FAMILI KAEDAH TAK TERSIRAT KUMPULAN BERSELANG-SELI DUA PARAMETER BAGI MENYELESAIKAN PERSAMAAN TERBITAN SEPARA KABUR

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FAKULTI SAINS DAN SUMBER ALAM
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PENGAKUAN

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DISAHKAN OLEH

1. PENYELIA
Prof. Madya Dr. Jumat Sulaiman Tandatangan

PENGHARGAAN

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ABSTRACT

FAMILY OF TWO PARAMETER ALTERNATING GROUP EXPLICIT METHODS FOR SOLVING FUZZY PARTIAL DIFFERENTIAL EQUATIONS

Numerical solutions involving fuzzy partial differential equation (PDE) problems play an important role for solving various problems in field of sciences, engineering, and computer networks. In this study, the use of Seikkala finite difference discretization scheme has been considered to discretize fuzzy elliptic and parabolic PDE problems for one and two dimension respectively. The discretization process being implemented over the proposed problems seeks to derive Seikkala finite difference approximation equations. Then, these fuzzy approximation equations are used to generate a corresponding fuzzy linear system. Following to that, the fuzzy linear system needs to be reduced into two corresponding crisp linear systems. Due to the coefficient matrix of crisp linear system, which is extremely sparse and large scale, the effectiveness of family of Two Parameter Alternating Group Explicit (TAGE) method has been analyzed by solving the crisp linear system. For purposes of comparison, this study also considered the formulation and implementation for the family of Gauss-Seidel (GS) and Alternating Group Explicit (AGE) methods by applying together with the concept of full-, half- and quarter-sweep iterations in solving the crisp linear system. In the line to illustrate the effectiveness of the full-, half- and quarter-sweep proposed iterative methods, two examples for each proposed problems are considered. Based on numerical experiment towards all those three families, the results show that family of TAGE methods are more superior in terms of number of iterations and computational time as compared to the Full-Sweep GS iterative method. This is due to the concept of quarter-sweep iteration that has been applied to the TAGE method enable to reduce the computational complexity reduction approximately by 75% as compared to the full-sweep case. Overall, the accuracy of the approximate solutions for the proposed iterative methods are nearly similar compared to the full-sweep case.

ABSTRAK

Penyelesaian berangka yang melibatkan masalah persamaan terbitan separa kabur mempunyai peranan penting bagi menyelesaikan pelbagai permasalahan dalam bidang sains, kejuruteraan dan rangkaian komputer. Dalam kajian ini, penggunaan skema pendiskretan beza terhingga Seikkala telah dipertimbangkan untuk mendiskretkan masalah persamaan terbitan separa eliptik dan parabolik kabur masing-masing pada satu dan dua dimensi. Proses pendiskretan telah dilaksanakan ke atas permasalahan yang dipertimbangkan untuk menerbitkan persamaan penghampiran beza terhingga Seikkala dan kemudiannya digunakan untuk menjanakan sistem persamaan linear (SPL) kabur. Manakala SPL kabur tersebut diturunkan kepada dua SPL asli yang sepadan. Hakikatnya matriks pekali bagi SPL asli berkenaan adalah bersifat jarang dan berskala besar, maka keberkesanan famili kaedah Tak Tersirat Kumpulan Berselang-seli Dua Paramater (TAGE) dianalisis dengan menyelesaikan SPL tersebut. Bagi tujuan perbandingan, kajian ini mempertimbangkan perumusan dan pelaksanaan bagi famili kaedah Gauss-Seidel (GS) dan Tak Tersirat Kumpulan Berselang-seli (AGE) dengan mengaplikasikan bersama konsep lelaran sapuan penuh, separuh dan suku dalam menyelesaikan SPL tersebut. Sejajar dengan usaha mendemonstrasikan keberkesanan ketiga-tiga famili menerusi pendekatan lelaran sapuan penuh, separuh dan suku, dua contoh bagi setiap permasalahan telah dipertimbangkan. Berdasarkan ujian berangka ke atas ketiga-tiga famili tersebut, keputusan menunjukkan bahawa famili kaedah lelaran TAGE adalah paling berkesan dari segi bilangan dan masa lelaran berbanding dengan kaedah lelaran GS sapuan penuh. Hal ini adalah disebabkan oleh konsep lelaran sapuan suku yang diaplikasikan ke atas kaedah TAGE dapat mengurangkan kekompleksan pengiraan sekitar 75% berbandingan dengan kes sapuan penuh. Secara keseluruhannya, kejituan penyelesaian hampiran bagi kaedah-kaedah lelaran dicadangkan adalah setara berbanding dengan kes sapuan penuh.

