RELATIONSHIP AMONG SOIL PROPERTIES AND MYCORRHIZAL FUNGI-LIANA ASSOCIATION FROM THE NORTHERN REGION OF PENINSULAR MALAYSIA AND THE SCREENING OF THEIR PHYTOCHEMICAL PROPERTIES

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by

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ACKNOWLEDGEMENT

Five years is not a short period of time. In five years, a baby who can only scream can now sing along to a nursery rhyme, and who was unable to say what's on their mind can now state their opinion. Surely things have changed so much within five years. A person's will to finish what they have started can also fade within this time. Five years is the time I took to complete this journey. Nonetheless, Alhamdulillah. Alhamdulillah, for a continuous and endless support together with prayers from my family, friends and lecturers. I managed to submit this long overdue dissertation. Thus, I would like to commemorate my deepest gratitude to Allah s.b.t for guiding me a path to work with such wonderful people. To Prof. Madya Dr. Rahmad Zakaria, thank you for sharing your knowledge and giving me a full trust in finishing this project. To Dr. Hasnuri Mat Hassan, thank you for believing in me and not giving up on me when I have already given up on myself. I thank you both for understanding my condition and for your excellent supervision. Special thanks to the staff members of PPSKH who have helped me directly or indirectly throughout my journey to finishing this research. To Zhafarina, Fasehah, Nurfariza, Aziera, Diana, Fatin Izzati, Zahidah, Izzuddin, Syafiq and the rest of you whom I have encountered during my time in USM as a student, thank you. Thanks to 4th Royal Belum-Temegor Scientific Expedition 2015. To Encik Abdul Rahim Abd Hamid and Puan Samiyah A. Hamid, I love you both for the utmost understanding and endless moral support. Heartiest gratitude towards them both as I would have not been where I am today without the sacrifice and prayers that they have made for me. Alhamdulillah.

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LIST OF ABBREVIATIONS

cm	Centimeter
µg	Microgram
mg	Milligram
mL	Milliliter
min	Minute
M	Molarity
psi	pounds per square inch
rpm	Revolution per minute
g	Gram
kg	Kilogram
n	number of replications
P	Phosphorus
N	Nitrogen

HUBUNGKAIT SIFAT TANAH DENGAN ASOSIASI KULAT MIKORIZA-LIANA DARI WILAYAH UTARA SEMENANJUNG MALAYSIA DAN SARINGAN SIFAT FITOKIMIANYA.

ABSTRAK

Fokus utama kajian ini adalah untuk mengenal pasti hubungkait antara liana dan mikoriza. Seperti yang telah dihuraikan daripada penulisan lain, liana mempunyai adaptasi yang baik ketika musim kemarau, manakala mikoriza diketahui membantu pengambilan nutrien dan air bagi tumbuhan perumahnya, maka hipotesis bahawa berkemungkinan kedua-dua organisma ini mempunyai hubungkait tercetus setelah teori tersebut digabungkan. Tanah rizosfera dan sampel liana telah diambil dari Kedah (Gunung Jerai), Pulau Pinang (Cherok Tokun) dan Perak (Royal Belum, Temengor dan Teluk Senangin) dan pemilihan liana ditumpukan kepada enam spesis: *Coscinium fenestratum, Strychnos ignatii, Luvunga crassifolia*, *Fissistigma manubriatum*, *Ampelocissus cinnamomea* dan *Smilax* sp. Kaedah pengesanan mikoriza seperti pengiraan spora dan peratusan jangkitan akar telah digunakan bagi mengesahkan asosiasi perumah dan kulat serta mengesahkan hipotesis yang telah dibuat. Penentuan tekstur tanah dan aspek fiziko-kimia seperti pH tanah dan kepekatan nitrogen dan fosforus (jumlah keseluruhan dalam tanah, tersedia, dan pengambilan nutrien dari pucuk) telah dijalankan bagi menilai kesan elemen tersebut terhadap bilangan spora dan jangkitan akar. Keputusan daripada kajian ini mendapati bahawa jangkitan kulat mempunyai korelasi positif dengan kepekatan jumlah keseluruhan P tanah ($r = 0.4510$) pada $P < 0.01$ dan peratusan tanah liat dalam tanah ($r = 0660$) pada $P < 0.01$ tetapi mempunyai korelasi negatif dengan peratusan pasir ($r = -0.614$) pada $P < 0.01$.

Sementara itu, bilangan spora berkorelasi secara negatif dengan kepekatan N tersedia ($r = -0.256$) dan pH tanah ($r = -0.319$) pada $P < 0.05$. Ekstrak akar, batang dan daun daripada sampel liana menunjukkan kehadiran fenol, fitokimia yang dijumpai dalam semua spesis yang dikaji selain *C. fenestratum*.

