

**LIQUID PHASE ADSORPTION OF MULTI-
COMPONENT ORGANIC SOLVENT MIXTURE ONTO
ACTIVATED CARBON**



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**FACULTY OF ENGINEERING
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**THESIS SUBMITTED IN FULFILLMENT FOR THE
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**FACULTY OF ENGINEERING
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2014

UNIVERSITI MALAYSIA SABAH

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16 June 2014

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ACKNOWLEDGMENT

BISMILLA HIRRAHMAN NIRRAHIM

(In the name of Allah, Most Gracious, Most Merciful)

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Norina Binti Yadin

16 June 2014

ABSTRACT

Liquid phase adsorption is a common technique in separation process. Most of the measurement and analysis of liquid phase adsorption are based on a single component adsorption. However, in the true process most of the adsorption process occurs in multi-component system. Hence, in this work a study of liquid phase adsorption at complete range of concentration for binary and multi-component onto adsorbent was conducted. Organic solvents such as methanol, propanol and acetone were selected as the adsorbate, whereas activated carbon was chosen as the adsorbent. Mixture of binary and three components of solvent were used as the liquid phase for the comparison. The experimental work was conducted by measuring the excess isotherm of azeotropic and non-azeotropic mixture. Result show that the excess isotherm can be explained according to the adsorption isotherm classified by Schay and Nagy. The analysis and interpretation of adsorption isotherm was carried out using Pseudo Ideal Adsorption Model for binary adsorption, Gibbs Dividing Plane Model and Langmuir-Freundlich Model for both binary and multi-component adsorption and Modified Competitive Langmuir Like Model for multi-component adsorption. Analysis of Pseudo-ideal monolayer adsorption shows that the total adsorption of Methanol-Propanol mixture was 11.79 mmole/g, Methanol-Acetone mixture 4.37 mmole/g, and Acetone-Propanol mixture 4.40 mmole/g. The result of adsorption isotherm was presented as adsorptivity of preference solvent. The adsorptivity of preference solvent from theoretical analysis was also compared with the directly measured adsorbed phase concentration. The trend of adsorption isotherms curve determined from the theoretical models was shown a monolayer adsorption behavior. However, the magnitudes of numerical values of adsorption from theoretical analysis methods used and directly measured adsorbed phase concentration were different from each other. Therefore it shows that the selection of theoretical analysis is important in the determination of adsorption isotherm. Besides that, the effect of binary adsorption and multi-components adsorption can be observed on the shape of excess adsorption isotherm and also on the magnitude of individual adsorption isotherm. Thus, assumption of single component adsorption in multi-components system may lead prediction of unrealistic process.

ABSTRAK

(FASA PENJERAPAN CECAIR UNTUK CAMPURAN PELARUT ORGANIK PELBAGAI KOMPONEN KE ATAS KARBON DIAKTIFKAN)

