/L (R2=0.89) vase solution. The vase solutions containing 5 (R2=0. 92) and 10 (R2=0. 91) mg/L DCLE-Pg+Ap each showed significant negative cubic relationships, with 60-68% lower L* values by day 10. The 15 (R2=0. 87) and 20 (R2=0. 86) mg/L DCLE-Pg+Ap vase solutions had significant negative linear trend of C* values, with a 48-54% decrease in L* values by day 10 compared to the initial colour (Figure 11C).

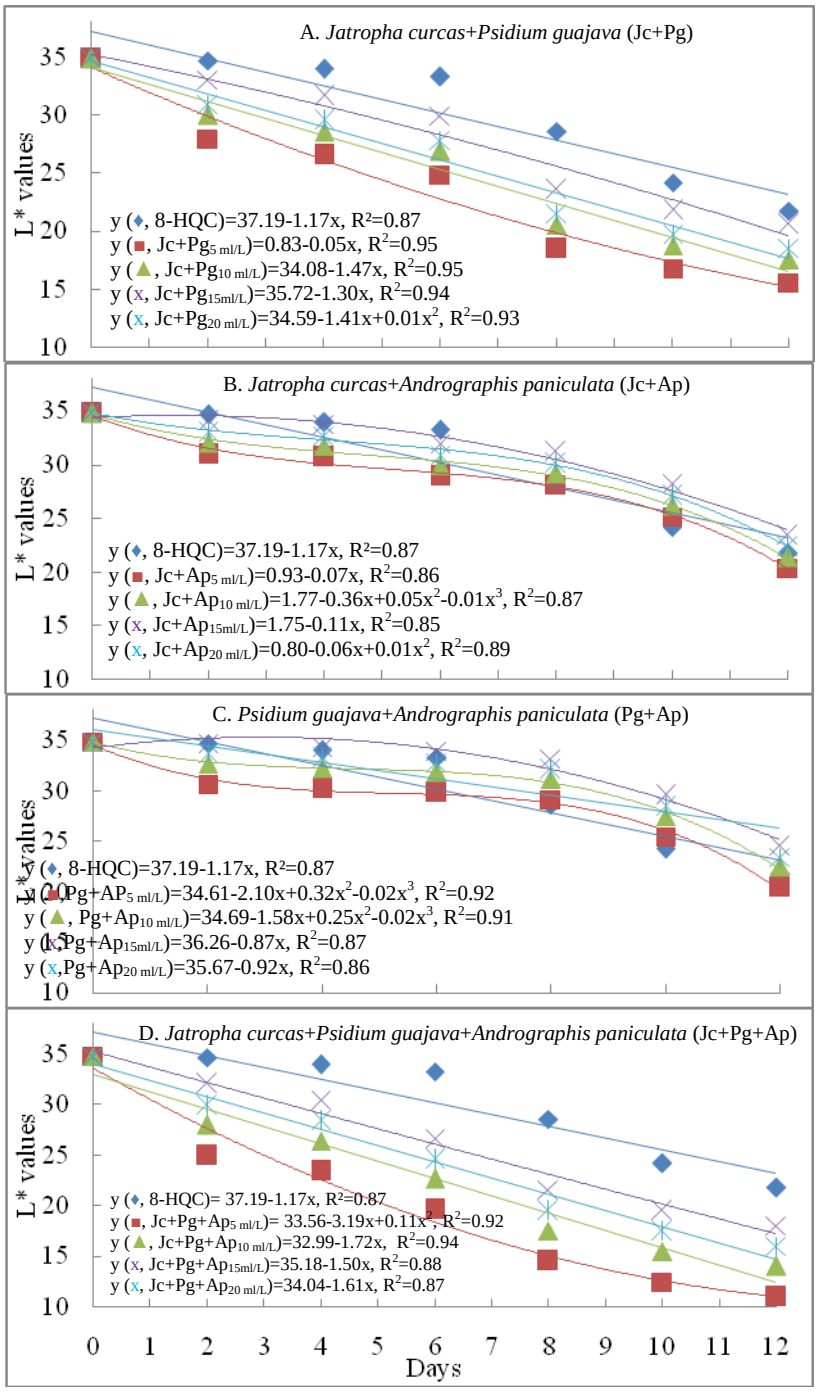

Figure 11. Relationship between L* colour values and days in floral preservative solutions containing (♦) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) of *J. curcas+P. guajava* (A), *J. curcas+A. paniculata* (B) and *P. guajava+ A. paniculata* (C), and triple combination leaf extracts (TCLE) of *J. curcas+P. guajava+ A. paniculata*(D) at concentrations of 5 (■), 10 (▲), 15 (x) and 20 (x) mg/L [Mokara Red orchid flowers; Each of the floral preservative solutions contains 2% sucrose and 3% citric acid; A solid line indicates a significant relationship $p \leq 0.05$. n=5].