ISI KANDUNGAN

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	Labisia
Species	*Labisia pumila*

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. *alata***	**var. *pumila***	**var. *lanceolata***
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*.

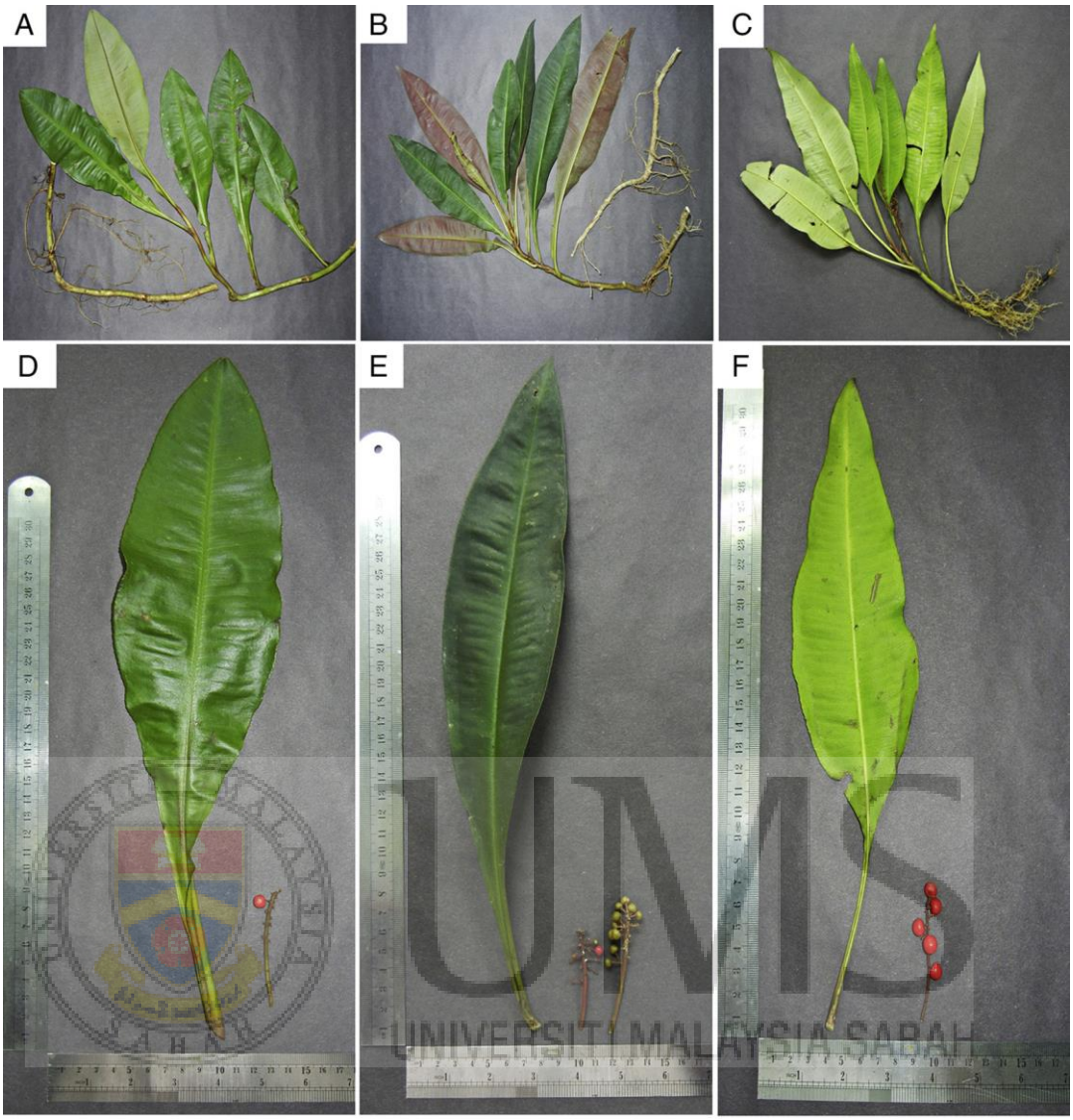

Figure 2.2: Three varieties of *L. pumila* (*ex vitro* conditions) (a) *L. pumila* var. *alata*, (b) *L. pumila* var. *pumila*, (c) *L. pumila* var. *lanceolata* and the macroscopic characteristics of leaf and fruit (d) *L. pumila* var. *alata*, (e) *L. pumila* var. *pumila*, (f) *L. pumila* var. *lanceolata*

Source : Aladdin *et al.* (2016)

2.1.3 Tissue culture of *Labisia pumila*

In the natural habitat, *L. pumila* propagates from its seeds (Mohd. Noh *et al.*, 2002). Zahari (2008) reported that *L. pumila* also can be propagated using its leaf, petiole and stem. Propagation of *L. pumila* var. *alata* high yielding clones using leaf cuttings had been conducted by Syafiqah *et al.* (2014). As *L. pumila* propagates in a slower rate in the wild (Mohd. Noh *et al.*, 2002), attempts have been made to cultivate this herb by using tissue culture techniques for the purpose of micropropagation and regeneration of healthy clones.

To date, there are only a few published studies on tissue culture of *L. pumila*. These *in vitro* studies include seeds germination and seedling development of *L. pumila* (Hartinie & Jualang, 2007), shoot regeneration (Hartinie, 2007; Ling *et al.*, 2013; Ozayanna, 2015; Shafiqah *et al.*, 2015), callus induction (Hartinie, 2007; Ling *et al.*, 2013; Ozayanna, 2015) and adventitious root induction (Hassan & Hussein, 2013; Ling *et al.*, 2013) on semi-solid medium. A recent study by Syafiqah *et al.* (2016) reported that the production of superior clone of *L. pumila* var. *alata* through tissue culture method is more feasible than using leaf cuttings for the production of future planting stocks of the herb.

2.1.4 Medicinal properties of *Labisia pumila*