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ABSTRACT

The main focus of this study was to determine the presence of association between liana and mycorrhiza. As liana was described by many literatures to have better adaptation during water scarcity season, meanwhile mycorrhiza was known to be helping in nutrient and water uptake of the host it associated with, a hypothesis that there could be a link between these two organisms was made after putting these theories together. Rhizosphere soil and liana samples were collected from Kedah (Gunung Jerai), Penang (Cherok Tokun) and Perak (Royal Belum, Temengor and Teluk Senangin) and the selection of liana were narrowed down to six species: *Coscinium fenestratum, Strychnos ignatii, Luvunga crassifolia*, *Fissistigma manubriatum*, *Ampelocissus cinnamomea* and *Smilax* sp. Mycorrhizal detection approaches such as spore count and percentage of root infection have been employed to confirm the association of host plant and the fungus thus to further verify hypothesis made. Soil texture and physico-chemical aspects such as soil pH and concentration of nitrogen and phosphorus (soil total, available and shoot) were determined to asses the effects of these elements on the number of spore and root infection. Results showed that mycorrhizal root infection from this study was positively correlated with the concentration of soil total P ($r = 0.4510$) at $P < 0.01$ and percentage of clay in soil ($r =$

0.660) at P < 0.01 but were negatively correlated with the percentage of sand (r = -0.614) at P < 0.01. Meanwhile, number of spore was negatively correlated with concentration of available N (r = -0.256) and soil pH (r = -0.319) at P < 0.05. Extract of roots, stems and leaves of sampled liana showed the presence of phenol, a phytochemical found in all species tested. In contradicting to phenol, alkaloid was found to be absent in all species tested except for *C. fenestratum*.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

One of the most recent changes in tropical forest is the immense growth of liana, while studies on their chemical properties are not well documented. Aboveground competition is one of the most prevalent effect of liana, as it is in their nature to ascend to the forest canopy via other trees. Nonetheless belowground competition between liana and trees still needed to be ascertained.

It is unanimously agreed that liana compete greatly with other trees through evidences from researches done in Amazonian Bolivia and Barro Colorado Island, Panama forest (Ingwell et al., 2010; Pérez-Salicrup, 2001; Schnitzer & Carson, 2010). Liana infestation on trees has becoming a common sight in tropical forest. They physically cause damage to their host by restraining its growth (Schnitzer et al., 2005). Liana maximizes their exposure to sunlight by climbing up the host thus reducing light accessibility to its host (Avalos et al., 2007; Bongers et al., 2002).

Liana are well rooted and will remain rooted throughout their lives (Bongers et al., 2002). This resulted in laborious root excavation activity, therefore explaining the lack of research being done on this matter. It is difficult to see with the naked eye the effects and severity of belowground competition. It can only be seen once its host had suffered from a serious damage. According to a study done by Lvarez-Cansino et al. (2015), the removal of lianas from its host immediately increases tree sap velocity. Other findings from this study shows that lianas compete under water stress and the degree of their competitiveness intensify during dry season.

Understanding the interaction in a sense of how lianas and trees compete is crucial as this would enlighten the influence of liana abundance in tropical forest. A recent study by Lvarez-Cansino et al. (2015) concluded that the effect of lianas varies and are non-host specific. From the same research, they found that lianas compete forcefully, notably during dry season, with water acting as limiting factor. Other study done by Schnitzer (2005), stating that during seasonal drought, lianas prevail remarkably against other trees. However how much does liana compete with its host? To what degree that we can agree that liana are in fact detrimental? It could be possible that the host itself grow in a poor habitat and in an infertile land.

With accretion of liana population in disturbed forest, data collected for this research may be useful especially in providing additional knowledge and understanding on the interaction of liana and its belowground environment. The confirmation of association between mycorrhiza and liana will further justify their ability to thrive against other trees. Identification of phytochemical compounds in liana particularly of those with medicinal value will encourage other researcher to reveal the potential of the remaining undervalued lianas in Malaysia.

This research is conducted to determine whether or not liana is associated with mycorrhiza which might link to the contribution towards the success of liana during water scarcity season. Conjointly, nutrient analysis will be done into finding out the influence of nitrogen and phosphorus on mycorrhiza. The outcome from these two findings will enable us to better understand the adaptability of liana in its natural habitat. The rationale behind the selections of a few liana species with medicinal value for this research is to make noticeable of the undervalued potential of lianas with great use. Thus, the objectives of this research are:

1.2 Research objectives

1. To determine the trends of mycorrhizal root infection and number of spores from different type of soil texture.

2. To determine the effects of nitrogen and phosphorus concentration on mycorrhizal percentage of root infection and number of spores.

3. To determine the presence of phytochemical compounds of selected liana species.

1.3 Hypothesis statements

Hypothesis 1:

Null (H_0): There is no significant in the trends of mycorrhizal root infection and number of spores from different type of soil texture

Alternative (H_a): There is a significant in the trends of mycorrhizal root infection and number of spores from different type of soil texture

Hypothesis 2:

Null (H_0): There is no significant in the effects of nitrogen and phosphorus concentration on mycorrhizal percentage of root infection and number of spores.

Alternative (H_a): There is a significant in the effects of nitrogen and phosphorus concentration on mycorrhizal percentage of root infection and number of spores.

Hypothesis 3:

Null (H_0): There is no significant in the presence of phytochemical compounds of selected liana species.

