



UNIVERSITI PUTRA MALAYSIA

***BIOLOGY, CULTIVATION AND POTENTIAL USES OF PASSION FRUIT
PLANT, PASSIFLORA SPECIES***

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FSPM 2016 6



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PLANT, *PASSIFLORA* SPECIES**

By

SHIAMALA DEVI RAMAIIYA

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

February 2016

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DEDICATION

For my dad,

MR. RAMAIYA KALIMUTHU

Thank you for telling me what I'm capable of.
For giving me the support that I needed to build a dream to chase after.
And for believing that I've the talent to reach my goals.

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

BIOLOGY, CULTIVATION AND POTENTIAL USES OF PASSION FRUIT PLANT, *PASSIFLORA* SPECIES

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February 2016

Chair : Japar Sidik Bujang, PhD
Faculty : Agriculture and Food Sciences (Bintulu)

Passiflora fruits are widely known for their unique flavour, fruity aroma, desirable organoleptic properties and essential nutrient contents. *Passiflora* plant is not native to Malaysia and it is cultivated in a small scale. The evolutions of cultivated *Passiflora* plants have also not been extensively investigated and there have been inconsistencies in the identification suggesting more research needs to be conducted. Hence, the present research was carried out to examine the adaptability, biology and phenology of five *Passiflora* species, i.e., *Passiflora edulis* Sims (Purple), *Passiflora edulis* Sims (Frederick), *Passiflora maliformis* Linn., *Passiflora quadrangularis* Linn. and *Passiflora incarnata* Linn. The morphology and molecular characterization were also examined for the five species with additional accessions from other geographical locations (i.e., *Passiflora edulis* Sims (Pink), *Passiflora edulis* Sims (Yellow) and *Passiflora foetida* Linn. for species identification and confirmation. In addition the nutrition properties of the fruit juices were also evaluated. Apart from their juices, the plants' agro by-products; leaves, stems and seeds were also examined for their utilization.

All species have the ability adapt to the local condition with plants continuously grew and produced flowers and fruits all the year around. The flowering and fruiting were not synchronized and showed a marked phenological pattern with species. Different flowering peaks were observed, e.g., 4 peaks in *P. edulis* compared to a single peak in *P. quadrangularis*. This was followed by fruiting with two months after anthesis. Good fruit yields were observed throughout the year which was attributed to *Passiflora* species ability for self-pollination. The higher fruit yield was recorded in *P. edulis* (Purple) and *P. quadrangularis* compared to other species. In *P. edulis* (Purple), the annual fruit production were approximately, 119,174 fruits of 11,103.90 kg ha⁻¹ with the weight ranged 56.4-156.5 g. The production of *P. quadrangularis* which produced bigger fruits, was 18,800.62 kg ha⁻¹ (9585 fruits) with weight ranged 774.2-3034.4 g.

The morphological study provided a useful tool for identification of *Passiflora* species. Using Principal Component Analysis (PCA) and Discriminant Analysis (DA) the morphological traits could be distinguished by the *Passiflora* species. Cluster analysis based on Spearman correlation coefficient further supported the species separation. Based on genetic characterization using nuclear ribosomal Internal Transcribed Spacer, ITS1-4 provided high resolution at species level and useful for differentiating the major groups of *Passiflora* subgenus. The phylogenetic relationships were consistent with results obtained for morphological assessments. The ITS also confirmed that the *Passiflora* accession from different geographical regions showed varied fruit colours (e.g., *P. edulis* produces purple, dark purple, pink red and yellow fruits) are actually genetically similar and belong to the same species of *P. edulis*. Ancillary, *Passiflora foetida* which is placed in the Stipulata supersection evolved distantly from, *P. Caerulea* Linn. (same supersection), thus the placement of this species into a separate subgenus is supported by the present finding.

Passiflora species mainly enters international trade in the form of fruit juice. Demands for the juice are increasing because of the juice's exotic flavor and ample nutrient compositions. A cup of 247 mL *Passiflora* juices provided 6-21% of fiber, 3-7% of protein and ~34% of K, 60-80% Mg, >80% P and 90% Fe of daily recommended allowance of minerals. In addition, reducing sugars; glucose and fructose were the predominant sugar components. With respect to vitamin content, a serving of 247 mL *Passiflora* fruit juice offered 71 mg of vitamin C and 2000 I.U. of vitamin A, sufficient to fulfill the daily required amount. *Passiflora* juices also possessed higher total antioxidant activity (TAA), total phenolic content (TPC) and total flavonoid content (TFC).