The TCLE treated vase solutions containing 5 mg/L of each leaf extract showed significant negative quadratic relationships (R^2=0.83) and a decrease of 68% in the petal L* values by day 12 compared to day 0. The L* values of flower petals in the TCLE showed significant negative linear relationships at 10 (R2=0.94), 15 (R2=0.88) and 20 (R2=0.87) mg/L, with decreases of 48-60% in the petal L* values by day 12 (Figure 11D).

The DCLE-Pg+Ap leaf extracts were most effective in maintaining the petal colour values, reducing early wilting and prolonging vase life. Obviously, the reduction in colour of the flower petals affected the vase life of the flowers. Similarly, Rahman et al. (2012) reported an increase in colour values of carnation flowers indicating good colour retention with longer vase life. Cut flowers with sugar added maintain the respiratory substrates, resulting in better petals, thus promoting respiration and extending flower vase life (Chandran et al., 2006).

Bud Opening and Floret Drop

Bud opening of cut flowers in the 15 mg/L SLE-Jc, 10 and 15 mg/L SLE-Pg and 15 mg/L SLE-Ap vase solutions were not significantly ($p \leq 0.05$) different compared to bud opening of cut flowers in the 8-HQC control (Table 6). The bud opening of cut flowers in the 5 and 20 mg SLE/L treatments were significantly lower (30-67%) compared to bud opening of cut flowers in the control (78%) (Table 6).The lower percentage of bud opening of flowers in the SLE vase solutions could be attributed to insufficient nutrition or food source as reported by Nowak and Rudnicki (1990). Moreover, the presence of microbes in the vase solutions blocked the xylem vessels resulting in reduced rates of preservative solutions uptake. Nevertheless, flowers that did not get sufficient carbohydrate resulted in lower percentage of bud openings and reduced longevity of cut flowers.

The bud opening of cut flowers were not significantly ($p \leq 0.05$) different in the 10 and 15 mg/L DCLE vase solution treatments compared to bud opening of cut flowers in the control (Table 7). The bud opening of flowers in the 5 and 20 mg/L DCLE treated preservative solutions were significantly lower (16-48%) compared to bud opening of cut flowers in the control. All concentrations of TCLE treatments had significantly lower (12-44%) bud opening compared to flowers in the control. Furthermore, the bud opening of cut flowers in all concentrations of TCLE treatments were significantly lower compared to the DCLE treatments. Thus, the DCLE preservative solutions could be used to improve bud opening similar to the control because of the synergistic effects. The flower stems possessed high sucrose inversion capacity that promoted percentage of bud opening in *Dendrobium* inflorescences and decreased abscission of flower buds (Pattaravayo et al., 2013). In cut inflorescences, the flower buds often do not open as well as in uncut stems. This is often due to the problem with water relations (van Doorn, 1997) and inadequate supply of sugar to the growing buds (van Doorn, 2004). In the present study, the low rate of bud opening in the SLE treated preservative solutions could be due to one of these or both causes. Furthermore, the SLE preservative solutions could not control the abundant number of microbes in the solutions, while the DCLE treated preservative solutions could control the microbes, resulting in more bud opening and less floret drop, as well as extended vase life of flowers. The processes of flower bud opening and colour development require substrates and energy for their satisfactory development in cut flowers (Thwala et al., 2013). Moreover, sucrose that was added to the preservative solutions, supplies cut flowers with substrates that are needed for respiration, thus enabling harvested buds to open into flowers (Pun and Ichimura, 2003).

The floret drop of cut flowers, in all the 10 and 15mg /L SLE preservative solutions and 5, 10 and 15 mg/L SLE-Ap preservative solutions were not significantly ($p \leq 0.05$) different compared to the floret drop of cut flowers in the 8-HQC control (Table 6). The floret drop of cut flowers in all the 5 and 20 mg/L SLE preservative solutions were significantly higher (10-67%) compared to floret drop of cut flowers in the 8-HQC control (7%) (Table 6).The SLE treated preservative solutions could result in insufficient nutrients to plant cells, where carbohydrate is converted to energy, resulting reduced vase solution uptake (Filip et al., 2009).

