UNIVERSITI PUTRA MALAYSIA

VASE LIFE ENHANCEMENT OF MOKARA RED ORCHID WITH Jatropha curcas L., Psidium guajava L. AND Andrographis paniculata (Burm.f.) Wall. ex Nees LEAF EXTRACTS

MD. MUKLESUR RAHMAN

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By

MD. MUKLESUR RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2014

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DEDICATION

THIS THESIS IS DEDICATED TO

MY HONOURABLE PARENTS AND PARENTS IN LAW

AND

MY BELOVED WIFE SHAMMI AKHTER

WHO BELIEVED IN MY ABILITIES AND ALWAYS INSPIRED ME IN MAKING SOME OF MY GOALS COME TRUE

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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May 2014

Chairperson: Siti Hajar Binti Ahmad, PhD
Faculty: Agriculture

A study was conducted on the effect of leaf extracts from *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata* on longevity of cut Mokara Chark Kuan 'Red' orchid flowers. A major problem in cut flowers is shortening of vase life due to blockage of xylem vessels by microorganisms or air bubbles (bacteria and their products), thus, reducing water uptake. Mature green leaves, below the youngest shoot of each *J. curcas* and *P. guajava* branches, were collected and used for this experiment. In case of *A. paniculata*, 6 to 8 weeks-old leaves and stems were collected. Bioactive antimicrobial phytochemical compounds were identified in leaf extracts by gas chromatography-mass spectrometry (GC-MS). Export-grade cut Mokara orchid flowers, with 75% opened florets, were purchased from a commercial grower. Each flower was treated with vase solutions containing a commercial flower preservative, 8-Hydroxyquinoline citrate (8-HQC), and three natural flower preservatives from leaf extracts of *J. curcas*, *P. guajava* or *A. paniculata*. The flower preservative treatments comprised i) Control (125 mg 8-HQC/L) and, ii) Single leaf extract (SLE), iii) Double combinations leaf extracts (DCLE) and iv) Triple combinations leaf extracts (TCLE) at 5, 10, 15 and 20 mg/L. Sucrose (2%) and citric acid (3%) were added to each vase solution. The experiments were conducted using a completely randomized design, with five replications.

J. curcas leaf extract contained nine bioactive antimicrobial compounds identified by GC-MS. The five major antimicrobial compounds in the extract were 9-hexadecenoic acid; 10-octadecenoic acid, methyl ester; 9,12-octadecadienoicacid (Z,Z); 9,12-octadecadienoicacid, methyl ester; and n-hexadecoic acid. Sixty-six bioactive antimicrobial compounds were identified in the *P. guajava* leaf extract. The most active antimicrobial compounds contained in the leaf extract were squalene; phytol; bicyclo; and azulene. In the *A. paniculata* leaf extract, 29 antimicrobial compounds were

identified. The three unique antimicrobial compounds were hexadecanoic acid, methyl ester; 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z)-; and 9,12-octadecadienoicacid, methyl ester. These findings indicate that the leaf extracts of *J. curcas*, *P. guajava* and *A. paniculata* could be used in agricultural applications as a source for natural flower preservative or biocide against microbes in the vase solution of cut flowers.

The SLE treatments did not extend cut flower vase life due to high floret drop and fading of petals. Lower pH was found in the 15 mg DCLE-Pg+Ap/L treated vase solutions. Moreover, flowers in both vase solutions retained better petal colour than other treated flowers. However, in this study the SLE treatments had shorter flower shelf life compared to 8-HQC. Therefore, 15 mg DCLE-Pg+Ap/L were used for the subsequent experiments to evaluate longer shelf life of cut flower. The SLE treatments were not used for further experiments due to the short vase life of flowers. The 15 mg DCLE-Pg+Ap/L treated vase solution had lower bacterial count compared to the 15 mg DCLE-Jc+Ap/L. The 15 mg DCLE-Pg+Ap/L was more effective in vase solution uptake compared to other treatments. In *P. guajava* leaves extracts showed antimicrobial activities, while *A. paniculata* showed both antifungal and antibacterial activities. Therefore, DCLE-Pg+Ap had the potential as a natural preservative solution to extend vase life of cut flowers to 3 days compared to 8-HQC by reducing microbial growth.