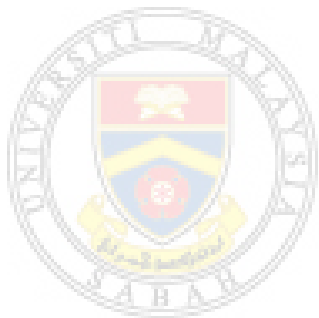
Fasa penjerapan cecair adalah satu teknik biasa dalam proses pemisahan. Kebanyakan pengukuran dan analisis fasa penjerapan cecair adalah berdasarkan penjerapan komponen tunggal. Walau bagaimanapun, dalam proses yang sebenar, proses penjerapan berlaku dalam sistem pelbagai komponen. Oleh yang demikian, dalam kajian ini, fasa penjerapan cecair untuk kepelbagaian kepekatan yang lengkap untuk dua campuran dan kepelbagaian campuran akan dijalankan. Pelarut organik seperti metanol, propanol dan aseton telah dipilih sebagai bahan terjerap, manakala karbon diaktifkan telah dipilih sebagai bahan yang menjerap. Campuran pelarut dua komponen dan tiga komponen telah digunakan sebagai fasa cecair untuk perbandingan. Kerja-kerja eksperimen telah dijalankan dengan mengukur isoterma lebih untuk campuran azeotrop dan campuran bukan azeotrop. Keputusan menunjukkan bahawa isoterma lebih dapat dijelaskan mengikut isoterma penjerapan yang diklasifikasikan oleh Schay dan Nagy. Analisis dan tafsiran penjerapan isoterma telah dijalankan dengan menggunakan model Pseudo Ideal Adsorption untuk penjerapan dua campuran, model Gibbs Dividing Plane dan model Langmuir-Freundlich untuk penjerapan dua campuran dan pelbagai campuran manakala model Modified Langmuir Like untuk penjerapan pelbagai campuran sahaja. Analisis yang dijalankan dengan menggunakan model Pseudo Ideal Adsorption menunjukkan bahawa jumlah penjerapan untuk campuran metanol-propanol adalah 11.79 mmole / g, campuran metanol-aseton ialah 4.37 mmole / g, dan campuran aseton-propanol adalah 4.40 mmole / g. Keputusan penjerapan isoterma telah dibentangkan sebagai adsorptivity untuk pelarut keutamaan. Adsorptivity pelarut keutamaan daripada analisis teori juga akan berbanding dengan kepekatan fasa terjerap yang akan diukur secara langsung. Bentuk lengkungan untuk isoterma penjerapan yang dihasilkan melalui model teori menunjukkan sifat penjerapan satu lapisan. Walau bagaimanapun, magnitud untuk nilai-nilai berangka penjerapan daripada kaedah analisis teori yang digunakan dan kepekatan fasa terjerap yang diukur secara langsung adalah berbeza antara satu sama lain. Oleh yang demikian, ianya menunjukkan bahawa pemilihan analisis teori adalah penting dalam penentuan penjerapan isoterma. Di samping itu, kesan penjerapan dua campuran dan pelbagai komponen dapat dilihat pada bentuk penjerapan isoterma yang berlebihan dan juga pada magnitud isoterma penjerapan individu. Oleh itu, andaian penjerapan komponen tunggal dalam sistem pelbagai komponen boleh membawa ramalan proses yang tidak realistik.

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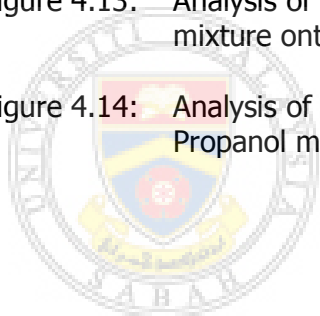
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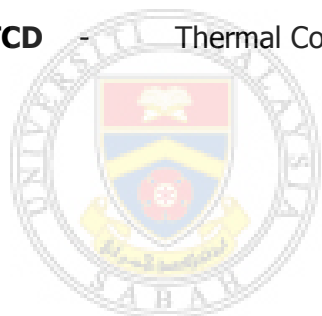
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LIST OF ABBREVIATION

- ACF** - Fibrous form of Activated carbon
- GAC** - Granular activated carbon
- GCMS** - Gas Chromatography Mass Spectrophotometer
- IAS** - Ideal adsorbed solution
- LF** - Langmuir-Freundlich
- LV** - LeVan-Vermeulen
- MCL** - Modified Langmuir like model
- PAC** - Powdered activated carbon
- SFE** - Supercritical Fluid Extraction
- TCD** - Thermal Conductivity Detector

