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Effects of Plant Growth Regulators on Root Culture and Yeast Extract Elicitation on Metabolite Profiles of *Polygonum minus*

(Kesan Pengawalatur Pertumbuhan ke atas Kultur Akar dan Elisitasi Ekstrak Yis ke atas Profil Metabolit Sekunder *Polygonum minus)*

MOHD AZHAR HASSAN*, MARIATULQABTIAH ABDUL RAZAK, AHMAD HAFIZ BAHAROM, MUHAMMAD SHAFIE MD SAH & MOHAMAD ZULKIFFELY A. RAHMAN

ABSTRACT

There are various secondary metabolites that have been identified in Polygonum minus *Huds. or* kesum *plant, but the production is often very low and depending on growth stage. Therefore, elicitation and* in vitro *techniques have been suggested as an effective way for inducing secondary metabolites production in plant. This study was conducted to determine the optimal conditions for* P. minus *root formation* in vitro *and to profile the metabolite content from* P. minus *root culture with and without elicitor treatment. From the root induction study, it was found that the fresh weight of induced root for nodal explant in MS liquid media supplemented with 0.5 mg/L NAA and shaken had the highest production (0.38±0.08 g) compared to other treatments including the control. The results from metabolite profile showed that the volatile compound of* P. minus *root produced without any elicitation contained 50.11% aliphatic (27.59% aldehide, 9.17% alkane and 13.35% others) and 19.39% sesquiterpene (β-caryophyllene, α-bergamotene, β-farnesene, α-caryophyllene dan β-curcumene) where the dodecanal compound (22.27%) and β-caryophyllene (8.09%) have the highest percentage value for aliphatic and sesquiterpene group, respectively. Moreover, elicitation of* P. minus *root culture using yeast extract at 100 mg/L concentration for 1 day demonstrated the ability to increase the production of secondary metabolites in many volatile compounds of* kesum in vitro *root including the sesquiterpene compounds compared to control treatment and other yeast extract elicitation treatments.*

Keywords: Aliphatic; elicitation; Polygonum minus*; secondary metabolite; sesquiterpene*

ABSTRAK

Terdapat pelbagai metabolit sekunder dikenal pasti di dalam Polygonum minus *Huds. atau* kesum *tetapi penghasilannya sangat rendah dan bergantung pada peringkat pertumbuhan. Oleh itu, teknik elisitasi dan* in vitro *telah dicadangkan sebagai cara yang berkesan untuk merangsang pengeluaran metabolit sekunder pada tumbuhan. Kajian ini dilakukan bagi menentukan keadaan yang optimum bagi penghasilan akar* P. minus *secara* in vitro *dan memprofil kandungan metabolit daripada kultur akar* P. minus *dengan dan tanpa perlakuan elisitor. Hasil kajian pengaruhan akar mendapati bahawa berat basah akar bagi eksplan nodal di dalam medium MS cecair yang ditambah dengan 0.5 mg/L NAA dan digoncang telah memberikan nilai hasilan yang paling tinggi (0.38±0.08 g) berbanding rawatan lain termasuk rawatan kawalan. Keputusan kajian profil metabolit pula menunjukkan bahawa sebatian meruap akar* P. minus *yang terhasil tanpa sebarang perlakuan elisitor terdiri daripada 50.11% alifatik (27.59% aldehid, 9.17% alkana dan 13.35% lain-lain) dan 19.39% sesquiterpena (β-kariofilena, α-bergamoten, β-farnesen, α-kariofilena dan β-curcumen) dengan sebatian dodekanal (22.27%) dan β-kariofilena (8.09 %) masing-masing menunjukkan nilai peratusan paling tinggi bagi kumpulan alifatik dan sesquiterpena. Manakala elisitasi kultur akar* P. minus *menggunakan ekstrak yis pada kepekatan 100 mg/L selama 1 hari didapati berupaya meningkatkan penghasilan metabolit sekunder dalam kebanyakan sebatian meruwap akar kesum yang terhasil termasuklah pada sebatian sesquiterpena berbanding dengan rawatan kawalan dan rawatan elisitasi ekstrak yis yang lain.*

Kata kunci: Alifatik; elisitasi; metabolit sekunder; Polygonum minus*; sesquiterpena*

INTRODUCTION

Plants can produce various secondary metabolites under specific conditions. These compounds play a role in adapting plants with environment including biotic and abiotic pressure (Rao & Ravishankar 2002). Secondary metabolites are usually produced by plants as defence systems against insects, herbivores and pathogens such as viruses, bacteria and fungi. They also protect plants from abiotic pressures such as drought, salinity, UV light, heavy metals, extreme temperatures and nutrient deficiency in the soil (Ismail et al. 2011). Other functions of secondary metabolites include as attractants of pollinators for plant

reproduction, signaling molecules and as hormones in secondary metabolism of plant cells (Korkina 2007). To date, thousands of different secondary metabolite structures have been identified in plants. Previous studies have identified 77 metabolites in the essential oil of *P. minus* leaf where most of them (76.59%) were aliphatic compounds, which contributes to the aroma and taste of the plant (Ahmad et al. 2014; Yaacob 1987).