In all the 10 and 15 mg/L DCLE preservative solutions treatments, floret drop of cut flowers were not significantly ($p \leq 0.05$) different compared to floret drop of cut flowers in the 8-HQC control (Table 7). In all the 5 and 20 mg/L DCLE treatments, the floret drop of cut flowers were significantly higher (26-65%) compared to floret drop of cut flowers in the control. All concentrations of TCLE treatments had significantly higher (48-80%) floret drop compared to flowers in the control (Table 7). Moreover, the floret drop of TCLE treated flowers were higher compared to DCLE treated flowers. This indicates that the DCLE treated flowers maintained sufficient nutrients and food supply compared to TCLE treated flowers. Similarly, preservatives with the combination of lemon, sprite, rite brand bleach; and lime, sugar and Listerine, reduced petal drop and floret wilting in *Epidendrum* orchids (Thwala et al., 2013). Pattaravayo et al. (2013) reported that sugar added with antimicrobial compounds promoted bud opening of cut *Dendrobium* flowers and largely prevented abscission of open flowers.

Selection of Double Combination Leaf Extracts (DCLE) on Microbial Populations in Floral Preservative Solutions of Cut Mokara Red Orchid Flowers

Vase Life as Affected by Microbes

The floral preservative solutions containing 15 mg/L DCLE-Pg+Ap of each extract, had double the bacterial counts, while preservative solutions containing DCLE-

Table 6. The effect of floral preservative solutions containing control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and single leaf extracts (SLE) on cut Mokara Red orchid flowers bud opening and floret drop [Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment		Bud opening (%)	Floret drop (%)
8-HQC	8-hydroxyquinoline citrate	78 a[z]	07 d
	Jatropha curcas (mg/L)		
SLE-Jc	5	27 cd	43 abc
	10	37 cd	30 bcd
	15	53 abc	13 cd
	20	10 d	67 a
	Psidium guajava (mg/L)		
SLE-Pg	5	37 cd	30 bcd
	10	53 abc	17 cd
	15	70 ab	10 d
	20	26 cd	60 ab
	Andrographis paniculata (mg/L)		
SLE-Ap	5	33 cd	37 abcd
	10	50 bc	27 cd
	15	67 ab	10 d
	20	13d	63 a

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0. [z]Means, followed by the same letters within each column, are not significantly different by DMTR ($p \leq 0.05$). n=5.

Jc+Ap had six times higher bacterial counts compared to the control containing 8-HQC preservative solutions (Figure 12).

The 8-hydroxyquinoline (8-HQ) is a conjugated system, and at the same time, it is a bi-functional hydrogen-bonding molecule, which as a protic solvent, simultaneously acts as an H donor at the O-H group and as a H acceptor at the N atom (Filip et al., 2009; Ketsa et al., 1992).Microbial growth in preservative solutions were found to be affected by 8-HQC, either in preservative solutions or as a pulse treatment. This could restrict microbial growth and subsequent vascular blockage, thus promoting water uptake (Anjum et al., 2001). However, 8-HQ may have physiological (temperature, water relations, carbohydrate supply and growth regulator) effects on the flowers (Marousky, 1971).

The 15 mg/L DCLE-Jc+Ap treated preservative solutions were significantly ($p \leq 0.05$) different in controlling fungal growth compared to DCLE-Pg+Ap and 8-HQC (Figure 13). The 15 mg/L DCLE-Jc+Ap treated preservative solutions had doubled the fungal growth compared to the 8-HQC control. The DCLE-Pg+Ap treated preservative solutions had five times higher fungal growth compared to DCLE-Jc+Ap preservative solutions (Figure 13).Traces amounts of fungi growth was found in the 8-HQC preservative solutions. Thus, by incorporating 8-HQC either in the preservative solutions or as a pulse treatment, would restrict the microbial growth and subsequent vascular blockage and thus promote water uptake (Chand et al., 2012). However, synthetic germicides containing a heavy metal could pollute the environment and has a harmful effect on human wellness. Flowers in the DCLE-Pg+Ap preservative solutions showed less bacterial counts. The *P. guajava* and *A. paniculata* leaf extracts showed antimicrobial and antibacterial activity (Suhaila et al., 2009; Singha et al., 2003). Bacterial populations developing in the stems of cut carnation flowers during the vase life leads to vascular occlusion, but it had small effects on flower longevity (van Doorn et al., 1991c). The uptake of solutions was inhibited only at bacterial concentrations higher than 10^7 cfu/mL, while rose stems containing a mixed population of bacteria was added to the vase water (van Doorn et al., 1991c).