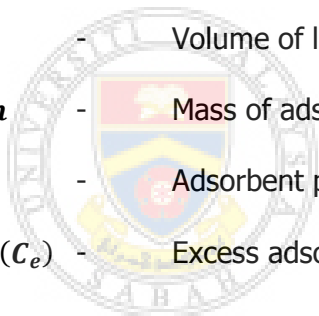


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LIST OF SYMBOL

$\%$	-	Percentage
(C_e)	-	Equilibrium concentration
C_0	-	Initial concentration of adsorbate
C_e	-	Final equilibrium concentration of adsorbate
$C_e V_a$	-	Equilibrium adsorbate concentration on the volume of adsorbed layer
N_s	-	Saturation adsorption capacity
V_a	-	Surface specific volume of adsorbed phase
W_0	-	Initial total mole of liquid mixture
W_0^w	-	Initial total mass of liquid mixture
W_s	-	Mass of adsorbent
a_{max}	-	Maximum adsorbate amount which could be adsorbed on 1 m ² on the adsorbent surface
a_{tot}	-	Total adsorbed amount in the adsorbed layer adsorbed on 1 m ² on the adsorbent surface
n_1^s	-	Amount of component 1 adsorbed
n_2^s	-	Amount of component 2 adsorbed
x_1	-	Equilibrium liquid mole fraction of preference adsorbate
x_{10}	-	Initial liquid mole fraction of preference adsorbate
x_{10}^w	-	Initial mass fraction of component 1
x_1^s	-	Adsorbed phase mole fraction of component 1
x_1^w	-	Equilibrium mass fraction of component 1
x_2	-	Equilibrium liquid mole fraction of component 2
x_2^s	-	Adsorbed phase mole fraction of component 2

Γ_1	-	Excess Adsorption of preference adsorbate
Γ_1^w	-	Relative adsorption of component 1
$^{\circ}\text{C}$	-	Degree Celsius
C	-	Carbon
CO₂	-	Carbon dioxide
e.g.	-	(exemple) means" for example" introduces example
<i>et al.</i>	-	Replace as list of names of persons
g	-	Gram
H	-	Hydrogen
O	-	Oxygen
K	-	Equilibrium constant
V	-	Volume of liquid mixture
m	-	Mass of adsorbent
q	-	Adsorbent phase concentration after equilibrium
$\Gamma(C_e)$	-	Excess adsorption value



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CHAPTER 1

INTRODUCTION

Labisia pumila or commonly known as Kacip Fatimah in Malaysia is a herbaceous plant widely used in folk medicine for facilitating childbirth and post-partum recovery (Bodeker, 2009). The phytochemical constituents of this herb have been well documented with phenolics and flavonoids being the main compounds (Norhanisah *et al.*, 2013). Several scientific studies reported that *L. pumila* possesses biological activities such as antioxidant (Norhaiza *et al.*, 2009; Karimi *et al.*, 2011), anti-carcinogenic (Pihie *et al.*, 2011), anti-microbial (Karimi *et al.*, 2011), antifungal and anti-inflammatory activities (Karimi *et al.*, 2013).

Considering the interesting pharmacological values that *L. pumila* has to offer, raw materials of this herb is highly demanded for commercial production. However, the propagation and growth rate of wild *L. pumila* is rather slow and time consuming (Mohd. Noh *et al.*, 2002; Jaafar *et al.*, 2009). Hence, a propagation system that can supply *L. pumila* continuously must be established to accommodate the demand of bioactive compounds synthesised by this herb.

Plant cell culture is an ideal biotechnological approach for secondary metabolites production as it produce continuous and reliable source of plant-based pharmaceutical products (Rao & Ravishankar, 2002; Yue *et al.*, 2016). Research to date has successfully produces high yielding cultures from various medicinal plants in either undifferentiated or differentiated cultures (Yue *et al.*, 2016). Undifferentiated cell suspension cultures lack stability and uniformity (Habibi *et al.*, 2017) which resulted in lower production of high value natural products (Yue *et al.*, 2016). In contrast, organ culture, especially adventitious root culture is more

favourable due to its fast growth and stable production of secondary metabolites (Murthy *et al.*, 2008; Habibi *et al.*, 2017).