The secondary metabolite content in the plant is often very low and depends on growth stage (Neumann et al. 2009; Poulev et al. 2003). Therefore, elicitation and *in vitro* techniques have been proposed as an effective way for generating high secondary metabolites in plants within a shorter time duration (Gor et al. 2011; Rao & Ravishankar 2002; Zhao et al. 2005). Elicitation of *Solenostemon scutellarioides* using methyl jasmonate, MeJA (50 µM) and salicylic acid, SA (50 µM) increased rosmarinic acid by 1.7 and 1.4 times, respectively, on the first day while elicitation using yeast extract (100 µg/mL) showed high content of rosmarinic acid (1.5 times) on the third day (Sahu et al. 2013). Few new compounds such as 2,2'-bioxirane, propanoic acid-2oxo-methyl ester, repandin A, 2-propanone, 1,3-dihydroxy-imidazolidine-2,4,5-trione and 2-acetyl-2-hydroxy-butyrolactone have been found in cell culture of *P. minus* treated with elicitors but not found in control culture (Vikram et al. 2014).

Polygonum minus Huds. or *kesum* is a popular aroma herb belongs to family of Polygonaceae. Various metabolites are produced by *P. minus* cell culture in a medium elicited by various concentrations of jasmonic acid (JA), SA, yeast extract and glass beads with different incubation times including 2-furancarboxaldehyde, 5-hydroxymethylfurfural and 2-cyclopenten-1-one-2-hydroxy (Shukor et al. 2013). However, no studies were conducted on elicitation of *P. minus* root culture using yeast extract and determination of optimum culture media for producing *P. minus in vitro* root. Therefore, this study was carried out aiming at determining the optimal conditions for *P. minus* root formation and profiling the metabolite content from the *in vitro* root culture of *P. minus* resulted from yeast extract elicitation and without elicitation.

Materials and Methods

In vitro Propagation of *Polygonum minus*

The sterile plants of *Polygonum minus* were obtained from the Plant Biotechnology Laboratory, Universiti Kebangsaan Malaysia. The selected nodal of sterile plant was cut by 2.0 cm using scalpel and subcultured into a tissue culture jar bottle containing Murashige and Skoog (MS) media. Each bottle was placed with seven nodal explants. All subcultured explants were placed in a culture room of 25 ± 2ºC temperature with a 16 h photoperiod pendaflour light. Subcultures on fresh media were performed every 2 months to ensure sufficient explant sources.

In vitro Root Induction

In vitro root induction was performed by placing 2.0 cm sterile nodal explants on the MS liquid media containing different types and concentrations of auxin, which were α-naphthalene acetic acid (NAA: 0.5, 1.0, 1.5 and 2.0 mg/L) and Indole-3-butyric acid (IBA: 0.5, 1.0, 1.5 and 2.0 mg/L). Each bottle jar was placed with three nodal explants. Liquid and solid MS media without plant growth regulators were used as control treatments in the experiment. All these treatment and control cultures were grown in static or shake condition on an orbital shaker (80 rpm) in a culture room at 25 + 2°C under 16 h photoperiod pendaflour light. This study utilised a randomised complete block design (RCBD) with 3 replications where each replication contains 5 jar bottles per treatment.

Data Collection and Statistical Analysis

Data for root length (cm), root weight (g) and root quality were recorded after the explant reached 2 months old. Statistical analysis was determined by one-way Analysis of Variance (ANOVA) using Statistic Analysis Software 9.4 programme (SAS 9.4). Analysis was carried out using Duncan's Multiple Range Test (DMRT).

Elicitation of Yeast Extract

Two-month-old seedlings were transplanted into jar bottles containing MS liquid media with different concentrations (100 and 250 mg/L) of yeast extract for elicitation, with control seedlings having no elicitor (0 mg/L). At the same time, control seedlings were transferred into jar bottles containing MS liquid media without elicitor. Then, *P. minus* seedlings were picked up on day 1 and 3 to perform a volatile compounds analysis using Gas Chromatography - Mass Spectrometry (GC-MS). Each experiment comprised three replications. Factorial experimental design of 3 × 2 (yeast extract concentration × elicitation period) was applied in this study.

