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CHARACTERIZATION OF SECRETORY STRUCTURES AND ESSENTIAL OILS IN AERIAL PARTS OF TORCH GINGER [Etlingera elatior (Jack) R.M. Sm.] AT DIFFERENT DEVELOPMENTAL STAGES

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By

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June 2019

Chair : Associate Professor Phebe Ding, PhD
Faculty : Agriculture

Torch ginger (*Etlingera elatior*) is an aromatic plant popularly used as flavouring in food preparation. It has been a subject for many scientific studies aimed at investigating their chemical composition of the essential oils (EO). However, the lack of systematic analysis involving different plant parts and developmental stages may have contributed to variation on the major compounds reported by different groups of researchers. Furthermore, identification on the type of secretory structures responsible for accumulating and storing the EO have not been carried out hitherto. Therefore, this study was carried out to address the current research gaps. First, the presence of secretory cells, glandular and non-glandular trichomes were microscopically identified as the main secretory system for EO of torch ginger. The discovery of glandular trichomes is the first report on the EO secretory structure in Zingiberaceae. The histochemical tests indicated the secretory contents of the secretory structures comprised of terpenes and lipophilic that are typical characteristic of EO.

Second, the variation in chemical composition of aerial parts (leaves, peduncle and inflorescence head) of torch ginger at different developmental stages were analysed using GC-MS. The EO leaves from different position were also sampled repeatedly at 8 and 18 WAE to study the changes in EO content. The EOs in leaves, peduncle and inflorescence head were predominated by 1-dodecanol and *n*-dodecanal as the two major compounds irrespective of developmental stages. However, the *α*-pinene content influenced markedly on the EO in inflorescence head and peduncle during the initial stage of flowering (10.9 and 21.1%, respectively), which then decreased substantially in the following stages (content ranged between 0.3 to 0.7%; and 0.4 to 4.1%, respectively). Subsequent multivariate analysis using principal component analysis (PCA) was able to distinguish the differences among the EO samples. Most notably, *α*-pinene was identified as the key compound that characterized the EO in inflorescence head, while *n*-dodecanal, 2-undecanone and tetradecanoic acid were the key characterizing compounds in the peduncle.

Finally, the antioxidant activities of the EO were evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay and *β*-carotene bleaching method. Partial Least Squares (PLS) were employed to correlate the antioxidant activities and the chemical compositions of EO. The leaves at 18 WAE and peduncle at tight bud stage exhibited potent radical scavenging and inhibitory activities. Sesquiterpenes (*(E)*-*β*-farnesene, *α*-humulene and *β*-elemene) and monoterpenes (*α*-pinene and *α*-terpineol) were correlated as the active compounds that contributed towards the antioxidant activities. On the other hand, the inflorescence head at full bloom stage was shown to be potent inhibitory activities compared to radical scavenging with *(E)*-caryophyllene, *(E, E)*-*α*-farnesene, *n*-dodecanal and 1-dodecanol correlated as the active compounds. In summary, the findings were able to establish the variation in chemical composition of torch ginger as a function of plant developmental stages and their implication on the antioxidant activities.

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

CIRI STRUKTUR REMBESAN DAN MINYAK PATI DALAM BAHAGIAN ATAS POKOK KANTAN [*Etlingera elatior* (Jack) R.M. Sm.] PADA PERINGKAT PERTUMBUHAN YANG BERBEZA

Oleh

LEE YEE LING

Jun 2019

Pengerusi : Profesor Madya Phebe Ding, PhD
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Kantan (*Etlingera elatior*) merupakan pokok beraroma tempatan yang terkenal sebagai penggunaan perasa dalam penyediaan makanan. Ia merupakan subjek penyelidikan saintifik dalam penyiasatan komposisi kimia dalam minyak pati tersebut. Namun demikian, analisis sistematik jarang melibatkan bahagian pokok dan peringkat tumbesaran yang mungkin menyebabkan perbezaan dalam laporan sebatian utama oleh pelbagai pihak saintis. Tambahan pula, identiti jenis struktur perembesan yang bertanggungjawab sebagai tempat pengumpulan dan penyimpanan minyak pati masih belum dikenalpasti sehingga kini. Oleh itu, tujuan kajian ini dijalankan adalah untuk mengatasi jurang penyelidikan semasa. Pertama, kehadiran sel rembesan, trikom berkelenjar dan tidak berkelenjar telah dikenalpasti melalui mikroskop sebagai struktur rembesan utama minyak pati dalam pokok kantan. Penemuan trikom berkelenjar adalah laporan yang pertama sebagai struktur rembesan minyak pati dalam Zingiberaceae. Kajian histokimia menyatakan isi kandungan dalam struktur rembesan tersebut terdiri daripada lipofilik dan terpen yang merupakan ciri tipikal minyak pati.