Alternative (H_a): There is a significant in the presence of phytochemical compounds of selected liana species.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of sampling site

Five different locations have been chosen as the main sampling sites for this study. The selected locations were Gunung Jerai in Kedah, Cherok Tokun in Penang, Teluk Senangin, Temengor and Royal Belum in Perak. Gunung Jerai is located at the border of Kuala Muda and Yan district, 50.37 km away from Universiti Sains Malaysia. Its peak is at 1,175 meter above sea level and it is the highest geographical landmark in Kedah. Cherok Tokun is located in Bukit Mertajam, Penang (latitude 5.37 and longitude 100.48) with its height reaching at 541 meter above sea level. Teluk Senangin is located in Lumut, Perak (latitude 4.33 and longitude 100.58, altitude 27 meters).

Royal Belum State Park and Belum -Temengor Forest Reserves are both located in the Northern Perak. As mentioned by Chye (2010), it was once a single area divided into two parts as a 124 km road was built across this area causing the split up of BelumTemengor. Royal Belum State Park is located at the bound of north Perak near to Malaysia-Thailand border (latitude 5.68 and longitude 101.39, altitude 301 meters) Belum- Temengor Forest Reserves are located at the South of Belum (latitude 5.47 and longitude 101.34, altitude 315 meters) The area covering 266,000 ha of land consisting predominantly Sundaic lowland and hill dipterocarp forest. In 1977, the completion of Temengor Dam further causes drastic changes in that area where 18,000 ha of untouched forest were flooded thus creating one of the biggest man-made lake in Malaysia.

2.2 Liana

Liana are woody climber that thrive and seize its essential needs by getting a free lift, ascending to the forest canopy using nearby trees. This allows them to focus on stem and root elongation, reproduction and competing with other trees for maximum resources requirement (Bongers et al., 2002). Liana are usually mistaken for a taxonomic grouping of a plant. For instance, liana may arise from family Orchidaceae (vanilla), Arecaceae (rattan), Dioscoreaceae (yam) and Vitaceae (grape). These families are not related genetically to one another. Nevertheless, some species within these families possess the same climbing properties. Thus, they are group together and known as liana. Liana can be considered as a disturbance indicator (Roderick et al., 2003) as they are widely spread in a high canopy gap area in the forest (Schnitzer & Bongers, 2011). Liana are known for their adaptability. They are able to modify leaf, stem and branch to enable clasping on their hosts. This includes stem twining, formation of thorns, spine, adhesive hairs and adventitious roots (Bongers et al., 2002).

2.2.1 *Ampelocissus cinnamomea*

Taxonomy of *Ampelocissus cinnamomea* species is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Rosanae
Order	:	Vitales
Family	:	Vitaceae
Genus	:	*Ampelocissus*
Species	:	*Ampelocissus cinnamomea* (Wall. ex M.A.Lawson) Planch.

Taxonomy of *Ampelocissus cinnamomea* species (Integrated Taxonomic Information System, 2017; The Plant List, 2013)

Ampelocissus cinnamomea is a species found in the family Vitaceae. *A. cinnamomea* was previously known as *Vitis cinnamomea* in old plant catalogue (Rao, 2010). *Ampelocissus* was first described by Planchon (Ng et al., 2014). Study made by Ren et al. (2011) suggested that *Ampelocissus* has a high relatedness to *Nothocissus* and *Pterisanthes* (other genera found in the Vitaceae family). There are about 985 species names has been accepted as the members of Vitaceae in the The Plant List (2013), with each being classified accordingly within 16 plant genera. *Ampelocissus cinnamomea* falls in the genus *Ampelocissus* with other 96 species as stated in The Plant List (2013).

It is very likely to confuse *A. cinnamomea* with another species from the same genus, especially *Ampleocissus ascendiflora* Latiff as both species possess close morphological resemblance. It is to note that the leaves of *A. ascendiflora* Latiff only present in a simple form throughout their life, whereas for *A. cinnamomea*, young leaves are simple but will gradually outgrow into becoming a 3-foliolate leaves when matured (Ng et al., 2014; Yeo et al., 2013). The later appearance of leaves forms an obovate shape in the terminal leaflet's blade while lateral leaflet's blade is asymmetric oblong. The leaves appear in a white tomentose underneath at juvenile stage that will later turn brownish-bronze when older. Different form of leaves may occur on a single stem as shown in Figure 2.1 (i-iii). Whereas the inflorescence is leaf-opposed; petals of the flowers are red with green discs (Ng et al., 2014). It can be found in open spaces and along rivers.

It is used as tonic for men in a folklore remedies. However study done by Kusuma et al. (2014) discovered a good antibacterial activity manifested by *A. cinnamomea* against *Propionibacterium acnes*, which is a Gram-positive anaerobic bacteria, responsible for inflammatory diseases such as acne vulgaris on human skin. Another findings from this study showed that the ethanolic extract of *A. cinnamomea* contain antioxidative effects. Discovery made by Hazalin et al. (2009) unfold the presence of endophytic fungi isolated from the leaves, stems and roots of *A. cinnamomea* which could yield metabolites that can display antibacterial activities.