Passiflora plant parts, i.e., leaves, stems and seeds as agro by-products are sources of functional and bioactive compounds giving the plants value beyond that of their fruit juices. The leaves and stems possessed good antioxidant and antibacterial properties. The maximum antioxidant compound was from extracts of *P. maliformis* leaf and *P. quadrangularis* stem and correlated with their TAA and TPC. The gram-negative bacteria were generally less susceptible to *Passiflora* extracts than gram-positive bacteria. The seeds of *Passiflora* species were also rich in protein, dietary fiber with predominant insoluble dietary fiber (72%) and rich in oil with a yield of 24-30%. Based on the PCA, *Passiflora* seed oils possessed similar characteristics as sunflower and soybean oils.

The cultivated five species of *Passiflora* plants have a good adaptation to the local climatic condition as evident by the plants' growth, production of flowers and fruiting with good yield. Hence, the information gathered on the cultivation and management practices can be recommended to growers and farmers for large-scale cultivation. Furthermore, the ITS1-4 is useful in identifying genetically similar accessions. The finding revealed that, all above ground plant parts of *Passiflora* species can be utilized. The fruit juice is gaining visibility in drinks, food and health promoter and leaves, stems, and seeds could be utilized due to their nutritional and phytochemical attributes.

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

**BIOLOGI, PENANAMAN DAN POTENSI KEGUNAAN TUMBUHAN
BUAH MARKISA, SPESIES *PASSIFLORA***

Oleh

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Buah *Passiflora* sangat terkenal dengan rasa yang unik, aroma, sifat organoleptik dan kandungan nutrisi yang penting. Spesies *Passiflora* bukan berasal dari Malaysia dan ditanam dalam skala yang kecil. Kajian terperinci terhadap evolusi spesies *Passiflora* tidak meluas dikaji dan terdapat ketidakseragaman dalam pengenalanpastian, namun mencadangkan lebih banyak kajian perlu dijalankan. Oleh itu, kajian ini dijalankan untuk mengkaji keupayaan menyesuaikan diri, biologi dan fenologi bagi lima spesies *Passiflora*, iaitu, *Passiflora edulis* Sims (Purple), *Passiflora edulis* Sims (Frederick), *Passiflora maliformis* Linn., *Passiflora quadrangularis* Linn. dan *Passiflora incarnata* Linn. Morfologi dan pencirian genetik juga diperiksa untuk lima spesies bersama beberapa aksesori dari lokasi yang lain iaitu, *Passiflora edulis* Sims (Pink), *Passiflora edulis* Sims (Yellow) dan *Passiflora foetida* Linn. untuk pengesahan spesies. Tambahan pula, ciri-ciri khasiat daripada jus buah markisa juga dinilai. Selain daripada jus, produk agro; daun, batang dan biji juga telah diperiksa untuk penggunaannya.

Semua spesies *Passiflora* mempunyai keupayaan beradaptasi dengan keadaan tempatan dengan pertumbuhan berterusan dan penghasilan bunga dan buah belaku sepanjang tahun. Penghasilan bunga dan buah tidak seragam dan menunjukkan corak fenologi ketara dengan spesies. Puncak berbunga berbeza diperhatikan, contohnya, 4 puncak bagi *P. edulis* berbanding puncak tunggal bagi *P. quadrangularis*. Ini diikuti oleh berbuah selepas dua bulan berbunga. Penghasilan buah yang baik dapat diperhatikan sepanjang tahun yang telah dikaitkan dengan keupayaan spesies *Passiflora* untuk pendebungaan sendiri. Produktiviti yang lebih tinggi dicatatkan di *P. edulis* (Purple) dan *P. quadrangularis* berbanding dengan spesies lain. Penghasilan buah-buahan *P. edulis* (Purple), kira-kira 119 174 buah yang 11 103.90 kg ha⁻¹ dengan berat basah antara 56.4-156.5 g. Penghasilan *P. quadrangularis* yang menghasilkan buah yang lebih besar, adalah 18 800.62 kg ha⁻¹ (9585 fruits) dengan berat basah antara 774.2-3034.4 g.