Traditionally, *L. pumila* is consumed in the form of water decoction from its leaf, root or the whole plant. Between the three varieties of this herb, *L. pumila* var. *alata* is more commonly used in the Malay traditional medicine (Jamal, 2006). Indigenous Malay women drinks the water decoction in order to ease their childbirth as well as a post-partum medicine (Burkill, 1935). Other traditional usages of *L. pumila* are for treating flatulence, dysentery, dysmenorrhea and gonorrhoea, "sickness in the bones" (Burkill, 1935) and haemorrhoids (Rahman, 1998).

Various scientific studies have been carried out in order to investigate the biological properties of *L. pumila*. The herb was traditionally used for assisting childbirth and post-partum period thus *L. pumila* is speculated to exhibit phytoestrogenic activity (Jamal *et al.*, 2003). Research showed that the water extract of *L. pumila* var. *alata* exhibited estrogenic property as it displaced the binding of estradiol to the antibodies, suggesting the presence of oestrogen-like compounds (Husniza, 2002). Studies conducted by Wahab *et al*. (2011) and Jamal *et al.* (2012) further supported the claims that *L. pumila* has phytoestrogenic activities. This provides an opportunity for *L. pumila* to be an alternative to oestrogen replacement therapy (ERT) in postmenopausal inflammation-induced osteoporosis. As it originates from natural sources, no harmful side effects might be encountered as long as it is taken within the safe therapeutic dose (Nadia *et al.*, 2012).

The antioxidant activities in the leaves, stems and roots of three varieties of *L. pumila* was evaluated by Karimi *et al.* (2011). From the study, leaves of all varieties exhibited higher free radical scavenging activities compared to other parts of the plant. *L. pumila* var. *alata* has the lowest IC_{50} values (concentration required to inhibit 50% of DPPH radicals) of 340.13 µg/mL; followed by *L. pumila* var. *pumila* (364.17 µg/mL) and *L. pumila* var. *lanceolata* (388.29 µg/mL). The sequence of ferric ions reductive potential in *L. pumila* was in the order of *L. pumila* var. *alata* > *L. pumila* var. *pumila* > *L. pumila* var. *lanceolata* with respective values of 54.84%, 53.11% and 52.17% (Karimi *et al.*, 2011).

