



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF A TERPENE SYNTHASE  
GENE FROM BANGUN-BANGUN  
[*Plectranthus amboinicus* (Lour.) spreng.]**

**TEH KAH YEE**

**FBSB 2019 23**



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FROM BANGUN-BANGUN [*Plectranthus amboinicus* (Lour.) Spreng.]**

By

TEH KAH YEE

Thesis Submitted to the School of Graduate Studies, Universiti  
Putra Malaysia, in Fulfilment of the Requirements for the Degree of  
**Master of Science**

October 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
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**October 2019**

**Chairman : Janna Ong Abdullah, PhD**  
**Faculty : Biotechnology and Biomolecular Sciences**

Plant natural products play important roles in the pharmaceutical field. Based on World Health Organization, 80% of the world population uses herbal plants as their medications to treat diseases because herbal plants are commonly accessible, effective and considered safer to be consumed by humans. *Plectranthus amboinicus* or *bangun-bangun* in Malaysia is a herbal plant and its bioactive compounds like terpenoids contributed to the properties and potential applications in the pharmaceutical field. Plant-derived terpenoids were proven by research and clinical studies to have great potentials for use as medicine and drugs. However, commercialization of plant-derived terpenoids has been challenging as they are present at low amount. This study was aimed to isolate, clone and functionally characterize the transcript of a terpene synthase gene from *P. amboinicus*. A novel full length transcript sesquiterpene synthase (designated as *PamTPS2*) was isolated, cloned and identified with an open reading frame of 1653 bp that encoded a 551 amino acids protein. The molecular weight of the deduced protein was approximately 64 kDa with an isoelectric point (pI) value of 5.68. Conserved motifs that are typically present in terpene synthases were also found in deduced *PamTPS2* protein. The conserved motifs found were aspartate-rich DDXD motif that is critical for positioning substrates, a NSE/DTE motif that can fix pyrophosphate substrate, and a RXR motif involving in the complexation of the diphosphate group after substrate ionization. Thus, it was suggested that *PamTPS2* belongs to class I terpene synthase and of the TPS-*b* group. Besides, the 3-D protein structure of *PamTPS2* was constructed using SWISS-MODEL and MODELLER 9.21 and validated via *in silico* analysis. It was revealed that the 3D structure of *PamTPS2* was made up of 34  $\alpha$ -helices, 2  $\beta$ -sheets and 34 turns. Through molecular docking, it was further revealed that *PamTPS2* could be a multi-

substrate enzyme as it has affinity towards three potential substrates in the following order (highest to lowest), which are, farnesyl pyrophosphate, geranyl pyrophosphate and geranylgeranyl pyrophosphate.

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed on leaf and stems at three different timepoints 8AM, 2PM and 8PM. *PamTPS2* transcript was shown to be expressed 5 folds higher in the leaf than the stems at 8AM. However, at 2PM, it was expressed around 140 folds higher in stem than leaves. The *PamTPS2* cDNA was cloned into pMAL-c5x vector and expressed in *Escherichia coli* BL21 strain. Functional enzymatic assays were performed to analyze the potential products produced from the catalytic activity of *PamTPS2*. Based on *in vivo* enzymatic test on *E. coli* harbouring pMAL-c5x:*PamTPS2*, caryophyllene and  $\beta$ -sesquiphellandrene were produced as final products. However, *in vitro* enzymatic tested on purified *PamTPS2* did not show any targeted terpene products. The successful isolation of a novel functional sesquiterpene synthase from *P. amboinicus* would be beneficial to be applied in the pharmaceutical field.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMENCILAN DAN PENCIRIAN GEN TERPENE SINTASE DARIPADA  
BANGUN-BANGUN [*Plectranthus amboinicus* (Lour.) Spreng.]**