Kajian morfologi berguna untuk mengenal pasti spesies *Passiflora*. Menggunakan 'Principal Component Analysis' (PCA) dan 'Discriminant Analysis' (DA) sifat-sifat morfologi boleh dibezakan dengan spesies *Passiflora*. Analisis kelompok berdasarkan pekali korelasi 'Spearman' disokong lagi pengasingan spesies. Berdasarkan pencirian menggunakan ITS1-4 disediakan resolusi tinggi di peringkat spesies dan berguna untuk membezakan kumpulan utama *Passiflora* subgenus. Hubungan filogenetik konsisten dengan keputusan yang diperolehi untuk taksiran morfologi. ITS juga mengesahkan bahawa *Passiflora* dari kawasan yang berbeza menghasilkan warna buah-buahan yang berlainan (contohnya, *P. edulis* buah ungu, ungu gelap, merah jambu dan kuning) sebenarnya tergolong dalam spesies yang sama iaitu *P. edulis*. *Passiflora foetida* yang diletakkan di supersection *Stipulata* tergolong jauh dari *P. caerulea* Linn. (sama supersection), dengan itu penempatan spesies ini ke dalam subgenus berasingan disokong dalam kajian ini.

Spesies *Passiflora* popular di antarabangsa dalam bentuk jus buah-buahan. Permintaan untuk jus semakin meningkat kerana rasa yang eksotik dan komposisi khasiat yang mencukupi. Secawan 247 mL jus buah markisa mengandungi 6-21% serat, 3-7% protein, ~ 34% K, 60-80% Mg, 80% P dan 90% Fe kandungan mineral harian yang disyorkan. Di samping itu, gula penurun; glukosa dan fruktosa adalah komponen utama. Bagi kandungan vitamin, satu hidangan 247 mL jus menawarkan 71 mg vitamin C dan 2000 I.U. vitamin A, yang mencukupi untuk memenuhi jumlah yang diperlukan setiap hari. Jus *Passiflora* mempunyai kandungan antioksidan lebih tinggi, TAA, TPC dan TFC.

Bahagian tumbuhan *Passiflora* iaitu daun, batang dan benih adalah sumber sebatian bioaktif dan memberi nilai tambahan selain daripada jus mereka. Daun dan batang spesies *Passiflora* juga mempunyai antioksidan yang baik dan ciri-ciri antibakteria. Sebatian antioksidan maksimum adalah daripada ekstrak daun *P. maliformis* dan batang *P. quadrangularis* dan dikaitkan dengan kandungan TAA dan TPC. Bakteria gram-negatif secara amnya kurang rentan kepada ekstrak *Passiflora* daripada bakteria gram-positif. Biji benih spesies *Passiflora* juga kaya dengan protein, serat pemakanan dengan serat tidak larut (72%) dan kaya dengan hasil minyak sebanyak 24-30%. Minyak juga mengandungi asid lemak dengan kandungan tinggi asid lemak tak tepu >80%; ivncarnat dan oleic asid. Berdasarkan PCA minyak biji *Passiflora* mempunyai ciri-ciri yang sama seperti minyak bunga matahari dan kacang soya.

Lima spesies tumbuhan *Passiflora* yang telah ditanam mempunyai adaptasi yang baik kepada keadaan iklim tempatan seperti yang dibuktikan oleh pertumbuhan dan jumlah penghasilan buah. Oleh itu, maklumat yang dikumpulkan mengenai kaedah penanaman dan pengurusan boleh disyorkan kepada petani untuk penanaman berskala besar. Tambahan pula, ITS1-4 berguna dalam mengenal pasti aksesori ivncarna yang sama. Kajian menunjukkan, semua bahagian tumbuhan ini boleh digunakan. Jus buah *Passiflora* popular dalam penghasilan produk minuman dan kesihatan serta daun, batang dan biji boleh digunakan kerana sifat nutrisi dan fitokimianya.

ACKNOWLEDGEMENTS

I am grateful to God almighty who has been giving me patience, health, wisdom and blessing to accomplish this thesis. This research would not have been possible without the support of many people. Firstly, I would like to express my sincere gratitude to my supervisor Prof. Dr. Japar Sidik Bujang for his patience, motivation and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. My sincere thanks also goes to Assoc. Prof. Dr. Muta Harah Zakaria for her insightful comments and guidance and not forgetting my co-supervisor, the late Dr. Wong Sing King, I will be ever grateful for his assistance and his memory will be with me always.

I am so thankful to UPM and Ministry of Higher Education (MOHE) for awarding me the scholarship and grant under the RUGS-01-01-12-1592RU. Also extend my appreciation to all the academic and non-academic staffs of the Faculty of Agriculture and Food Sciences for providing facilities, for their help, advice and contribution in sharing knowledge during my study years.