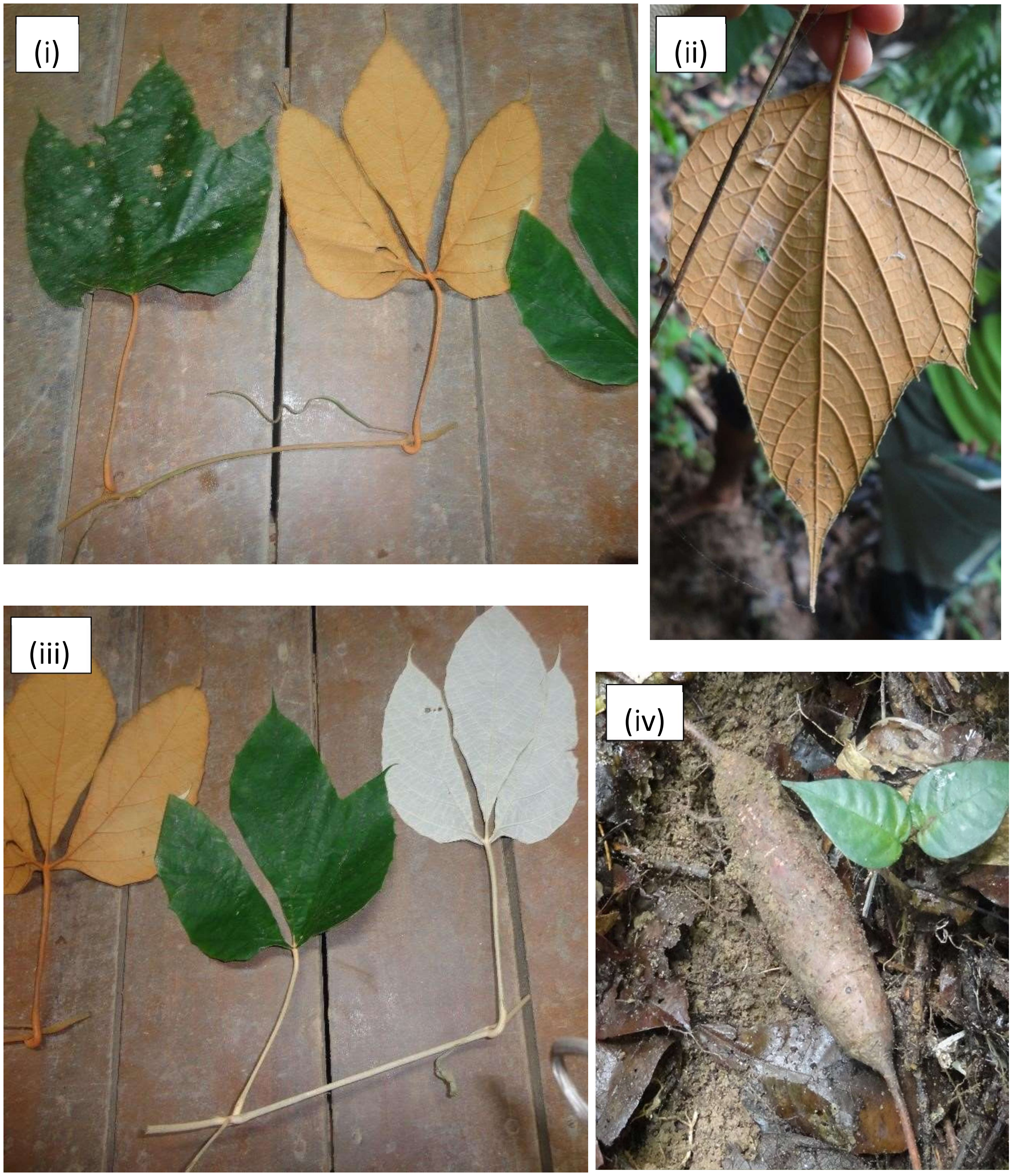

Figure 2.1: (i) Old leaves of *A. cinnamomea* with two different shapes, palmately lobe and trifoliate attached to the same stem side by side. (ii) Abaxial surface of a simple palmately lobed leaf of *A. cinnamomea* with brownish-bronze underneath. (iii) Two juvenile leaves of *A. cinnamomea* front and back with glaucous underneath. (iv) Rhizome of *A. cinnamomea.* Image source: Norahizah Abd Rahim.

2.2.2 *Coscinium fenestratum*

Taxonomy of *Coscinium fenestratum* is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Ranunculanae
Order	:	Ranunculales
Family	:	Menispermaceae
Genus	:	*Coscinium*
Species	:	*Coscinium fenestratum* (Goetgh.) Colebr.

Taxonomy of *Coscinium fenestratum* (Integrated Taxonomic Information System, 2017; The Plant List, 2013)

Coscinium fenestratum from the Menispermaceae family are known as akar mengkunyit and tongkat ali emas by the local. The vibrant yellow inner flesh reminded the local of the turmeric colour, thus the name akar mengkunyit came about. There are 68 of plant genera that have been listed in The Plant List (2013) under this family. At present, 448 species name are accepted, belonging to the family of Menispermaceae. The Plant List (2013) includes 10 scientific species name for the genus *Coscinium*, but only 2 are acknowledged to date.

C. fenestratum can be commonly found in high rainfall forest across Sri Lanka, Peninsular Malaysia, Thailand, Cambodia, Vietnam, Sumatra and Borneo (Selvam, 2012). Although it is common in certain places, *C. fenestratum* requires an extensive time to grow as they only reached their reproductive stage 15 years after germination. This is threatening as they could not comprehend with the excessive collection of their bark and root for medical purposes in addition to exuberant forest destruction (Selvam, 2012). This will impose great damage to the survivability of *C. fenestratum* in the wild.