The pre-treatment pulsing with 5 mg AgNP/L for 24 h, was placed in leaves extract of *P. guajava* and *A. paniculata* (AgNP5+DCLE-Pg+Ap) treated vase solution effectively controlled microbial xylem blockage. AgNP5+DCLE-Pg+Ap had the potential as a natural preservative in minimizing microbial populations, and extending vase life of cut flowers. The results indicated that flowers treated with vase solution containing AgNP5+DCLE-Pg+Ap had a higher rate of solution uptake compared to flowers treated with 8-HQC. The 8-HQC vase solution contained trace amount of bacterial suspension of 5×10^8 colony-forming units (CFU)/mL of gram positive and gram negative bacteria and fungi as well as in AgNP5+DCLE-Pg+Ap treatment vase solution. Flower stems in AgNP5+DCLE-Pg+Ap treated vase solution showed better petal colour, as indicated by the L*, C* and h° values compared to 8-HQC. Thus, leaf extracts of *P. guajava* and *A. paniculata* have the potential as a cut flower preservative to minimize microbial populations and extend 7 more days vase life of cut flowers compared to 8-HQC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENINGKATAN JANGKA HAYAT KERATAN BUNGA ORKID MOKARA MERAH DENGAN EKSTRAK DAUN *Jatropha curcas* L., *Psidium guajava* L. AND *Andrographis paniculata* (Burm.f.) Wall. ex Nees

Oleh

MD. MUKLESUR RAHMAN

Mei 2014

Pengerusi: Siti Hajar Binti Ahmad, PhD
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Satu kajian telah dijalankan ke atas kesan ekstrak daun *Jatropha curcas, Psidium guajava* dan *Andrographis paniculata* terhadap jangka hayat bunga keratan orkid Mokara Chark Kuan 'Red'. Daun hijau matang yang berada di bawah pucuk *J. curcas* dan *P. guajava* telah dikumpulkan dan digunakan dalam eksperimen ini. Daun dan dahan *A. paniculata*, yang berumur enam hingga lapan minggu telah digunakan. Bioaktif sebatian antimikrob dikaji dalam ekstrak daun melalui kaedah gas kromatografi-spektrometri jisim (GC-MS). Bunga keratan Mokara orkid bergred ekspot dengan 75% bunga terbuka telah diperolehi daripada pengusaha komersial. Setiap bunga dirawat dengan larutan jambangan yang mengandungi pengawet bunga komersial iaitu 8-hidrooksikuilonin sitrat (8-HQC) dan tiga jenis pengawet semula jadi daripada ekstrak dedaun *J. curcas*, *P. guajava* atau *A. paniculata*. Rawatan pengawet bunga terdiri daripada: i) Kawalan yang mengandungi (125 mg 8-HQC/L), ii) Ekstrak daripada satu daun (SLE) tanpa kombinasi iii) Kombinasi ekstrak dua daun (DCLE) dan iv) Kombinasi ekstrak tiga daun (TCLE) *J. curcas*, *P. guajava* atau *A. paniculata* pada kepekatan 5, 10, 15 dan 20 mg/L. Sukrosa (2%) dan asid sitrik (3%) telah ditambah pada setiap rawatan. Kajian telah dilakukan dengan reka bentuk rawak penuh lengkap, menggunakan lima replikasi.

Berdasarkan analisis GC-MS, sejumlah sembilan sebatian antimikrob telah dikenal pasti dalam ekstrak daun *J. curcas*. Lima sebatian antimikrob utama iaitu asid 9-heksadesinoik; asid 10-oktadekanoik, metil ester; asid 9,12-oktadekadienoik (Z,Z); asid 9,12-oktadekadienoik, metil ester; dan asid n-heksadekoik. Dalam ekstrak daun *P. guajava*, sejumlah 66 sebatian antimikrob telah dikenal pasti. Di antaranya ialah empat sebatian antimikrob yang aktif iaitu squalena; fitol; bisiklo; dan azulena. Dalam ekstrak daun *A. paniculata*, sebanyak 29 bahan kimia anti-mikrob telah dikenal pasti. Tiga sebatian antimikrob iaitu asid heksadekanoik, metil ester; 9, 12,15-oktadekatrienoik,

metil ester, (Z,Z,Z)-; dan asid 9, 12-oktadekadienoik, metil ester merupakan sebatian yang unik ditemui daripada ekstrak tersebut. Penemuan ini menunjukkan bahawa ekstrak dedaun *J. curcas*, *P. guajava* dan *A. paniculata* boleh digunakan dalam aplikasi pertanian sebagai sumber pengawet bunga atau biosida semula jadi terhadap mikrob dalam larutan jambangan bagi bunga keratan.