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Kini, produk natural dari tumbuh-tumbuhan memainkan satu peranan yang penting dalam bidang farmaseutikal. Berdasarkan Pertubuhan Kesihatan Sedunia (WHO), sebanyak 80% populasi dunia menggunakan tumbuhan herba dalam ubat mereka untuk merawat penyakit kerana tumbuhan herba mudah untuk dijumpai, berkesan dan lebih selamat dimakan oleh manusia. *Plectranthus amboinicus* atau *bangun-bangun* dari Malaysia merupakan salah satu tumbuhan herba dan sebatian bioaktifnya seperti terpenoid telah menyumbang kepada potensi penggunaanya di dalam bidang farmaseutikal. Terpenoid dari tumbuhan telah terbukti melalui penyelidikan dan kajian klinikal bahawa ia mempunyai potensi hebat untuk digunakan sebagai ubat. Tetapi, penghasilan terpenoid daripada tumbuhan adalah sangat mencabar kerana jumlah terpenoid yang rendah. Oleh itu, kajian ini bertujuan untuk mengasingkan, mengklon dan mencirikan fungsi transkrip cDNA terpene sintase daripada *P. amboinicus*. Satu transkrip penuh sesquiterpene sintase (*PamTPS2*) yang baru telah dikenalpasti dengan rangkaan baca terbuka 1653 bp yang terkod 551 protein asid amino. Berat molekul *PamTPS2* dijangkakan dalam 64 kDa dengan takat isoelektrik 5.68. Motif-motif seperti DDXXD, NSE/DTE dan RXR, yang terpelihara dalam terpene sintase juga dijumpai dalam transkrip *PamTPS2*. Maka, *PamTPS2* dicadangkan sebagai terpene sintase kelas I dan dalam kumpulan TPS-*b*. Pembentukan protein tiga dimensi (3D) juga dibentukkan dan disahkan menggunakan analisis *in silico*. Melalui penyatuan molekul, *PamTPS2* juga menunjukkan bahawa ianya merupakan enzim multi-substrat yang mempunyai afiniti dengan tiga substrat, iaitu, farnesil pirofosfat (FPP), geranil pirofosfat (GPP) dan geranilgeranil pirofosfat (GGPP).

Tindak balas berantai polymerase kuantitatif masa sebenar (RT-qPCR) telah dijalankan ke atas daun dan batang pada tiga masa yang berbeza seperti 8AM, 2PM dan 8 PM. Ianya menunjukkan kadar pengekspresan transkrip *PamTPS2* dalam daun adalah 5 kali ganda lebih tinggi daripada batang pada jam 8AM. Namun, pada jam 2PM, kadar pengekspresan transkrip *PamTPS2* dalam batang adalah 140 lipat tinggi daripada daun. Transkrip *PamTPS2* telah diklonkan dan diekspresikan dalam *Escherichia coli* BL21. Cerakin kefungsian enzim telah dijalankan untuk mengesahkan produk potensi dari aktiviti pemangkin *PamTPS2*. Ujian enzim *in vivo* terhadap *E. coli* yang mempunyai pMAL-c5x:*PamTPS2* menunjukkan bahawa kariofilena dan  $\beta$ -sesquiphellandrene dihasilkan sebagai produk akhir. Akan tetapi, tiada produk terpene yang disasarkan hasil dalam ujian enzim *in vitro* terhadap *PamTPS2* tulen. Kesimpulannya, isolasi sesquiterpene sintase yang baru ini akan memanfaatkan bidang farmaseutikal.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

bp	Base pair
DMAPP	Dimethylallyl pyrophosphate
dNTP	Deoxyribonucleotide triphosphate
DTT	1,4-Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FPP	Farnesyl diphosphate
GGPP	Geranylgeranyl diphosphate
GPP	Geranyl diphosphate
HMGR	3-hydroxy 3-methylglutaryl Co-A reductase
IPP	Isopentenyl diphosphate
IPTG	Isopropyl $\beta$ -D-1-thiogalactopyranoside
MEP	2-C-methyl-D-erythritol 4-phosphate
MVA	Mevalonate
rpm	Revolutions per minute
S.O.C	Super optimal broth with catabolite repression
TAE	Tris-acetate-EDTA
TPS	Terpene synthase
Tris	Tris(hydroxymethyl)aminomethane
x g	Relative centrifugal force (times gravity)
X-GAL	5-bromo-4-chloro-3-indolyl-D-galactopyranoside