One would easily indentify *C. fenestratum* by making a small cutting on its stem to see a yellow vibrant colour inside. The leaves are simple, alternate, ovate in shape, cordate with truncated base, acuminate apex; petioles are clearly swollen at both ends with prominent five to seven main nerves as shown in Figure 2.2 (i-iii). The surfaces of the leaves are glabrescent above somewhat coriaceous with glaucous, sericeous underneath. Twigs are densely grayish tomentose when young but became less tomentose as it grew older.

Inflorescence grow on old leafless stems with flower that looks almost like a takraw ball with colour ranging from pale green to yellow (Figure 2.2 (iv)). Fruits are globose to subglobose in shape with brown, orange or yellow tomentellous in accordance to the maturity stage of the fruit (Selvam, 2012). Its stem and roots are being use in treating ulcers and wound. However, there are claims in folk medicine that *C. fenestratum* is being use for treating jaundice, diabetes, fever and tonic to reduce stomachache (Selvam, 2012).

A study conducted by Shirwaikar et al. (2005) unveil the ability of alcoholic extract of the stem of *C. fenestratum* in lowering blood glucose level. The study was further supported by Yin et al. (2002) where they discovered the ability of berberine, an alkaloid which is also found in *C. fenestratum*, to influence glucose-lowering effect in a liver cell by decreasing the amount of sugar produced by the liver.

Figure 2.2: (i) Adaxial leaf of *C. fenestratum* with glabrescent surface. (ii) Abaxial leaf surface of *C. fenestratum* with glaucous, sericeous underneath. Arrow showing swollen petioles with grayish tomentose twigs. (iii) Free-standing *C. fenestratum* saplings on the forest floor. (iv) Inflorescence of *C. fenestratum* growing on old stem. Image source: Norahizah Abd Rahim.

2.2.3 *Fissistigma manubriatum*

Taxonomy of *Fissistigma manubriatum* is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Magnolianae
Order	:	Magnoliales
Family	:	Annonaceae
Genus	:	*Fissistigma*
Species Merr.	:	*Fissistigma manubriatum* (Hook.f. & Thomson)

Taxonomy of *Fissistigma manubriatum* (Integrated Taxonomic Information System, 2017; The Plant List, 2013)

Fissistigma manubriatum is one of the members of Annonaceae family. One hundred and twenty-eight plant genera were listed in The Plant List (2013) under the family of Annonaceae. There were 5,130 scientific plant names of species submitted to The Plant List (2013) to be recorded under the Annonaceae family but only 2,106 were accepted as species names with 65 species falls into the genus of *Fissistigma*.

One of the pronounce feature of plants under this family is the flower which comes in a whorl of leathery petals that will eventually developed into a club-shaped fruits (Wiart, 2006). The inner bark is often teeming with guava-like fragrant and do not contain latex or sap. As mentioned by Wiart (2006) it is estimated that there are almost 50 species from this family possess medicinal value, unfortunately the use of it is not notable to many.

F. manubriatum was known as *Melodorum manubriatum* Hook. f. and Thomson in 1855 and were later changed to *F. manubriatum* (Hook. f. and Thomson) Merr (Singh et al., 2013). They are widely distributed in the Sumatra, Malay Peninsula, Borneo, Philippines and was later discovered in Meghalaya, India during 2008 exploration (Singh et al., 2013). In various parts of Malaysia and Indonesia, *F. manubritum* is known by numerous of different names.

Some of the local names given to *F. manubriatum* are subjected to its ability and nature. For instance, *F. manubriatum* is known as akar sembelit for its ability to treat or ease upset stomach. This is in agreement with Wiart (2006) whom, had remarked the influence of isoquinoline, an alkaloid found in *F. manubriatum,* to soothe stomachache by interfering with the production of gastric juices in the lining of the stomach. *F. manubritum* is also known as akar larak, akar kenchon, akar jangkar and akar mempisang among the locals stretching across Malaysia and Indonesia (Irawan, 2005).

This species is found in lowland to sub mountain. The leaves are alternately arranged, about 5 – 18 cm long with rounded base, pointed tips with sericeous (covered with fine silky hair) underneath as shown in Figure 2.3 (i-iv). The midrib of its leave is brownish orange with dull green underneath with young twigs tomentose (densely matted woolly hair almost velvety) (Figure 2.3 (i-ii)). The fruits are ovoid-globose in shape and are tawny tomentose (velvety orange-brown).