Microscopic Observations of Floral Preservative Solutions

More gram-positive *Coccus* spp., and gram-negative *Coccus* spp. bacteria and three fungi, *Fusarium* spp, *Penicillium* spp. and *Alternaria* spp. were observed in the of SLE treated floral preservative solutions compared to

Table 7. The effect of floral preservative solutions containing control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), double combination leaf extracts (DCLE) and triple combination leaf extract (TCLE) on bud opening and floret drop [The DCLE combination contains two leaf extracts and TCLE combination contains three leaf extracts; Leaf extracts comprised of *Jatropha curcas* (Jc), *Psidium guajava* (Pg) and *Andrographis paniculata* (Ap)].

Treatment				Bud opening (%)	Floret drop (%)
8-HQC	8-hydroxyquinoline citrate			78 a[z]	7 h
	Jatropha curcas (mg/L)	*Psidium guajava* (mg/L)			
DCLE-Jc+Pg	5	5		24 fgh[z]	64 abc
	10	10		52 abcdef	36 cdefgh
	15	15		73 ab	24 efgh
	20	20		16 gh	76 ab
	Jatropha curcas (mg/L)	*Andrographis paniculata* (mg/L)			
DCLE-Jc+Ap	5	5		40 defgh	44 cdef
	10	10		56 abcde	32 defgh
	15	15		77 ab	16 fgh
	20	20		24 fgh	56 abcd
	Psidium guajava (mg/L)	*Andrographis paniculata* (mg/L)			
DCLE-Pg+Ap	5	5		48 bcdef	40 cdef
	10	10		64 abcd	24 efgh
	15	15		80 a	12 gh
	20	20		28 efgh	56 abcd
	Jatropha curcas (mg/L)	*Psidium guajava* (mg/L)	*Andrographis paniculata* (mg/L)		
TCLE-Jc+Pg+Ap	5	5	5	16 gh	76 ab
	10	10	10	28 efgh	64 abc
	15	15	15	44 cdef	48 bcde
	20	20	20	12 h	80 a

To each floral preservative solutions treatment, 2% sucrose was added as a carbohydrate source and 3% citric acid was added to maintain the floral preservative solutions at pH 3.0. [z]Means, followed by the same letters within each column, are not significantly different by DMTR ($p \leq 0.05$). n=5.

the 15 mg/L DCLE-Pg+Ap preservative solutions (Figure 14). Similarly, Gerbera and rose cut flowers have shown access by microorganisms *Bacillus, Fusarium, Kluyveromyces* and *Pseudomonas* spp into xylem vessels (Put, 1990). Additionally, Put (1990) found gram positive *Streptococcus* (*S. lactis*) groups in the micro-flora from freshly harvested, cut chrysanthemums and Gerbera flowers in the vase water after 12-15 days of vase life. In the vase solutions of cut roses, many different kinds of bacteria, yeasts and fungi have been identified (Put, 1990; van Doorn and Witte, 1997). In the vase solutions in the present study, six different microorganisms were found which were two fungi and four bacteria. In carnation vase solutions, Zagory and Reid (1986) identified 25 different microorganisms. The isolated fungi were two different strains of *Fusarium solani* which were observed in CA solutions (Jowkar et al., 2012). While in an experiment on *Narcissus tazetta*, the only fungus found in CA vase solutions was *Aspergillu s*sp. (Jowkar, 2006). This indicates that the CA is not capable of efficiently preventing fungus growth. Among the six different separated bacterial colonies, two were *Bacillus*, one was *Coccus* and one colony was *Streptomyces* spp. The *Pseudomonads* and *Enterobacteria* were the dominant bacterial strains in stems of cut 'Sonia' roses (van Doorn and Witte, 1997).