The antimicrobial activities of *L. pumila* was also reported by Karimi *et al.* (2011). Methanol extracts of the aerial parts of *L. pumila* were tested against both Gram positive (*Micrococcus luteus*, *Bacillus subtilis* B145, *Bacillus cereus* B43, *Staphylococcus aureus* S1431) and Gram negative (*Enterobacter aerogenes*, *Klebsiella pneumonia* K36, *Escherichia coli* E256, *Pseudomonas aeruginosa* PI96) pathogens. Although the antibacterial activities of *L. pumila* were lower than the control (kanamycin), this herb still showed potential in the inhibition of several pathogenic microbes.

Other than that, *L. pumila* also exhibited significant anticarcinogenic activity. A study by Pihie *et al.* (2011) reported that ethanol extract of *L. pumila* was able to reduce tumour incidence, tumour burden and tumour volume in 7, 12-dimethylbenz(a)anthracene (DMBA)/croton oil-induced mouse skin carcinogenesis. In addition, the skin tumour growth was also delayed as compared to carcinogen control group. Further studies should be carried out to identify the active compound responsible for the anticarcinogenic of *L. pumila.*

The antifungal and anti-inflammatory activities of the leaf and root extracts of *L. pumila* were evaluated by (Karimi *et al.*, 2013). The leaf and root extracts displayed moderate antifungal activity against three fungi species (*Fusarium* sp., *Candida* sp. and *Mucor* sp.) compared to streptomycin (positive control). The activity profiles of the extracts in terms of percentage of nitric oxide inhibition ranges from 45.66±1.29 to 75.68±1.70%. This result suggested that the leaf and root extracts of *L. pumila* also exhibited potential anti-inflammatory activity.

2.1.5 Phytochemicals in *Labisia pumila*

The biological activities of *L. pumila* could be attributed to the presence of various phytochemicals in the respective extracts. A reversed phase-high performance liquid chromatography (RP-HPLC) analysis of *L. pumila* extracts which was obtained by microwave extraction method showed that the main flavonoids found in all three varieties of *L. pumila* are kaempferol, apigenin, myricetin, naringin and rutin. The same study also revealed that gallic acid and caffeic acid were the major phenolic compounds in all *L. pumila* varieties (Jaafar & Karimi, 2011). It was alleged that phenolic and flavonoid compounds possess diverse biological activities such as antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities (Ghasemzadeh & Ghasemzadeh, 2011). Table 2.3 lists the identified phenolic and flavonoids from the herb as reviewed by Norhanisah *et al.* (2013). Other phytochemicals such as saponin, beta-carotene, ascorbic acid, alkenyl compounds and benzoquinones derivates were also found in *L. pumila* (Norhanisah *et al.*, 2013).

Table 2.3: Phenolic acids and flavonoids from *L. pumila*

Category	Compound name	Reference
Phenolic acids	Caffeic acid	Chua *et al.* (2011), Jaafar *et al.* (2011)
	Chlorogenic acid	Chua *et al.* (2011)
	Coumaric acid	Chua *et al.* (2011)
	Gallic acid	Chua *et al.* (2011), Jaafar *et al.* (2011)
	Protocatechin acid	Chua *et al.* (2011)
	Pyrogallol (Pyrogallic acid)	Jaafar *et al.* (2011)
	Vannilic acid	Chua *et al.* (2011)
	Syringic acid	Chua *et al.* (2011)
	Salicylic acid	Chua *et al.* (2011)
Flavonoids	Apigenin	Jaafar *et al.* (2011)
	Catechin	Chua *et al.* (2011)
	Daidzein	Jaafar *et al.* (2011)
	Epigallocatechin	Chua *et al.* (2011)
	Genistein	Jaafar *et al.* (2011)
	Kaempferol	Chua *et al.* (2011), Jaafar *et al.* (2011)
	Myricetin	Chua *et al.* (2011), Jaafar *et al.* (2011)
	Naringin	Jaafar *et al.* (2011)
	Quercetin	Chua *et al.* (2011), Jaafar *et al.* (2011)
	Rutin	Jaafar *et al.* (2011)

2.2 Plant Tissue Culture System for Medicinal Plant Propagation

Plants are an important source of secondary metabolites which have been used in the development of drugs, flavours, fragrances and pesticides (Rodríguez-Sahagún *et al.*, 2012). Although plant secondary metabolites have no significant role in its fundamental life processes, they are essential in the interaction of the plant with its environment (Smetanska, 2008). In addition, the production of these compounds depends heavily on plant species and plant's physiological and developmental stage (Namdeo, 2007).