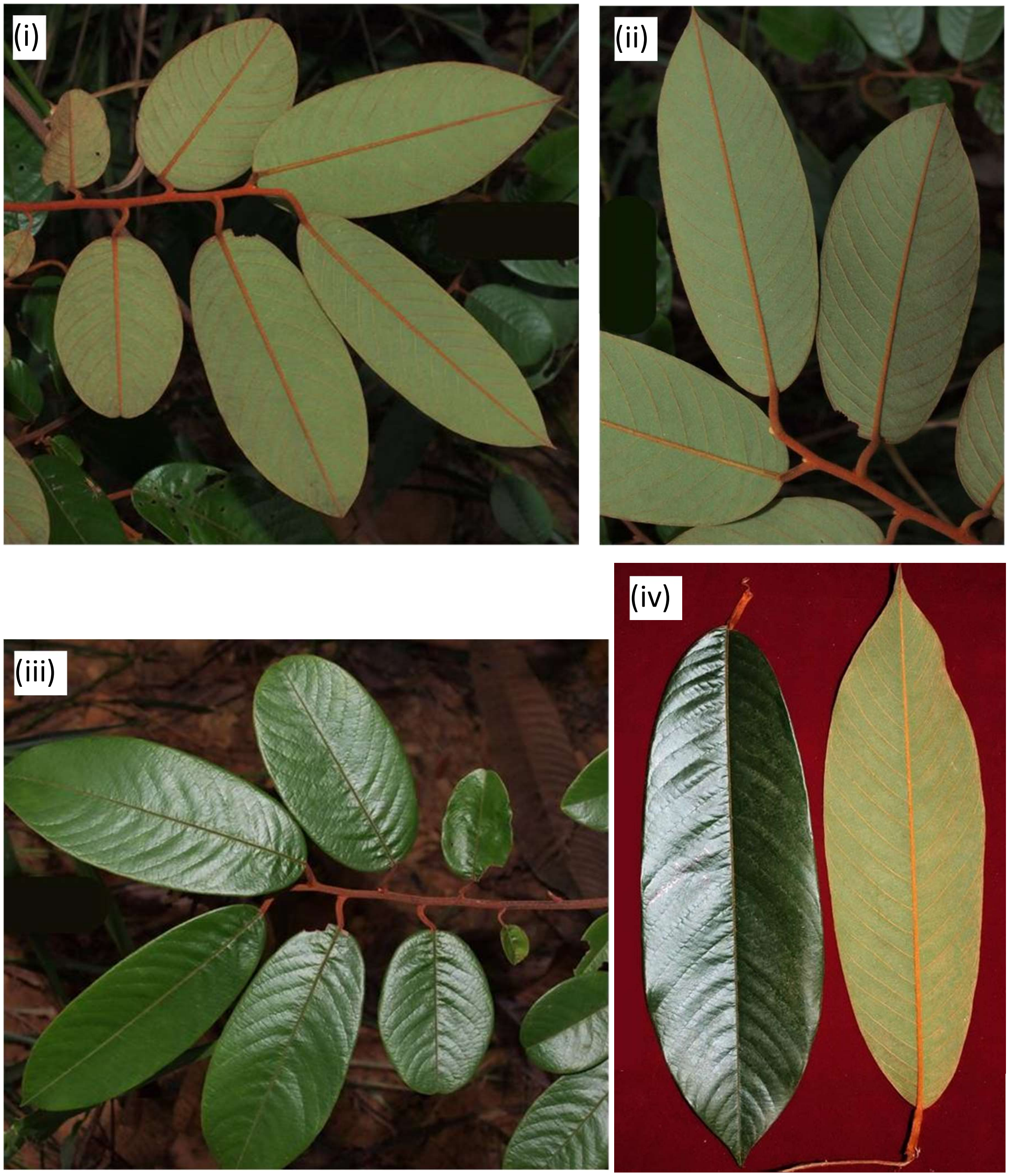

Figure 2.3: (i) Abaxial leaves of *F. manubriatum*. (ii) The closeup image of abaxial leaves surface of *F. manubriatum*. (iii) Adaxial leaves of *F. manubriatum*. (iv) Single leaf of *F. manubriatum* side by side. Image source: Assoc. Prof. Dr. Rahmad Zakaria.

2.2.4 *Luvunga crassifolia*

Taxonomy of *Luvunga crassifolia* species is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Rosanae
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	*Luvunga*
Species	:	*Luvunga crassifolia* Tanaka

Taxonomy of Luvunga *crassifolia* (Integrated Taxonomic Information System, 2017; Roskov et al., 2017)

Luvunga crassifolia Tanaka belongs in the Rutaceae family. There are 158 genera recorded in The Plant List (2013). Over 1,000 species names have been accepted with 2 species names falls into the genus of *Luvunga*. Some species within this genus are still being revised to this date. As mentioned by Stone (1985), although the attribute of *Luvunga* is recognizable, it is challenging to identify sterile collections.

L. crassifolia is a big woody climber with prominent spine at juvenile and develop into a hook when matured as shown in Figure 2.4 (ii). Leaves are alternate, trifoliate with thick coriaceous surface (Figure 2.4 (iii)). They are broad, elliptic oblong with apiculate leaf apex; terminal inflorescences or sometimes basal to leaves. Flowers are bisexual with 3-5 lobed cup-shape calyx; 3-5 petals; 6-10 equally length stamens (Dianxiang & Hartley, 2008).

An interview done with Jahai people of Royal Belum during 4th Royal Belum scientific expedition done in 2015, described that the root of *L. crassifolia* is being used as ointment to relief muscle pain. Ong et al. (1996) also included *L. crassifolia* as one of a medicinal plant found in the Botanic Garden of University Malaya. According to Setyowati et al., (2005) a Dayak tribe from Central Kalimantan, Indonesia, have been incorporating nature in their daily basis and not forgetting being dependent on natural remedies for better health. By boiling the roots of *L. crassifolia* together with roots of *Eurycoma longifolia* (tongkat ali), the concoction is being consumed as a tonic to maintain the well being of the body.