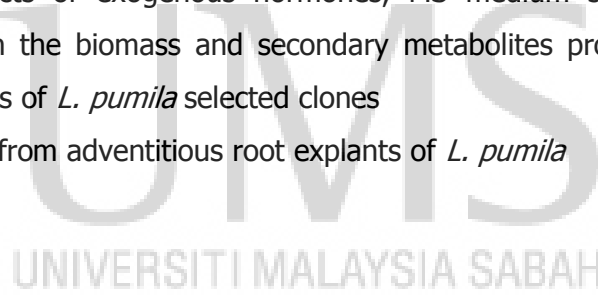
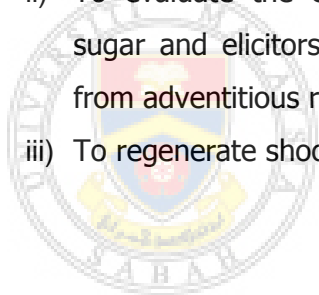
Establishment of organ cultures that produce large amounts of biomass with increased accumulation of secondary metabolites is possible through specific strategies (Murthy *et al.*, 2014a). These includes the selection of high-yielding clones, optimisation of medium composition such as type of basal medium, carbon source and plant growth regulators; and physical factors such as temperature, medium pH, agitation and aeration. Other approaches such as elicitation, precursor feeding, permeabilisation and immobilisation could also assist with the accumulation of metabolites (Abouzid, 2014; Malik *et al.*, 2014; Murthy *et al.*, 2014a; Ali *et al.*, 2016; Yue *et al.*, 2016; Andrews & Robert, 2017).

Through optimisation of *in vitro* culture conditions of adventitious root culture, high product concentration and efficacy can be achieved from the continuous source of secondary metabolites of root cultures (Murthy & Praveen, 2012). This study will highlight some of the strategies undertaken to increase *L. pumila* adventitious root metabolites yield including selection of clones, optimisation of plant growth regulators, MS medium strength and carbon source; and also elicitation. Initiation of organ cultures began with selecting parent plants that showed higher contents of the desired secondary product for organ induction (Murthy *et al.*, 2014a). The selection of a specific organ for the induction of *in vitro* adventitious roots is essential as the accumulation of metabolites varies in different organs of the same species. Following selection of high performing organ lines, another key consideration is to establish optimum media and culture composition (Ochoa-Villarreal *et al.*, 2016). Typical modifications to the adventitious root culture medium include the addition of phytohormones (Wu *et al.*, 2006; Baque *et al.*, 2010a; Fazal *et al.*, 2014), modification of the salt strength (Baque *et al.*, 2010b; Li *et al.*, 2015; Deepthi & Satheeshkumar, 2017) and sugar concentration (Baque *et al.*, 2012; Yin *et al.*, 2013; Li *et al.*, 2015). In addition, metabolite production in organ cultures can be stimulated *in vitro* by adding elicitors into the culture medium as metabolites are produced by plants in response to the imposed stresses (Naik & Al-Khayri, 2016; Andrews & Robert, 2017).

Apart from producing secondary metabolites, adventitious root can also serve as a reliable micropropagation method in tissue culture especially when numerous small shoots arise rapidly from each explant, hence leading to high rate of propagation. Previous studies on shoot regeneration of *L. pumila* only focused on leaf and stem explants (Hartinie, 2007; Ling *et al.*, 2013; Ozayanna, 2015; Syafiqah *et al.*, 2016). No attempt was done to explore the potential of adventitious roots explants of *L. pumila* for shoot regeneration purpose.

Therefore, the present study has focused on the aforementioned strategies to produce bioactives from adventitious root cultures of *L. pumila* with antioxidative properties. In addition, the potential of adventitious root explants of *L. pumila* for producing new shoots will also be investigated. The objectives of the study are;

- i) To select superior *in vitro* source materials from each variety of *L. pumila* (var. *alata*, var. *pumila* and var. *lanceolata*) for high antioxidative properties
- ii) To evaluate the effects of exogenous hormones, MS medium strength, sugar and elicitors on the biomass and secondary metabolites production from adventitious roots of *L. pumila* selected clones
- iii) To regenerate shoots from adventitious root explants of *L. pumila*



CHAPTER 2

LITERATURE REVIEW

2.1 *Labisia pumila* (Bl.) Fern. Vill

2.1.1 Origin, distribution and taxonomy of *Labisia pumila*

Labisia pumila (Bl.) Fern. Vill is herbaceous plant which grows wildly in the rain forest of Malaysia, Indochina, Thailand and Papua New Guinea (Sunarno, 2005). The distribution of *L. pumila* is shown in Figure 2.1. In Malaysia, this herb is usually known as Kacip Fatimah. Other local names of *L. pumila* include Selusoh Fatimah, Kacit Fatimah, Tadah Matahari and Mata Pelanduk Rimba (Sunarno, 2005; Jamal, 2006).