Rawatan SLE tidak melanjutkan jangka hayat bunga keratan disesabkan oleh keguguran kudup bunga yang tinggi dan kelopak bunga pudar. Selain itu, pH yang rendah telah diperolehi daripada larutan 15 mg DCLE-Pg+Ap/L. Warna bunga daripada kedua-dua larutan dikekalkan dengan lebih baik daripada rawatan lain. Walau bagaimanapun, rawatan SLE mempunyai jangka hayat jambangan bunga yang lebih singkat daripada 8-HQC. Oleh itu, 15 mg DCLE-Pg+Ap/L telah digunakan untuk eksperimen berikutnya untuk menilai jangka hayat yang lebih lama bagi bunga keratan. Rawatan SLE tidak digunakan untuk uji kaji seterusnya oleh kerana prestasinya yang tidak memuaskan. Larutan 15 mg DCLE-Pg+Ap/L mempunyai kiraan bakteria yang lebih rendah berbanding larutan dengan 15 mg DCLE-Jc+Ap/L. DCLE sebanyak 15 mg DCLE-Pg+Ap/L adalah lebih berkesan berbanding rawatan lain. DCLE-Pg+Ap didapati berpotensi sebagai larutan untuk bunga keratan dengan memanjangkan jangka hayat bunga melebihi tiga hari lebih berbanding dengan 8-HQC.

Pra-rawatan seketika dengan 5 mg AgNP/L selama 24 jam pada kepekatan, ditambah ke dalam ekstrak daun *P. guajava* dan *A. paniculata* (AgNP5+DCLE-Pg+Ap) didapati mengawal mikrob di dalam xilem yang tersumbat dengan berkesan. AgNP5+DCLE-Pg+Ap mempunyai potensi sebagai bahan pengawet semula jadi untuk bunga keratan dalam mengurangkan populasi mikrob dan memanjangkan jangka hayat bunga. Keputusan kajian menunjukkan bahawa bunga yang dirawat dengan larutan jambangan AgNP5+DCLE-Pg+Ap mempunyai kadar pengambilan larutan jambangan yang lebih tinggi berbanding dengan rawatan 8-HQC. Larutan 8-HQC mengandungi sejumlah bakteria 5×10^{8} koloni-membentuk unit (CFU)/mL gram positif dan gram negatif bakteria dan juga kulat, yang juga terdapat didalan larutan AgNP5+DCLE-Pg+Ap. Selain daripada itu, bunga yang dirawat dalam AgNP5+DCLE-Pg+Ap memberi kelopak warna yang lebih baik, seperti nilai yang ditunjukkan oleh L*, C* dan h° berbanding 8-HQC. Oleh itu, ekstrak de daun *P. guajava* dan *A. paniculata* mempunyai potensi sebagai bahan pengawet bunga keratan untuk mengurangkan populasi mikrob dan dapat memanjangkan jangka hayat bunga melebihi 7 hari berbanding 8-HQC.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

The members of the Supervisory Committee were as follows:

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8-HQC	8-hydroxyquinoline citrate
AgNP	Silver nanoparticles
ANOVA	Analysis of variance
Ap	*Andrographis paniculata*
Ba	Bacteria
C*	Chroma
CA	Citric acid
CFU	Colony forming units
CRD	Completely randomized design
CW	Cell wall
DCLE	Double combination leaf extracts
DMRT	Duncan's multiple range test
eV	Electron volts
GC-MS	Gas chromatography-mass spectrometry analysis
h	Hour
h°	Hue angle
ISHS	International Society for Horticultural Science
Jc	*Jatropha curcas*
kV	Accelerating voltage
L*	Lightness
MAS	Malaysian Agricultural Statistics
mg/L	Milligram/liter
mM	Millimolar
µm	Micrometer
m/z	Mass-to-charge ratio value
NA	Nutrient agar
NIST	National Institute of Standards and Technology
NS	Nanosilver
p	Probability value of test statistics
PDA	Potato dextrose agar
Pg	*P. guajava*
ppm	Parts per million
RH	Relative humidity
RHS	Royal Horticultural Society
SAS	Statistical Analysis System
SEM	Scanning electron microscopy
SLE	Single leaf extract
STS	Silver thiosulphate
Suc	Sucrose
TEM	Transmission electron microscopy
UPM	Universiti Putra Malaysia
V	Xylem vessel
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