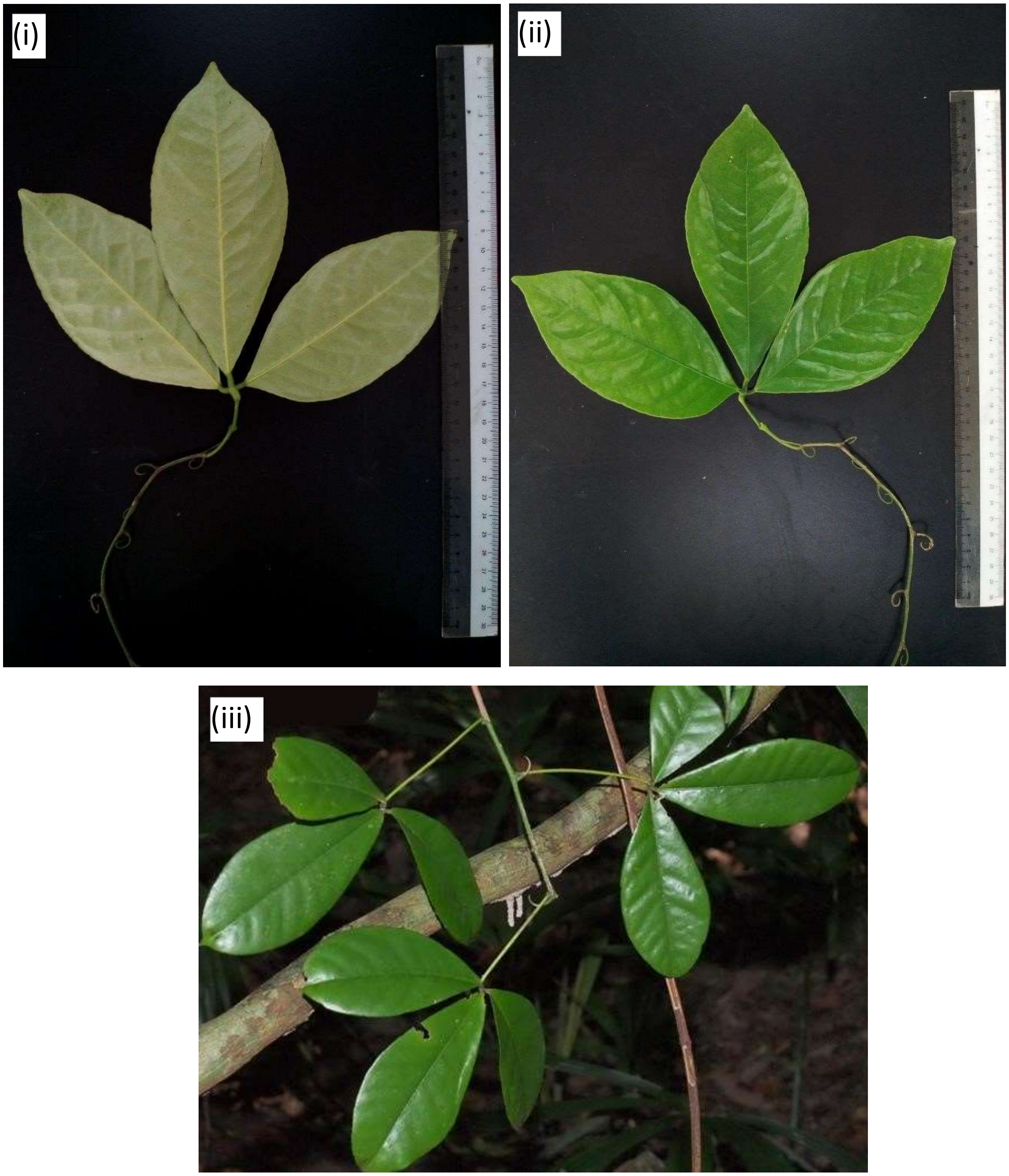

Figure 2.4: (i) Abaxial leaves of *L. crassifolia*. (ii) Adaxial leaves of *L. crassifolia.* Arrow showing the hook structure of *L. crassifolia*. (iii) Image of *L. crassifolia* in the forest. Image source: Norahizah Abd Rahim.

2.2.5 *Smilax* sp.

Taxonomy of *Smilax* species is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Lilianae
Order	:	Liliales
Family	:	Smilacaceae
Genus	:	*Smilax*
Species	:	*Smilax mysotiflora*

Taxonomy of *Smilax mysotiflora* (Integrated Taxonomic Information System, 2017; The Plant List, 2013)

There are two genera listed in The Plant List (2013) which belongs to the Smilacaceae family, *Smilax* and *Heterosmilax.* There are 261 species names have been recorded as a member of this family and 248 species names are listed as the species of the genus *Smilax* (The Plant List, 2013). *Smilax* can be found abundantly in eastern Asia. However, that doesn't mean that they could not be found in the temperate region. There are species of *Smilax* recorded from North America and Europe (Tian et al., 2017). One of the most well recognized *Smilax* species in Malaysia is *Smilax mysotiflora* also known as ubi jaga. It is known for its ability to improve sexual functions in men.