Extraction of Secondary Metabolites

Approximately, 2 g of *P. minus* root from the control and the treatment samples were separated from the seedlings for each elicitation. The roots of *P. minus* were chopped with a knife until it turned into very small pieces. Then, the roots were put into the SPME (Solid Phase Microextraction) vial and closed with a sealed lid. After that, the root samples were heated at 65°C for 15 min in the heating block. The vapour formed was collected by penetrating the SPME needle through the sealed lid. The SPME fibre (100 µm polydimethylsiloxane, PDMS) contained in the needle was extended into the space of sample closure area for absorbing the volatile compounds released from *P. minus* roots.

GC-MS Analysis

The presence of secondary metabolites or volatile and semi-volatile compounds in the control and treated *P. minus*

roots were determined by Gas Chromatography - Mass Spectrometry (GC-MS). The gas chromatography system used was Agilent Technologies Model 5975C with non-polar DB-5MS columns (30 m length × 0.25 mm diameter) and film thickness of 0.25 µm. The GC-MS analysis parameters were set as in Table 1. The compounds detected by the mass spectrometry was compared with the data in GC-MS NIST library based on similarity index (SI) unit and retention time (RT). Only volatile compounds with SI unit higher than 80 and were consistently present in two or more replications accepted for subsequent analysis.

Results and Discussion

Effects of plant growth regulators on *in vitro* root production of *Polygonum minus*

In general, auxin additions in growth media cause changes in protein synthesis and RNA production that can stimulate growth and rapid cell division to increase the number of roots (Davies 2013; Husen & Pal 2007). The effects of various types and concentrations of auxin (NAA and IBA) have been studied to determine and develop optimal culture media for producing *P. minus* roots *in vitro*.

The fresh weight of induced root produced by the explant is influenced by the type and concentration of plant growth regulators (PGR) used in media. For instance, fresh weight of induced root for nodal explants in liquid MS media supplemented with 0.5 mg/L NAA and shaken demonstrated the highest production (0.38±0.08 g) compared to other treatments including control treatment (Table 2). Similar results have been reported in the *Ophiorrhiza prostrata* (Martin et al. 2008), *Kaempferia galanga* and *Kaempferia rotunda* (Geetha et al. 2015).

Like NAA, IBA also showed the highest fresh weight of induced root in the concentration of 0.5 mg/L on shaking media condition, which was 0.34±0.07 g. However, its fresh weight value was lower than that of NAA. The same result also recorded between NAA and IBA with 1.0 mg/L concentration where the NAA showed higher root production than IBA. According to Hartmann et al. (2007), IBA is the strongest, stable and less toxic of auxin that is widely used as a root booster hormone for most species such as *Clinacanthus nutans* (Chen et al. 2015) and *Wattakaka volubilis* (Vinothkumar & Senthilkumar 2015). However, NAA is stated to be the most suitable auxin for *in vitro* rooting of nodal explants for some plant species including *Ophiorrhiza prostrata* (Martin et al. 2008) and *Citrus tangerina* (Nwe et al. 2014). There were also some species showing the same rooting response to NAA and IBA like *Punica granatum* where MS media at full strength containing 0.5 mg/L NAA and 0.5 mg/L IBA showed the best rooting results, respectively (Singth et al. 2014). The combination of both NAA and IBA hormones is very successful for some species in *in vitro* root production; for example, on the rooting of *Eriobotrya japonica* (Abbasi et al. 2013).

Normal weight of fresh roots that decreased when the NAA concentration increases from 0.5 to 2.0 mg/L indicates that the effect of inhibition from using high concentration of plant growth regulator. This phenomenon has been also reported in *Mentha piperita* (Ghanti et al. 2004) and *Citrus tangerine* (Nwe et al. 2014). Observation by Baker and Wetzstein (1994) and Rai et al. (2009) suggested that auxin at high concentration causes the production of degradative metabolites to increase and inhibit root growth processes.

The liquid media at static or shaking condition affects the root production of nodal explants. This was evidenced by the production of low fresh root weight in the liquid media, which was static rather than shaking for each NAA and IBA concentration used in this experiment including the control treatment. IBA showed a significant decrease in root weight for media that are in a static condition rather than shaking for each concentration used in the experiment. Mehrotra et al. (2007) stated that growth rate of shoot and root can be enhanced *via* forced aeration in liquid culture media that are continuously shaken. Continuous shaking on liquid media can produce enough oxygen supply until it finally affects the fast and plentiful growth. It also facilitates the distribution of nutrient evenly for the whole explants, which results in the best root growth.