I also would like to express my sincere appreciation to my daddy Mr. Ramaiya, my mother Mrs. Vasanthi, my siblings Ramani and Shalini for their endless love, guidance and supports that enabled me to complete the thesis. My heartfelt gratitude to my laboratory colleagues Shafiq, Fakhurulddin, Anuar, Poova and Sutha for the stimulating discussions and all the fun we have had. Special thanks also goes to my dearest friends Palani, Puru, Thiban, Phene, Hui Hong and Rakib for always being there for me.

I certify that a Thesis Examination Committee has met on 1st February 2016 to conduct the final examination of Shiamala Devi Ramaiya on her thesis entitled "Biology, Cultivation and Potential Uses of Passion Fruit Plant, *Passiflora* Species" 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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LIST OF ABBREVIATIONS

e.g.	for example
i.e.	that is
No.	Number
DA	Discriminant Analysis
DNA	Deoxyribonucleic acid
ha	Hectare
I.U.	International unit
ITS	Internal Transcribed Spacer
Mag.	Magnification
ML	Maximum Likelihood
MP	Maximum Parsimony
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RDA	Recommend Daily Allowance
UPMKB	Universiti Putra Malaysia Bintulu Sarawak Campus



CHAPTER 1

INTRODUCTION

Passiflora plants generally known as passion fruit may well be the most fascinating plant of the tropics. Their unique, almost lavishly beautiful flowers have a mysterious look and convey an exotic ambiance (Vanderplank, 2000). Their common names besides passion fruit include granadilla (English), markisa (Malaysia, Indonesia), linmangkong (Thailand), limangkan (Laos), maracuja (Brazil, Spanish), maracuya (Portuguese), parcha (Philippines) and lilikoi (Hawaii). Passion fruit plants belong to the family Passifloraceae consists of 18 genera including genus *Passiflora* (Ocampo *et al.*, 2007). The main dispersal area extends over Central America and South America (Vanderplank, 2000; Krosnick and Freudenstein, 2005).

Passiflora plants cultivation is emerging as a major income earner in almost every Brazilian state. Although 50 species bear edible fruits, only two forms of *P. edulis*; i.e., *P. edulis* (Purple passion fruit) and *P. edulis* f. *flavicarpa* (Yellow passion fruit) are widely cultivated in commercial scale for fresh fruit and juice market (Bernacci *et al.*, 2008). The other species, e.g., *P. quadrangularis*, *P. incarnata*, *P. ligularis* and *P. laurifolia* are also cultivated in small scale for local consumption in certain countries. The world production of passion fruits increased from 1.05 million MTs in 2005 to 1.27 million MTs in 2010, with Brazil as the larger producer and consumer in the world (FAO, 2012).

In Peninsular Malaysia, the first record reported on the growing of *Passiflora* vines was in 1914 when the Department of Agriculture grew some vines in Gunung Angsi, Negeri Sembilan. Growing of this plant for commercial purposes was not considered seriously. In 1960s the *Passiflora* plants were grown in Ayer Hitam (Johor) and Cameron Highlands (Pahang) which were extended to be a commercial scale and the vines were successfully fruited (Chai, 1979). *Passiflora* fruit production and acreage data in Peninsular Malaysia are not well documented. Thereafter, the *P. edulis* f. *flavicarpa* fruit production in these regions has been affected by a passion fruit woodiness diseases (PWD) which discouraged further expansion in commercial planting (Chai, 1979). However, this fruit is still cultivated on a small scale due to the prevalence of suitable growing conditions (Ramaiya *et al.*, 2013). There is an immense potentiality of boosting passion fruit industry in Malaysia. The region has good climate with sufficient rainfall and warm temperature with little variation in the photoperiod for growing a variety of horticultural crops very successfully. Although, there is an increase in awareness of the fruit's health benefits but information on their propagation techniques, adaptability and biology are scarce. Thus, more trials and information is required to successfully cultivate the plants on a larger scale and to improve the production in the country.

As *Passiflora* plants have long been of interest to botanists, horticulturalists and taxonomists, their taxonomical classification and evolutionary history has long been debated and is still undergoing revision as more species are continuing to be recognized. The species of the *Passiflora* provides a remarkable example of floral complexity and diversity (Krosnick and Freudenstein, 2005). The variations in morphology were attributed to their adaptation to various habitats that could produce plants phenotypically different from their native environment. Taxonomic studies on *Passiflora* are based on the morphological (Crochemore *et al.*, 2003a; Viana *et al.*, 2010; Santos *et al.*, 2011) leading to a classification of this genus but the existing inter- and intra-species dissimilarity among the *Passiflora* species makes understanding the link between morphological plasticity, genotypic diversity and speciation challenging.