The conventional system for the propagation of medicinal plant with pharmacological interests often encountered problems such as low propagation rate and the occurrence of plant infections (Campbell *et al.*, 2001). As the demand of plant-based products became more prominent, a continuous and reliable source of medicinal plant is in greater need as the secondary metabolites yields are influenced by the genetic factors, geographic and climatic condition of the cultivation site and also the post-harvesting processes (Rodríguez-Sahagún *et al.*, 2012).

Plant tissue culture is an attractive alternative to that of conventional propagation of medicinal plants. One of the advantages of this biotechnological approach over the conventional production is its independency of geographical and environmental factors. This means that the secondary metabolites are synthesised in a controlled environment and this eliminates the negative biological influences that affect secondary metabolites production in nature. Other than that, a defined production system is also offered through plant cell, tissue and organ culture as products are supplied in a continuous rate and uniform quality. Moreover, this technology makes it possible to obtain novel compounds that are not normally found in parent plant (Rao & Ravishankar, 2002).

To date, there are many medicinal plants which have been established for production of secondary metabolites using plant tissue culture techniques. There are four main approaches of secondary metabolites production in *in vitro* cultures namely callus, cell suspension, immobilised cell and organ cultures. Among these approaches, cell suspension cultures are most widely applied in studies of secondary metabolites production (El Meskaoui, 2013). However, many studies involving undifferentiated cell culture reported on low accumulation of the desired compounds. This situation frequently occurs when the metabolite of interest was only synthesised in specialised plant tissues or glands in the parent plant (Hussain *et al.*, 2012). For example, *Hypericum perforatum* have not demonstrated the ability to accumulate hypericins and hyperforins in undifferentiated cells as these compounds accumulated in foliar glands (Smetsanka, 2008).

Other than that, the biosynthesis of certain compound remains organ or tissue specific, hence it is not able to be produced via cell culture (Campbell *et al.*, 2001). This might be due to the similarity in secondary metabolites production pattern in organ cultures and intact plants (Filová, 2014). Therefore, in such circumstances the organ cultures, namely shoot or root cultures, may offer a better alternative than undifferentiated cell cultures. In addition, organ cultures show biochemical and genetic stability in many instances compared to undifferentiated cells cultures. Organ cultures thus offer a predictable, high-productivity system which does not necessarily involve extensive optimisation (Campbell *et al.*, 2001; El Meskaoui, 2013; Filová, 2014).

2.2.1 Adventitious organogenesis

Adventitious organogenesis refers to the production of new organs on explants of different plant tissues such as leaves, stems and roots (Varshney & Anis, 2014). The emergence of root from leaf explant and shoot from roots are classified as adventitious structures. The formation of adventitious structures follows two pathways; either through direct organogenesis from established cell types or from callus tissues formed following mechanical damage (Casson & Lindsey, 2003). The major advantage of establishing a direct organogenesis pathway is that clones multiplication are achieved in a much faster rate compared to regeneration via an intermediate callus phase. Moreover, the occurrence of somaclonal variation can be precluded through direct organogenesis (Lakshmanan *et al.*, 2006; Abahmane, 2011).

As shown in Figure 2.3, there are three phases involved in adventitious organogenesis, namely dedifferentiation, induction and realisation. In the dedifferentiation stage, cells acquire competence to respond to hormonal signals of organ induction. Next, in the induction phase, cells are determined to form a specific organ. Therefore, the hormonal composition is highly critical during this stage. After the cells are determined, the new program of realisation is initiated and morphological differentiation and development of the nascent organ occur (Loberant & Altman, 2010; Varshney & Anis, 2014).