Control treatment, which is a liquid MS media without any plant growth regulator placed in a shaking condition (C1) produced the highest fresh weight of root (0.26±0.04 g) compared to other two control treatments (C2 and C3). There were only two treatments in this study that produced fresh weight of root higher and significantly different compared to C1, which were T1 and T5 with the weights

TABLE 1. Parameters for GC-MS analysis of *Polygonum minus* root

Parameter	Unit
Injector temperature	220°C
Detector temperature	280°C
Column temperature	50°C, 3 min; 20°C/min - 100°C, 3 min; 30°C/min - 250°C, 3 min.
Flow rate	1.3 mL/min
Injection volume	1 µL
Injection method	Splitless
Mass spectrometry	Scan mode (m/z range = 55-355)

TABLE 2. Means from DMRT for length and fresh weight of *in vitro* root of *Polygonum minus* in different types and concentrations of plant growth regulators with different media condition

Code	Treatment				Score	
	Plant growth regulator (mg/L)		Media form	Media condition	Mean of *in vitro* root fresh weight (g)	Mean of *in vitro* root length (cm)
	NAA	IBA				
T1	0.5	0	Liquid	Shake	0.38±0.08^{a}	7.60±1.61^{f}
T2	1.0	0	Liquid	Shake	0.28±0.06^{c}	8.20±0.86^{ef}
T3	1.5	0	Liquid	Shake	0.24±0.06^{c-e}	5.57±0.82^{g}
T4	2.0	0	Liquid	Shake	0.18±0.05^{fg}	5.07±0.62^{g}
T5	0	0.5	Liquid	Shake	0.34±0.07^{b}	10.87±1.05^{bc}
T6	0	1.0	Liquid	Shake	0.21±0.05^{ef}	10.67±1.06^{c}
T7	0	1.5	Liquid	Shake	0.25±0.05^{cd}	11.73±1.39^{b}
T8	0	2.0	Liquid	Shake	0.17±0.02^{g}	9.40±1.06^{d}
T9	0.5	0	Liquid	Static	0.21±0.06^{ef}	4.77±0.59^{g}
T10	1.0	0	Liquid	Static	0.22±0.04^{de}	7.73±0.90^{f}
T11	1.5	0	Liquid	Static	0.17±0.03^{g}	5.10±0.78^{g}
T12	2.0	0	Liquid	Static	0.13±0.03^{hi}	4.77±1.33^{g}
T13	0	0.5	Liquid	Static	0.07±0.03^{j}	2.63±0.95^{hi}
T14	0	1.0	Liquid	Static	0.08±0.02^{j}	3.20±0.56^{hi}
T15	0	1.5	Liquid	Static	0.07±0.03^{j}	3.53±0.85^{h}
T16	0	2.0	Liquid	Static	0.03±0.02^{k}	2.43±1.25^{i}
C1	0	0	Liquid	Shake	0.26±0.04^{cd}	7.47±0.83^{f}
C2	0	0	Liquid	Static	0.16±0.04^{gh}	8.77±0.78^{de}
C3	0	0	Solid	Static	0.10±0.03^{ij}	13.80±1.29^{a}

Means within a column with the same letters are not significantly different at $p<0.0001$ according to DMRT

of 0.38±0.08 and 0.34±0.07 g, respectively. Treatments that produced a weight of roots that is not significant or lower than C1 were considered unsuitable as treatments of root production for *P. minus* nodal explant due to loss of cost, time and energy. Data of root fresh weight for C3 control treatments was lower compared to C1 and C2 suggesting that the root production of *P. minus* nodal explant was more suitable in liquid rather than solid media. Liquid media allows a closer relationship between media and tissue in which it can stimulate and facilitate nutrient and hormone uptake resulting in improved shoot and root growth (Sandal et al. 2001).

Table 2 also illustrates that root length was not the only factor contributing to the fresh weight of the root produced. For instance, the C3 control treatment produced the longest root of 13.8±1.29 cm compared to the other treatments, but the fresh weight of its root was recorded among the lowest. Although T1 treatment produced a relatively moderate root length of 7.6±1.61 cm compared to the other treatments, the fresh weight of the root recorded was the highest among the others. This proved that other factors such as the number and thickness of the root produced can give an impact on the fresh weight of the root recorded.