Kedua, variasi dalam komposisi kimia dari bahagian atas pokok (daun, tangkai pokok dan bunga) kantan pada tahap tumbesaran yang berbeza dikaji dengan menggunakan GC-MS. Minyak pati daun daripada kedudukan yang berbeza juga disampel secara berulang pada minggu 8 dan 18 selepas kemunculan untuk mengkaji perubahan dalam minyak pati. Minyak pati dari daun, tangkai pokok dan bunga terdiri daripada 1-dodekanol dan *n*-dodekanal sebagai sebatian utama dalam komposisi kimia pada semua tahap tumbesaran pokok. Namun demikian, kandungan *α*-pinen mempengaruhi kandungan minyak pati di dalam bunga dan tangkai pokok semasa tahap pembungaan awal (masing-masing 10.9 dan 21.1%), dan kemudian menurun dengan ketara pada tahap pembungaan seterusnya (kandungan masing-masing antara 0.3 hingga 0.7% dan 0.4 hingga 4.1%). Analisis data multivariat menggunakan prinsipal komponen analisa (PCA) berjaya membezakan kandungan antara sampel minyak pati kantan. *α*-Pinen dikenalpasti sebagai sebatian utama yang mencirikan minyak pati dalam bunga, manakala *n*-dodekanal, 2-undekanone and tetradekanoic asid merupakan sebatian pencirian dalam tangkai pokok.

Akhirnya, aktiviti antioksidan daripada minyak pati ditentukan dengan menggunakan kaedah pemerangkapan radikal 1,1-difenil-2-picrylhydrazil (DPPH) dan pelunturan *β*-karotena. Separa analisis kuasa dua (PLS) digunakan untuk kajian korelasi antara aktiviti antioksidan dengan kandungan komposisi kimia dalam minyak pati. Daun pada 18 minggu selepas kemunculan dan tangkai pokok pada peringkat kudup menunjukkan potensi aktiviti pemusnahan radikal dan perencatan. Sesquiterpen (*(E)*-*β*-farnesen, *α*-humulen and *β*-elemen) dan monoterpen (*α*-pinen and *α*-terpineol) menunjukkan korelasi sebagai sebatian aktif yang menyumbangkan terhadap aktiviti antioksidan. Selain itu, bunga pada tahap pembungaan penuh menunjukkan potensi perencatan berbanding pemerangkapan radikal dimana ‘*(E)*-caryophyllene’, *(E, E)*-α-farnesen, *n*-dodekanal and 1-dodekanol merupakan sebatian aktif. Kesimpulannya, penemuan dalam kajian ini dapat menunjukkan variasi dalam komposisi kimia minyak pati daripada kantan sebagai fungsi peringkat tumbesaran pokok dan implikasi terhadap aktiviti antioksidan.

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I certify that a Thesis Examination Committee has met on 20 June 2019 to conduct the final examination of Lee Yee Ling on her thesis entitled "Characterization of Secretory Structures and Essential Oils in Aerial Parts of Torch Ginger [*Etlingera elatior* (Jack) R.M. Sm.] at Different Developmental Stages" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy).

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5.6 Partial least square analysis of antioxidant activities determined using DPPH and *β*-carotene bleaching assay. Biplot illustrating the correlation among the chemical compounds of essential oils from peduncle at different developmental stages. Evident group separation of the samples with *X*-point labelled indicates the tentatively identified compound that exert antioxidant activities. 104

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BHT	butylated hydroxytoluene
DPPH	1,1-diphenyl-2-picrylhydrazyl
EO	Essential oil
FAA	Formaldehyde-acetic acid-alcohol
FFNSC	Flavour and Fragrance Natural Synthetics and Compounds
GC-MS	Gas chromatography-mass spectrometry
GT	Glandular trichomes
IC_{50}	Inhibition concentration at 50%
nGT	Non-glandular trichomes
NIST	National Institute of Standards and Technology
PC	Principal component
PCA	Principal component analysis
PLS	Partial least squares
Q^2	Predictive ability
R^2	Goodness of fit
SC	Secretory cells
SD	Standard deviation
SEM	Scanning electron microscope
VIP	Variable Importance in Projection
UV-Vis	Ultraviolet-visible

CHAPTER 1

INTRODUCTION

Essential oils (EO) are natural products made up of complex mixtures of volatile to semi-volatile compounds formed by aromatic plants as secondary metabolites (Zuzarte and Salgueiro 2015). The composition of EO often varies substantially, considering the presence of constituents can be about dozens to potentially hundreds occuring at different concentrations that define the quality of EO. That being said, characterizing the chemical composition of EO pose several challenges due to the inherent factors involved in regulating the production of secondary metabolites in plant (Maietti *et al.* 2013).