Figure 2.1 : Distribution of *L. pumila*

Source : Global Biodiversity Information Facility (GBIF) Secretariat (2016)

According to Sunarno (2005), there are eight varieties of *L. pumila* namely var. *alata*, var. *discoplacenta*, var. *gladiata*, var. *lanceolata*, var. *pumila*, var. *malintangensis*, var. *neriifolia* and var. *sessilifolia*. Among these eight varieties, only var. *alata*, var. *pumila* and var. *lanceolata* are well-known in Malaysia (Stone, 1990). These three varieties can be distinguished from each other via their petiole and leaf physical appearances (Sunarno, 2005).

The taxonomy of *L. pumila* is shown in Table 2.1. *Marantodes pumilum* (Blume) Kuntze is a heterotypic synonym of *L. pumila* that has been accepted by The Plant List (2013). This name was originally found in Post and Kuntze (1903) as accepted taxon in the genus *Marantodes* (family Primulaceae). Myrsinaceae and Primulaceae are two best known families in Ericales. The taxon limits of Myrsinaceae and Primulaceae have been substantially changed, therefore the limits of Primulaceae was extended based on numerous synapomorphies within the group (Mabberly, 2008; Bremer *et al.*, 2009).

Table 2.1: Taxonomy of *L. pumila*

Taxonomy	
Domain	Eukaryota
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Ericales
Family	Myrsinaceae
Genus	<i>Labisia</i>
Species	<i>Labisia pumila</i>

Source: Global Biodiversity Information Facility (GBIF) Secretariat (2016)

2.1.2 Morphological description

Wild *L. pumila* usually grows in habitat with humus-rich soils, sandy loam and sometimes in deep clay soil or granite soils. This plant is able to grow until 60 cm in height and carries four to twelve leaves per plant. Its leaf size is approximately around 5 to 35 cm long and 2 to 8 cm wide. In addition, *L. pumila* also produced flower and fruits. Their whites to pinkish flowers are quite small which grow in spike like panicle or small clusters. Meanwhile, the size of the fruit is about 0.5 cm in diameter which changes colour from green to red or purple when ripen (Stone, 1988; Zhari *et al.*, 1999; Sunarno, 2005). The comparison of morphological characteristics and the habitat of the three varieties of *L. pumila* are shown in Table 2.2. Figure 2.2 shows the three varieties of *L. pumila* which were grown in the field.

Table 2.2: Morphological characteristics and habitat of *L. pumila*

Variety	var. <i>alata</i>	var. <i>pumila</i>	var. <i>lanceolata</i>
Petiole shape	Broad winged	Slightly winged	Terete
Length of petiole	5-12 cm	4-15 cm	6-21 cm
Length of anther	0.8 mm	1.2 mm	0.8 mm
Habitat	Lowland primary forests, shady secondary forest	Shady rain forests, edge of swampy forests	Shady primary forests, secondary and mossy forests

Source: Sunarno (2005)

Aladdin *et al.* (2016) conducted a comparative study of var. *alata*, var. *pumila* and var. *lanceolata* using microscopic technique to identify the anatomical characteristics presents in the leaf and stem parts of the plant. Based on the anatomical investigation; anisocytic stomata, scale and capitate glandular trichomes were present in all three varieties of *L. pumila*. From the study, Aladdin *et al.* (2016) concluded that the identification of anatomical features in terms type of stomata and trichomes, outline structure of stem and leaf margin, petiole and midrib, organisation of vascular system, areolar venation, pattern of anticlinal walls, the distribution of secretory canals and cell inclusion can be used to differentiate each variety of *L. pumila*.