A study conducted by Wan et al. (2013) to prove the methanolic effects of *S. mysotiflora* tubers in regard to improving aphrodisiac potential on male rats has been proven effective. Indeed, the introduction of *S. mysotiflora* tubers extract, elevate fertility and excite sexual behavior on the tested rats (Wan et al., 2013).

Most medicinal preparation of *S. mysotiflora* incorporated the rhizome and tubers of this plant (Tian et al., 2017; Wan et al., 2013). However, the leaves and fruits of *S. mysotiflora* as described by Isaac Henry Burkill are able to treat syphilis and rheumatism (Ang et al., 2004). As mentioned by Dasuki et al. (2012), some of the phytoconstituents found in *S. mysotiflora* tubers are; alkaloids, saponins, flavonoid, tannins and coumarins. However, according to a review by Tian et al. (2017) more than 100 steroidal saponins have been extracted from more than 20 different *Smilax* species over the past 49 years and the influence of steroidal saponins is notable in antifungal, cytotoxic and anti-inflammatory.

According to Laitonjam & Kongbrailatpam (2010), the root decoction of *Smilax lanceifolia,* has been used by the people in North-East India to assuage stomach and extremities pain. Other than that, boiled extract of S. *lanceifolia* is being consume in similar ways to herbal tea in order to eradicate cystoliths in bladder and renal calculi in kidney (Laitonjam & Kongbrailatpam, 2010). A record made by Lin (2005) stated that the leaves of *S. lanceifolia* is being used by the Ja Hut people in Jerantut, Pahang, to relief pricking pain by applying crushed leaves to the affected area. An experiment conducted by Laitonjam & Kongbrailatpam (2010) suggested that the methanolic extract of *S. lanceifolia* posses an antioxidative property.

S. lanceifolia is a climbing vine with tendrils. Simple, alternate leaf with lanceolate to ovate-oblong leaf blade; petiole of 1-2.5 cm. The inflorescence is present in a form of axillary umbel; flowers are usually small in green to greenish white colour. It has yellowish red to black globose berries (Ba Qia, 2008).

Figure 2.5: (i) Abaxial leaf of *Smilax* sp. (ii) Adaxial and abaxial surface of *Smilax* sp. leaf. Arrow showing the tendrils of *Smilax* sp. Image source: Norahizah Abd Rahim

2.2.6 *Strychnos ignatii*

Taxonomy of *Strychnos ignatii* is as follows:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Asteranae
Order	:	Gentianales
Family	:	Loganiaceae
Genus	:	*Strychnos*
Species	:	*Strychnos ignatii* P.J. Bergius

Taxonomy of *Strychnos ignatii* (Integrated Taxonomic Information System, 2017; The Plant List, 2013)

Strychnos ignatii is an indigenous plant in the Philippines but are widely spread throughout Asia. It belongs to the Loganiaceae family. There are 15 plant genera within this family with 351 species. Up to 168 species are from the genus *Strychnos* as recorded in The Plant List (2013). In Malaysia *Strychnos ignatii* is known as “gajah tarik”.

It is a large woody climber with tapering, smooth stem. It has prominent ring-like outline throughout its stem. Leaves are oblong, smooth, leathery and opposite. They are usually rounded at the base and pointed at the tip with notable tri-nerve (Figure 2.6 (iii)). On average, the size of leaves may range from 8 to 20 centimeters long. Fruits of *S. ignatii* are round with yellowish to brown colour having diameter on average of about 10 cm. Twelve to twenty seeds are embedded in pale yellow pulps. They are bitter tasting and odorless. The seeds are being utilized for its medicinal benefits. They are about 2.5 cm wide, about the size of an olive, hard and compact. Its colour may vary from dull green to pale brown in accordance to its freshness.

It is believed by the Jahai people of the Royal Belum that the seed and bark of *S. ignatii* can be used as tonic. As mentioned in the study carried out by Marzotto et al. (2012), the seed of *S. ignatii* was utilized to treat anxiety related symptoms in mice. Strychnine is the main chemical that contributed towards this healing property. It is a strong alkaloid which needs to be used in a very small dosage otherwise it can be toxic and poisonous. Unattended exposure to strychnine by a non-practitioner may results in fatality.

Ignatia (from *Strychnos ignatii)* is commonly associated with Nux Vomica (from *Strychnos Nux Vomica)* which is a potent poison that attacks the spinal cord when used in large quantity (Kress, 1995). Both consist of strychnine but there might be slight incongruousness in term of molecular constitution in both drugs which lead to different therapeutic exposition (Kress, 1995). According to Felter & Lloyd (1900), curarine is another form of alkaloid similar in certain extent to strychnine but possessed a greater poisonous intensity. It has been extracted from *Strychnos toxifera* that was used as curare by a Yanomami tribe from Brazil.

Figure 2.6: (i) Adaxial leaves of *S. ignatii*. (ii) Abaxial leaves of *S. ignatii.* (iii) Image of the adaxial and abaxial leaves of *S. ignatii* side by side Image source: Assoc. Prof. Dr. Rahmad Zakaria.