CONCLUSION

In the present study, SLE treatments did not extend the cut flowers' vase life, because of the higher pH values of preservative solutions, resulting in lower rates of preservative solutions uptake, flower fresh weights and bud opening. In addition, fading petals was high, resulting in more floret drop. The DCLE of *J. curcas*, *P.*

guajava or *A. paniculata* had synergistic effects on the preservative solutions pH values. The DCLE-Jc+Ap and DCLE-Pg+Ap had synergistic effects and could hold the pH values on preservative solutions, resulting in extended vase life of the cut flowers. Concurrently, low pH values were found in 15 mg/L DCLE-Jc+Ap and DCLE-Pg+Ap preservative solutions. Moreover, flowers in the above two preservative solutions retained better petal colour than the other treated flowers. Hence, leaf extracts of *A. paniculata* in combination with *P. guajava* and *J. curcas* has the potential to minimize pH values in preservative solutions, thus extending the vase life. Nevertheless, in this study SLE had shorter vase life in preservative solutions compared to the control containing 8-HQC. Therefore, DCLE-Pg+Ap was used in subsequent experiments to evaluate the long vase life of cut flowers with higher rates of preservative solutions

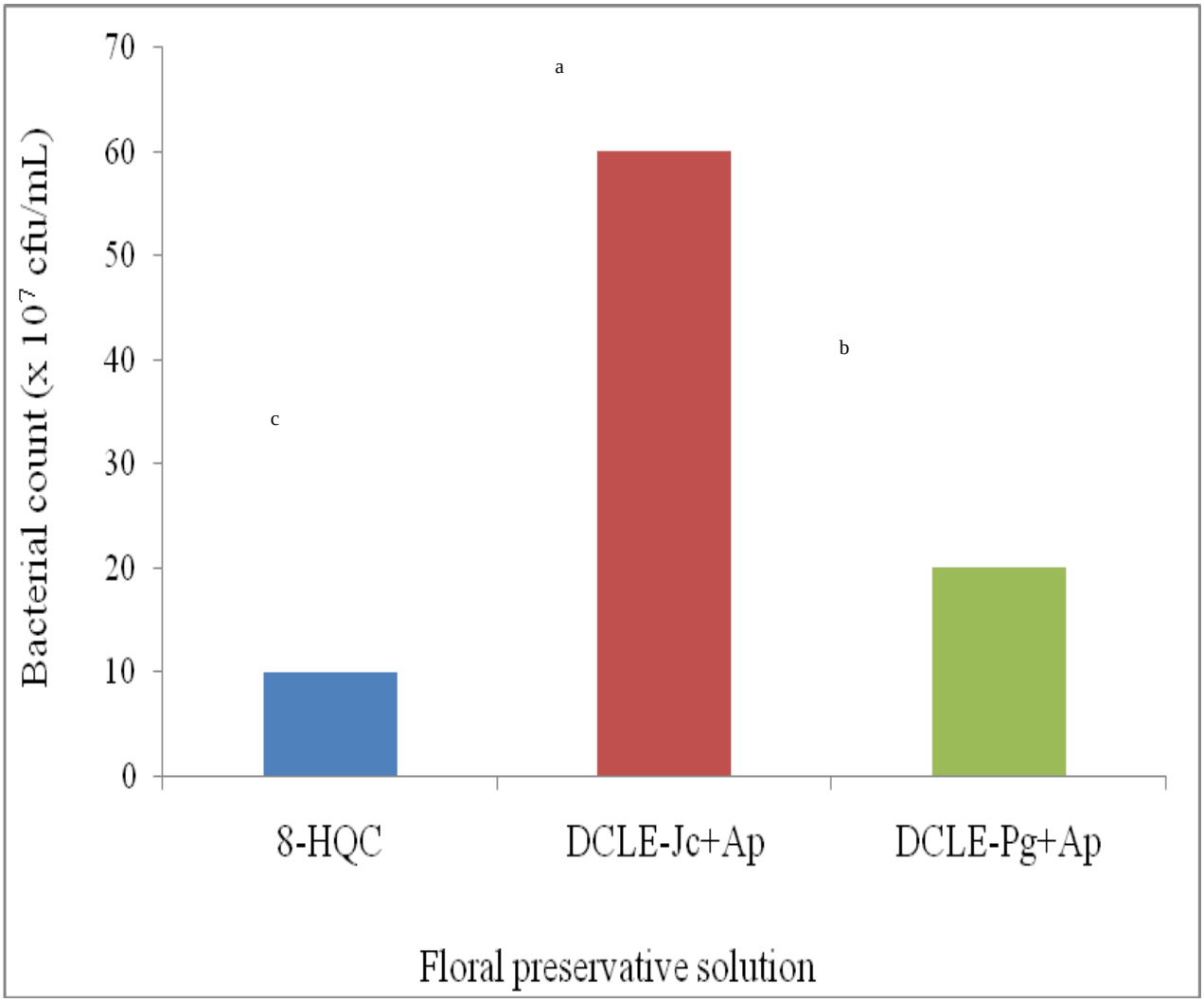