In recent years, there have been increasing efforts made by several authors using various approaches to better understand the germplasm characterization of *Passiflora* genus based on molecular phylogeny (Viana *et al.*, 2003; Cerqueira-Silva *et al.*, 2010; Mader *et al.*, 2010; Ortiz *et al.*, 2012). Although insights into *Passiflora* phylogeny at the subgeneric level have been gleaned, genetic information and evidence for monophyletic groups below this level is limited (Hansen *et al.*, 2006; dos Santos *et al.*, 2011). Thus, more researches need to be carried out to accurately define this phylogeny and classification of the species through morphology and molecular approaches.

In addition, *Passiflora* fruit mainly enter international trade in the form of juice. The juice is very popular and accepted worldwide due to its fresh and unique aroma and flavor which the results of natural combinations are of volatile constituents in a well-balanced system including of sugar, organic acids and phenolic compounds. Demands for *Passiflora* fruit juice are increasing not only because of the juice's exotic flavor but also due to its essential nutrients content. In recent year, more attention has also been drawn to vitamin content and antioxidant activity in *Passiflora* fruits (Janzantti *et al.*, 2012; Macoris *et al.*, 2012).

Apart from its juice, all the *Passiflora* plant parts are potentially valued for their uses, giving the plants value beyond that of their fruits, which are processed as juice. The agro-byproducts of *Passiflora* plants parts; i.e., leaves, stems and roots have long been used in folk medicine and become an increasingly important in modern medicine. In Europe, the *Passiflora* has been used in homeopathic medicine to treat pain, insomnia and nervous exhaustion (Patel *et al.*, 2011). The *Passiflora* fruits juice industrial wastes i.e., rind and seed rich in pectin (Yapo and Koffi, 2006) and fiber content (Chau and Huang, 2004), respectively. Developing technologies for value-added approach to waste management would be the best option in passion fruit industry. There is a great possibility of expanding the *Passiflora* plants cultivation for commercial scale production in Malaysia and has tremendous scope of market both at national and international level because of its unique and excellent flavor and aroma. Simultaneously, *Passiflora* plant's parts having numerous organoleptic and phytotherapeutic properties has great scope for domestic and export market.

Despite its great importance, scientific research has not kept up with this expansion and recently greater attention is being directed to other lesser known *Passiflora* species as well. Thus, this led to the interest in researching on this plant species. Therefore, the objectives of this study are:

1. to examine the cultivation practices, growth, adaptability, biology, phenology and production of *Passiflora* species grown under local condition;
2. to evaluate the morphological characteristics of *Passiflora* species;
3. to examine the molecular polymorphism among the *Passiflora* species;
4. to evaluate the nutritional properties of the *Passiflora* fruits juices and
5. to examine plants' agro by-products; leaves, stems and seeds for their utilization.

A summary of the experimental approach and introduction to each of the major sections of the main study is given in Figure 1.1.