Orchids have a very special place along with all ornamental crop plants in Malaysia. A total of 214 million ornamental crop plants was grown in 2011 (Malaysian Agricultural Statistics, 2012). The number of cultivated orchids had increased by 11 times in the last 2007 to 2011. The orchid industry occupied 46% of the total floriculture industry in Malaysia. The most popular orchid types cultivated are 11.17% *Dendrobium*, 8.0% Aranda, 5.01% Oncidium and 3.5% Mokara, generally grown in Johor and Selangor (Malaysian Agricultural Statistics, 2012). Mokara Chark Kuan (Pink, Orange, Red), with a generic name Mokara, is a hybrid that progenitors from Singapore in 1969. The Grex epithet is Chark Kuan, and the cultivar epithet is Orange. Mokara is a trigeneric produced from the hybridization of *Arachnis flosaeris*, *Ascocentrum ampullaceum* and *Vanda peduncularis* (Yew-Hwa, 1995; Yam and Thame, 1999). The plant has a monopodial growth habit and continues to grow infinitely from the tip or crown of the plant. The Mokara flowers are very appealing with substantial colours such as red, pink, purple, yellow, or white.

The postharvest life of a cut orchid flower is limited by several elements like yellowing or discolouration and wilting and shedding of individual unopened buds and florets. Floret shedding has been reported to occur when the rate of water loss fell below 1.0 g/day per spray (Dai and Paul, 2003). Microbial contamination at the stem base or in the vase solutions has been reported to cause xylem blockage, especially when microorganisms such as bacteria and fungi build up in the vase solutions or in the sap-conducting tissue of xylem (Botelho *et al.*, 2007; Martinez-Romero *et al.*, 2007; Yahyazadeh *et al.*, 2008). This xylem blockage has not been documented well in the locally grown cut orchid industry. Besides, when a stem is cut, air is immediately aspired into all opened xylem conduits. This air will be restricted to the opened conduits. Since vase water bacteria cannot move from one xylem vessel to the other, and polysaccharides excreted by bacteria only moves partially up the stem, the blockage that occurs further up the stem is mainly due to air bubbles in the xylem conduits (Heleen *et al.*, 2003). Usually, a floral vase solution is acidified and includes a biocide (8-hydroxyquinoline citrate, 8-HQC) to inhibit bacterial proliferation. The effects of biocides on flower life of *Alstroemeria pelegrina* L. was 20.4 days when treated with 0.05 g acetyl pyridinium chloride/L and 0.2 g 8-HQC/L. Although, flower vase life of carnation was 22 days when treated with 0.05 g acetyl pyridinium chloride/L and 0.05 g dantogard/L. Moreover, the vase life of cut rose flower was 9.6 days when treated with 0.05 g isocil/L (Knee, 2000). However, there is limited information in the literature on the relative effectiveness of different natural biocide, derived from plants, which can be environmentally friendly and not harmful to human health.

Malaysia is one of the countries in Asia that has high diverse biological resources. In Malaysia, *Jatropha curcas*, *Psidium guajava* and *Aandrographis paniculata* are also known as jarak pagar, jambu batu and hempedu bumi, respectively (Gübitz *et al.*, 1999; Chen *et al.*, 2006; Savitha and Rathnavijaya, 2011). *J. curcas*, *P. guajava* and *A. paniculata* belongs to the family of Euphorbiaceae, Myrtaceae and Acanthaceae, respectively. They are widely cultivated in Central and South America, Southeast Asia, India and Africa. Previous studies have shown that none of the compounds had a consistent and high anti-bacterial effect on concentrations that are not toxic to flowers. *J. curcas* leaf extracts showed antimicrobial activity (Okoh *et al.*, 2009). *P. guajava* leaf extracts showed antibacterial activity, and polyphenolic compounds are the active antimicrobial components in the leaves (Suhaila *et al.*, 2009). Leaf extracts of *A. paniculata* inhibit both gram-positive and gram-negative bacteria by the active compound andrographolide (Singha *et al.*, 2003). Presently, extraction is the vital step for the recovery and separation of bioactive chemicals from plant resources. Plant derived metabolites are key sources of different phytochemicals used for the production of pharmaceuticals. These natural products make obtainable input to produce new structural types of antimicrobial and antibacterial chemicals. In addition, there are no recognized criteria for the selection of a particular compound.