## CHAPTER 1

### INTRODUCTION

According to the World Health Organization (WHO), approximately 80% of world population is depending on herbal plants as medications to treat diseases since it is cost effective, easier accessibility, safer and gentler to be consumed (Arumugam et al., 2016; Palhares et al., 2015; Swamy & Sinniah, 2015). Nowadays, natural products from plant are the main material being greatly applied in pharmaceutical field. The global market for medicinal herbs and their products had a turnover at US\$ 60 billion in 2010 and it is expected to reach US\$ 5 trillion by 2050 (Nirmal et al., 2013). Hence, the high demand for herbal plants has encouraged the finding of new products from natural origin.

*Plectranthus amboinicus*, also known as *bangun-bangun* in Malaysia, is a medicinal herb that is categorized under the plant family of Lamiaceae. It was found to be prominent in medicinal usage and it was commonly used in traditional medicine for common diseases treatment such as asthma, cough, cold, headache, fever, constipation, skin infection, mucus and sore throats (Arumugam et al., 2016; Saad et al., 2015; Yuthistran et al., 2015). This herbal plant has increasingly gained interest of researchers due to its pharmacology properties. Studies had been conducted to understand the bioactive compounds identified in *P. amboinicus* that contributed to the properties and its potential applications in the pharmaceutical field (Sabrina, Ahs, & Shukri, 2014; Hassani, Zainati, Zrira, Mahdi, & Oukessou, 2012; Chang, Cheng, Hung, Chung, & Wu, 2010).

Generally, from research studies, *in vitro*, preclinical and clinical studies, it was proven that terpenoids (also known as isoprenoids) were greatly applicable in the pharmaceutical field. Examples of terpenoids that had been applied in pharmaceutical field were, antimalarial drug artemisinin from *Artemisia annua* (sweet wormwood), anti-cancer drug Taxol from *Taxus brevifolia*, carotenoids as a potential anti-cancer drug, and sterols to reduce cholesterol level (Milani, Basirnejad, Shahbazi, & Bolhassani, 2017; Weaver, 2014; Ro et al., 2006). However, the main source of terpenoids is naturally originated from plants and it is challenging to mass produce plant-derived terpenoids for commercialization purpose because many plant derived terpenoids are present at low quantities. Therefore, alternative ways such as microbial biosynthesis and chemical biosynthesis are favourable in enhancing the production of terpenoids or precursors of terpenoids.

To date, there are limited molecular studies been done on isolating full length terpene synthase from *P. amboinicus*, since recent studies related to *P. amboinicus* has been focusing on their pharmacology, nutrional and phytochemical properties (Arumugam, Swamy, & Sinniah, 2016; Fu & Fu, 2015; Ajitha, Ashok Kumar Reddy, & Sreedhara Reddy, 2014). Therefore, the data obtained from this study is useful as a reference for future research.

Hence, this study was conducted:

1. to determine the full length cDNA of a terpene synthase from *Plectranthus amboinicus*, and
2. to obtain the isolated terpene synthase in *Escherichia coli* and functionally characterize the expressed terpene synthase.