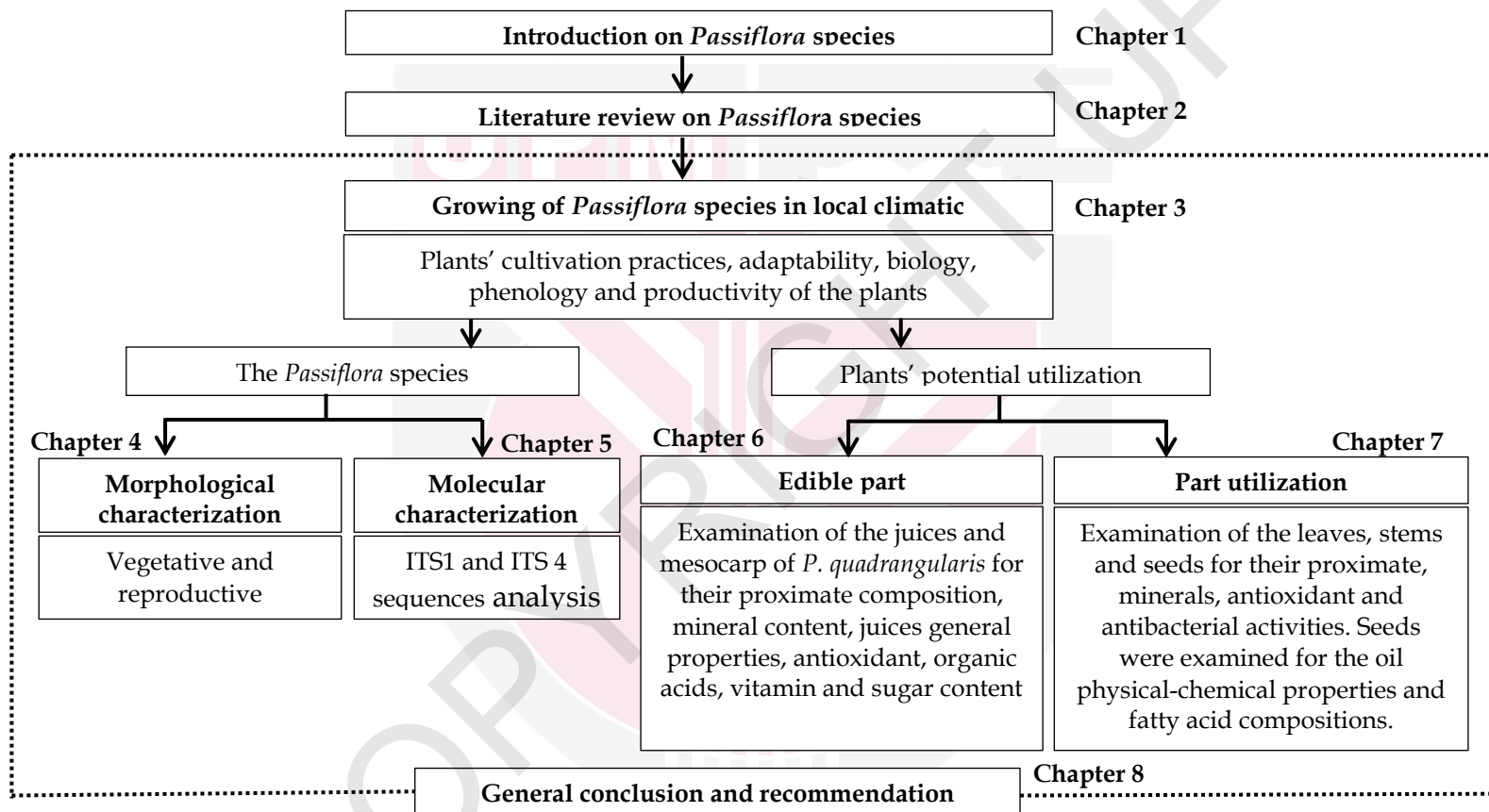


Figure 1.1: Schematic framework of the study.

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LIST OF PUBLICATIONS

Publication from the present thesis

- Ramaiya, S.D.**, Bujang, J.S., Zakaria, M.H., Wong, S.K. and Sahrir, M.A.S. (2013). Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (*Passiflora*) cultivars. *Journal of the Science of Food and Agriculture*. 93: 1198-1205.
- Ramaiya, S.D.**, Bujang, J.S. and Zakaria, M.H. (2014). Assessment of total phenolic, antioxidant and antibacterial activities of *Passiflora* species. *Scientific World Journal*. Article ID 167309: 1-10.
- Ramaiya, S.D.**, Bujang, J.S. and Zakaria, M.H. (2014). Genetic variations in *Passiflora* species based on morphological and ITS sequence analysis. *Scientific World Journal*. Article ID 598313: 1-11.

Other publication during the study period

- Sahrir, M.A.S., Bujang, J.S., Zakaria, M.H. and **Ramaiya, S.D.** (2013). Marketable wild fruit of Sarawak, Borneo: their mode of consumption, uses and sugar profile. *Indian Journal of Traditional Knowledge*. 12: 195-201.
- Khairul, A.M.S., Bujang, J.S., Zakaria, M.H., Aziz, A. and **Ramaiya, S.D.** (2014). *Halophila ovalis* plants' responses under laboratory condition. *Bio-Science Research Bulletin*. 30: 15-25.

Conference paper presented:

- Ramaiya, S.D.**, Bujang, J.S. and Zakaria, M.H. (2010). Vegetative and reproductive morphology of passion fruit, *Passiflora* species. Paper presented at the *Universiti Brunei Darussalam 1st Graduate Science Student Research Conference*. December 2010. Universiti Brunei Darussalam, Brunei.
- Ramaiya, S.D.**, Bujang, J.S., Zakaria, M.H. and Wong, S.K. (2012). Evaluation of sugars, ascorbic acid, total phenolic content and antioxidant capacity of giant passion (*Passiflora quadrangularis* L.). Paper presented at the *International Agriculture Congress 2012*. September 2012. Marriott Putrajaya, Malaysia.
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