In vase solutions of cut *Dendrobium* 'Pompadour' flowers, silver nitrate ($AgNO_3$), 8-HQC, sucrose and citric acid (CA) have been used for enhancing the longevity of cut flowers (Ketsa *et al.*, 1995). The sucrose acts as a food source while CA (stabilizes pH to 3-4), $AgNO_3$ and 8-HQC act as antimicrobial agents preventing the blockage of xylem vessel (Meman and Dabhi, 2006). Currently, $AgNO_3$ is no longer used in commercial vase solutions because it is a synthetic germicide containing silver, a heavy metal that can pollute the environment and cause damage to human health (Damunupola and Joyce, 2006). Silver nanoparticles (AgNP) have been found to be ten times less toxic than the soluble $AgNO_3$, a soluble silver salt (Griffith *et al.*, 2008). The AgNP, or nanosilver (NS), is a cluster of silver atoms that range in diameter from 1-100 nm. It is the most commonly used in nano material for microbial control (Morones *et al.*, 2005; Chaloupka *et al.*, 2010) because it has large surface area-to-volume ratio, great efficacy against a large number of bacterial species (Jiang *et al.*, 2004) and low toxicity to human (Foldbjerg *et al.*, 2009). AgNP has the ability to anchor to the bacterial cell wall and subsequently penetrate it, thus causing structural changes in the cell membrane like the permeability of the cell membrane and cell death. There is a formation of 'pits' on the cell surface, and accumulation of the nanoparticles on the cell surface (Sondi and Salopek-Sondi, 2004). The AgNP in a preservative solution that extended the vase life of Gerbera, thus, each of the compounds has the potential to act as novel alternatives to common chemicals as floral preservatives. The 5, 10, 20 and 50 mg AgNP/L in preservative solutions showed promising prospects for the utilization of natural plant extracts in extending flower vase life (Li *et al.*, 2012). Pre-treatment pulse with 50 mg NS/L for 1 h had significantly alleviated bacteria related blockage in the stem-ends of cut Movie Star roses due to its strong antibacterial efficacy (Li *et al.*, 2012).

However, *J. curcas*, *P. guajava* and *A. paniculata* leaf extracts reduce xylem blockage with enhancer of AgNP in cut Mokara Red orchid flower that are new and not clear. So, more research is required to reach a clear understanding regarding the safety of AgNP. Thus, it is important to develop a new substance of floral solutions from a biological origin, leaf, stem, and/or root as an alternative biocide for the floriculture industry. Therefore, this research is determined to find out the effectiveness of biocide formulations from leaf extracts of *J. curcas*, *P. guajava* and *A. paniculata* and their combinations as natural biocide. This study also seeks to find out if pre-treatment pulse with the AgNP in vase solutions could extend the vase life and maintain quality of cut flowers. Finally, the leaf extracted compounds restricted the growth of microorganisms (bacteria and fungi), which destroyed the vessel cells and, as a result, no xylem occlusion took place. Consequently, leaf extracts inhibit the growth of bacteria in the vase water or inside the xylem vessels of the flower stem. According to Siva *et al.* (2008), Kalimuthu *et al.* (2010) and Rahman *et al.* (2011) the inhibition of fungal growth by *J. curcas* extracts. *P. guajava* leaves extracts showed antimicrobial activities, while *A. paniculata* showed both antifungal and antibacterial activities (Kumar *et al.*, 2010; Long *et al.*, 2010).

The main objective of this study is to evaluate the efficacy of plant extracts from *J. curcas, P. guajava* and *A. paniculata* leaves in prolonging longevity of cut Mokara Red orchid flowers. Therefore, this study was carried out with the following specific objectives:

1) To determine the antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata*.
2) To determine optimum concentration of *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata* leaf extracts on postharvest performance of cut Mokara Red orchid flowers.
3) To determine the efficacy of silver nanoparticles as an enhancer of *Psidium guajava* and *Andrographis paniculata* leaf extracts in alleviating xylem blockage in cut Mokara Red orchid flowers.

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