Figure 12. Bacterial counts on floral preservatives solutions containing (■) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and double combinations leaf extracts (DCLE) of (■) *J. curcas+A. paniculata* (Jc+Ap) and (■) *P. guajava+A. paniculata* (Pg+Ap). [Mokara Red orchid flowers; each of the floral preservative solution contains 2% sucrose and 3% citric acid. The DCLE of Jc+Ap and Pg+Ap contain 15 mg/L leaf extracts each. Means on each column, followed by different letters, are not significantly different by DMRT ($p \leq 0.05$). n=5.

uptake. The preservative solutions contained two groups of gram-positive *Coccus* spp. and gram-negative *Coccus* spp. bacteria. There were three fungi, *Fusarium* spp., *Penicillium* spp. and *Alternaria* spp., in the preservative solutions. The 15 mg/L DCLE-Pg+Ap preservative solutions had a lower bacterial count compared to the 15 mg/L DCLE-Jc+Ap. Nevertheless, the DCLE-Pg+Ap had higher fungi growth compared to DCLE-Jc+Ap and 8-HQC. Therefore, DCLE-Pg+Ap had the potential as a natural preservative solutions to extend the vase life of orchid flowers by 3 days compared to the control treatment with 8-HQC.

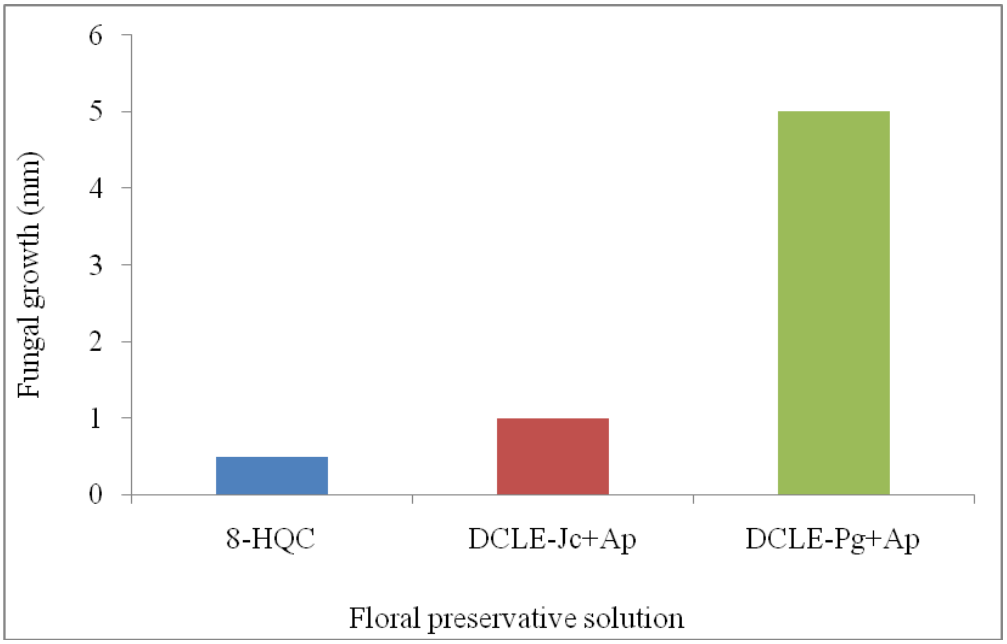

Figure 13. Fungal growth on floral preservative solutions containing (■) control (125 mg/L 8-hydroxyquinoline citrate, 8-HQC), and double combinations leaf extracts (DCLE) of (■) *J. curcas*+*A. paniculata* (Jc+Ap) and (■) *P. guajava*+*A. paniculata* (Pg+Ap). [Mokara Red orchid flower; each of the floral preservative solutions contains 2% sucrose and 3% citric acid. The DCLE of Jc+Ap and Pg+Ap contain 15 mg/L leaf extracts each. Means on each column, followed by different letters, are not significantly different by DMRT ($p \leq 0.05$). n=5.