First, synthesis and storage of EO normally occurs in specialized secretory tissues in vascular plants (Maffei 2010). The presence of such structures is a typical characteristic of aromatic plant and has been for some time, played a key role in plant taxonomy, particularly trichomes (Behnke 1984; Wagner *et al.* 2004). For example, presence of peltate and capitate glandular trichomes are generally associated with mint family Lamiaceae (Werker *et al.* 1985; Fahn 1988; Ascensão *et al.* 1995) and secretory cavities in family Myrtaceae and Rutaceae. In addition, structural diversity of secretory cells also account for certain chemical class of the secretory products. Studies have demonstrated that peltate glandular trichomes is indeed the major site of monoterpene biosynthesis, particularly in *Mentha* species (Turner *et al.* 2000). Occasionally, the distribution of secretory type within a plant varies substantially and the process of oil accumulation could affect the oil yields and quality (Li *et al.* 2013).

Second, a marked dissimilarity in the composition of EO is strongly associated with plant developmental stages (Figueiredo *et al.* 2008). As plant progresses from juvenile to mature stage, modification in the metabolic system takes place resulting in higher rates of cyclization and dehydration of the compounds (Máñez *et al.* 1991), hence the changes in chemical composition of EO. A classic instance has been demonstrated in developing leaves of peppermint (*Mentha* × *piperita* L.) where composition of monoterpenes, specifically limonene and menthone, significantly altered with plant age. The two major monoterpene compounds are present in the young leaves of peppermint, but soon decline as plant age advances and menthol becomes the dominant monoterpene constituent (Gershenzon *et al.* 2000).

The metabolic modifications stemmed from the presence of certain biosynthetic precursors that regulate different compounds dominating at certain plant age. Investigation on monoterpene variation in white micromeria (*Micromeria fruticosa*) demonstrates that formation of monoterpene alcohols (i.e. geraniol, citronellol) are probably blocked in young leaves, and the enzymes involved in the formation of the monoterpene alcohols only appear late in leaf development (Dudai *et al.* 2001). Consequently, characterizing the EO chemical composition as a function of developmental stages is imperative given the timeline of plant growth and development also crucially determines the proper time for harvesting raw material, which in this case, the aromatic plant intended for EO production (Sangwan *et al.* 2001; Chalchat and Ozcan 2008; Verma *et al.* 2012).

Third, the plant organ(s) from which EO are derived: green parts (leaves and stems), flowers, bark, wood, fruits, pericarp or seed only, or roots also largely affect the chemical composition of a given EO (Novak *et al.* 2005; Ogunwande *et al.* 2005). The variability is particularly evident and comprehensible given that production of those secondary metabolites are naturally functional oriented clues as part of the plant's mechanism maneuvering towards survival of the fittest. For example, metabolites produced in vegetative parts (leaves and stems) are exclusively associated with plant defence. On the contrary, floral metabolites which are associated with reproductive system are involved in both attractions of pollinators, as well as defence against florivores and pathogens (Muhlemann *et al.* 2014). Hence, variation in chemical composition is expected from which the EO are derived from.

Integration of these three facets in characterizing the chemical composition are lacking especially in torch ginger (*Etlingera elatior*). It is an aromatic herb whose numerous appeals include an ostentatious bloom with attractive ornamental display, along with reputed medicinal properties and nutritions that have been proven scientifically. An extensive review on the literature regarding the EO analysis of torch ginger resulted in an equivocal outcome where variation exists over the reports of compounds predominating in the torch ginger's EO. Clarification accounting for the differences have to be resolved to justify the plant's merit as emerging natural source of chemical and pharmaceutical feedstocks (Abdelwahab *et al.* 2010). Furthermore, the type of secretory structures responsible for accumulation and storage of EO for *Etlingera* have yet to be identified hitherto.

EO possesses wide range of biological activities and antioxidant activities is not only used as tool to understand and estimate their validity as remedies (Ilić *et al.* 2015), but most of the variety groups of volatile compounds have been physicochemically characterized as antioxidant (Guimarães *et al.* 2010). Studies have demonstrated that the attributes of the antioxidant activities are directed toward the chemical profile of EO where the presence of active compounds and their inteaction within the EO might contribute towards varying degree of activities.

While the laid out facets on characterization of torch ginger EO profile are built on fundamental basis of plant science, it is hope that this research could provide clarification to account for shortcomings from previous analyses while simultaneously produce resourceful information that can be exploited in the EO production and quality management. Furthermore, new discovery can also be benefited for scientific community.

Objectives

1. To identify the type of secretory structures and examine their distribution at different developmental stages,
2. To elucidate the histochemical content of the secretory structures,
3. To evaluate the chemical composition of essential oils from different plant parts at different developmental stages,
4. To evaluate the antioxidant activities of the extracted essential oils.

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LIST OF PUBLICATIONS

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Lee YL, Ding, P, Abas F, Ismail IS. 2019. Floral morphology and secretory structures of *Etlingera elatior* (Jack) R. M. Smith (Zingiberaceae). *Protoplasma* (under review).

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Lee YL, Ding P. Screening analysis of essential oil of *Etlingera elatior* – variations among plant parts and ontogeny. 3rd ISHS Southeast Asia Symposium on Quality Management in Postharvest Systems, Siem Reap, Cambodia, 13-15 August 2015.

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