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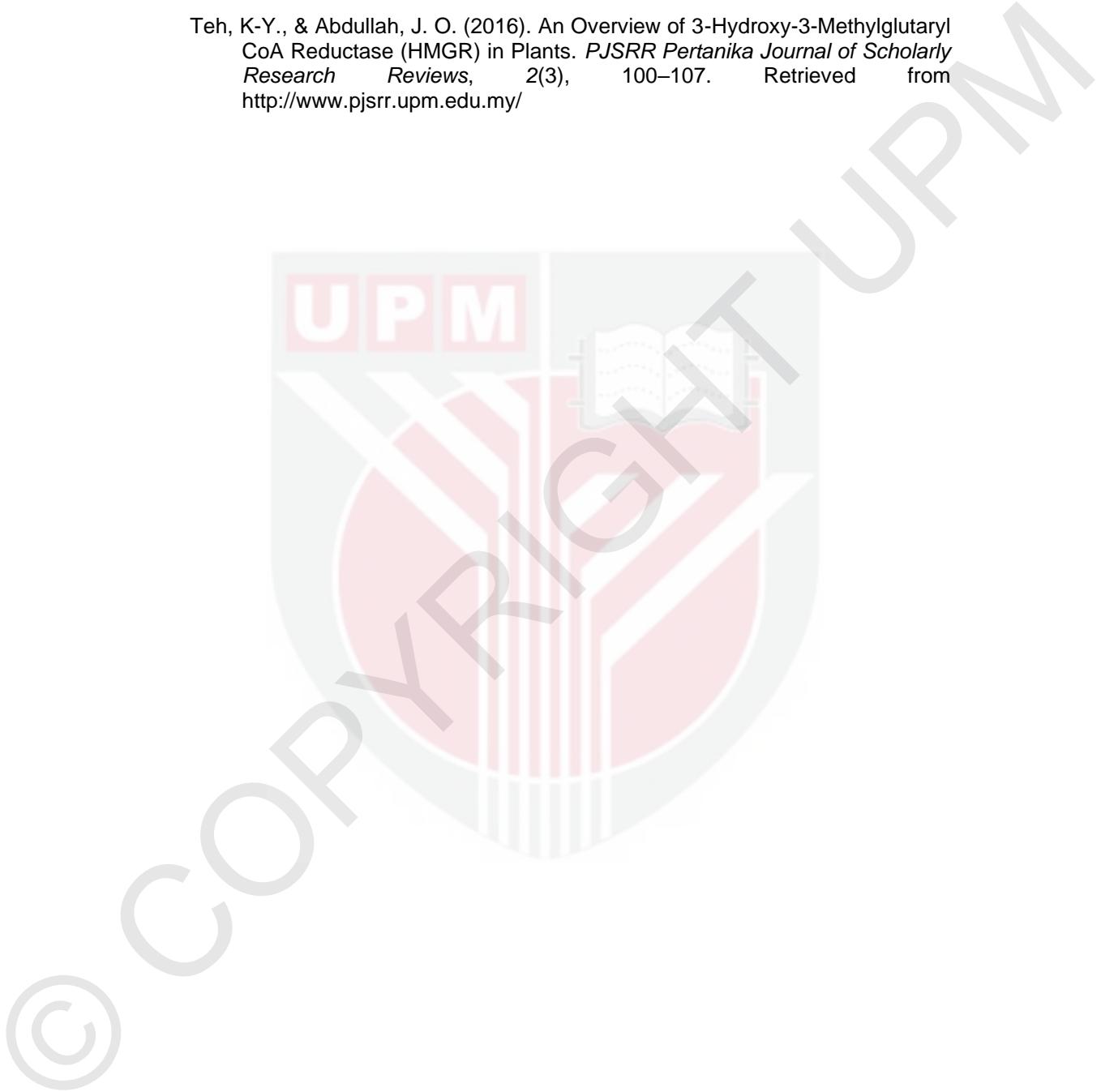
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Teh Kah Yee was born in Ipoh, Perak on 27<sup>th</sup> June 1991. She started her primary education in S.J.K (C) Ave Maria Convent from 1998-2003 and continued her secondary education in S.M.J.K Ave Maria Convent from 2004-2008. Then, she continued her sixth form at S.M.J. K Sam Tet Ipoh from 2009-2010. After that, she pursued her degree study in Universiti Malaysia Pahang with Bachelor Applied Sciences (Hons). in Industrial Biotechnology and graduated in the year 2015. In the same year, she started her Master degree in Plant Biotechnology under supervision of Assoc. Prof. Dr. Janna Ong Abdullah in Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia. During the period of her master study, she has been focussing her research interest in the area of plant molecular biology and assisted laboratory classes as a demonstrator for four semesters.

## PUBLICATION

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