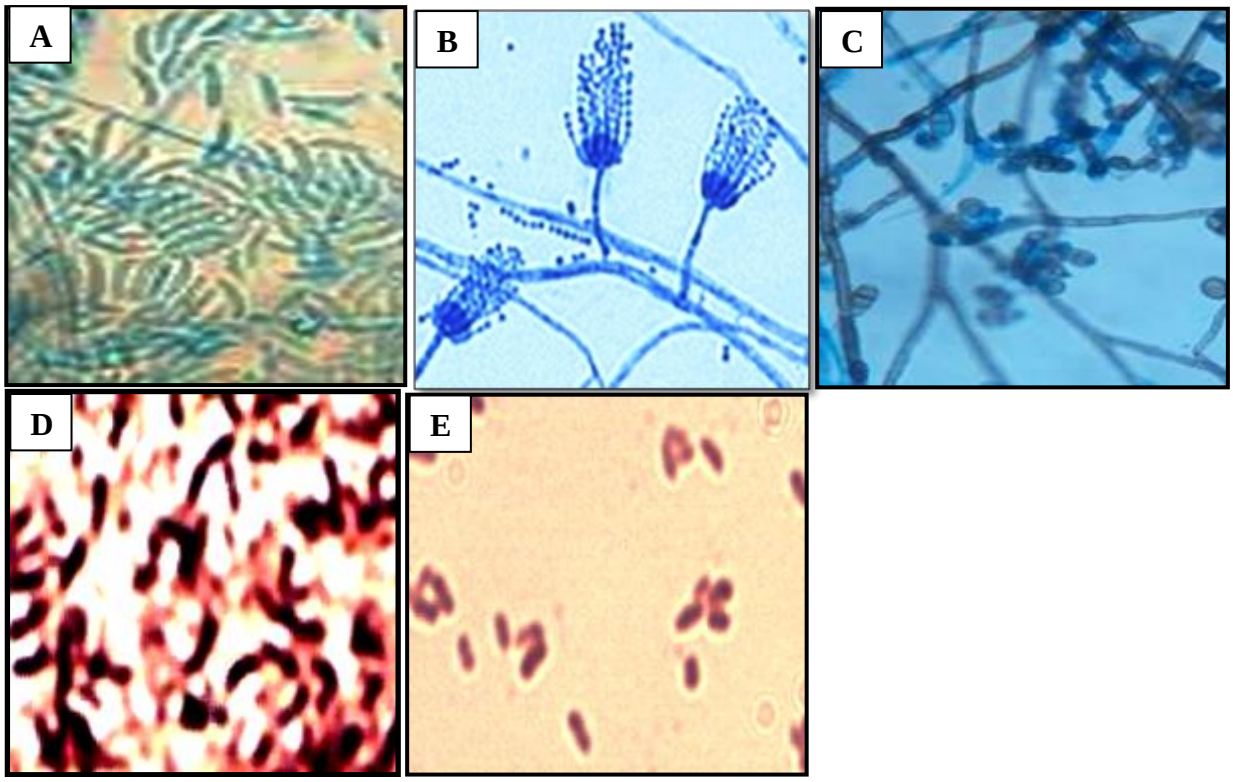

Figure 14. Microscopic observations of fungi and bacteria. [A) *Fusarium* spp. Morphological features represent micro conidia and a single chlamydo spore, B) *Penicillium* spp .represent single and branching of conidiophores, C) *Alternaria* spp. represents hyphae with conidiophores single or branching, and club-like appearance of the conidia, D) Gram-negative *Coccus* spp. represents pink-rod shaped or chain forming bacteria, and E) Gram-positive *Coccus* spp. represents a purple chain forming bacteria respectively; Scale bar 100 µm.].

LIST OF ABBREVIATIONS

8-HQC:	8-hydroxyquinoline citrate
$AgNO_3$:	Silver nitrate
CA:	Citric acid
cfu:	Colony forming units
SLE:	Single leaf extract
DCLE:	Double combination leaf extracts
Pg:	*Psidium guajava*
Ap:	*Andrographis paniculata*
Jc:	*Jatropha curcas*
TCLE:	Triple combination leaf extracts

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DISCLOSURE POLICY

The author(s) declare(s) that there is no conflict of interests regarding the publication of this paper.

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