The results in Table 3 displays that the roots characters in all treatments media including control in shacking condition were thick, long and dark in colour (Figure 1(A)) except for the treatment of T3 and T4 where the root character was clump (Figure 1(B)). When in static condition, the *in vitro* roots formed for all treatments media were thin, short and bright in colour (Figure 1(C)) except for treatment T12 where the root character was clump. According to Davies and Joiner (1980), high concentration of NAA can affect the quality and shape of the root produced. The root elongation phase is highly responsive to auxin concentration where it can inhibit root elongation at high concentration (Baker & Wetzstein 1994; Hu & Wang 1983) resulted from ethylene production within the root zone that acts as an inhibitor agent (Chang et al. 2013). Situation in the treatment of T3 and T4 also occurred in *Mentha piperita* where the resulting root length decreased when the NAA concentrations used exceeds 1.0 mg/L (Ghanti et al. 2004).

EXTRACTION OF VOLATILE COMPOUNDS FROM *POLYGONUM MINUS IN VITRO* ROOT

The chemical composition of volatile compounds from *P. minus* has been identified since 1987 (Yaacob 1987). Subsequently, more studies on *P. minus* have been conducted discovering more volatile compounds such as in the study of Huda-Faujan et al. (2009), Vikram et al. (2014) and Vimala et al. (2006). In addition, chemical composition for essential oils from other species such as *Polygonum odoratum* was studied by Vietnamese (Dung et al. 1995) and Australia chemists (Hunter et al. 1997). Some studies have been able to identify valuable compounds from the roots of two *Polygonum* genus such as phytoestrogens in *Polygonum cuspidatum* (Matsuda et al. 2001) and indigo in *Polygonum tinctorium* (Young-Am et al. 2000). To date, there is only one study reported on the chemical composition of the volatile compound

TABLE 3. Root characters of *Polygonum minus* in different types and concentrations of plant growth regulators with different media condition

Code	Treatment				Score
	Plant growth regulator (mg/L)		Media form	Media condition	Root characters
	NAA	IBA			
T1	0.5	0	Liquid	Shake	Thick
T2	1.0	0	Liquid	Shake	Thick
T3	1.5	0	Liquid	Shake	Thick and clump
T4	2.0	0	Liquid	Shake	Thick and clump
T5	0	0.5	Liquid	Shake	Thick
T6	0	1.0	Liquid	Shake	Thick
T7	0	1.5	Liquid	Shake	Thick
T8	0	2.0	Liquid	Shake	Thick
T9	0.5	0	Liquid	Static	Thin
T10	1.0	0	Liquid	Static	Thin
T11	1.5	0	Liquid	Static	Thin
T12	2.0	0	Liquid	Static	Thin and clump
T13	0	0.5	Liquid	Static	Thin
T14	0	1.0	Liquid	Static	Thin
T15	0	1.5	Liquid	Static	Thin
T16	0	2.0	Liquid	Static	Thin
C1	0	0	Liquid	Shake	Thick
C2	0	0	Liquid	Static	Thin
C3	0	0	Solid	Static	Thin

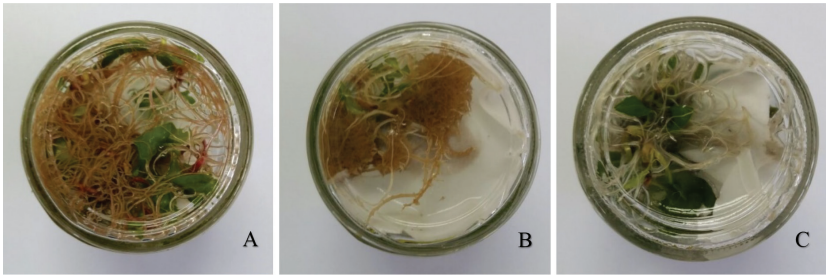


FIGURE 1. Root characters of *Polygonum minus*: A) Thick, long and dark colour; B) Clump; C) Thin, short and bright colour

on *P. minus in vitro* root, which uses jasmonic acid as the elicitor (Ismail et al. 2011). Therefore, in this study, the metabolite profile before treatment of the *P. minus in vitro* root was done for comparison purposes, in terms of type (qualitative) and composition (quantitative) of small molecules (MW<1000 Da) before different elicitation treatments are performed. Elicitors such as SA and MeJA have showed the induction of some compounds including galantamine in *Lycoris radiate* elicited by MeJA (Jiang et al. 2011) and taxol in the suspension culture of *Taxus chinensis* var. *mairei* elicited by SA (Wang et al. 2007). Our findings also allow metabolomics comparison between different organs or conditions of *P. minus*. For example, Shukor et al. (2013) identified 12 chemical compounds in *P. minus* leaf cell culture where these compounds do not exist in actual plant leaf.

GC-MS analysis was performed on volatile compounds of *in vitro* root which was collected from the *P. minus* root sample bottle through the SPME extraction technique. SPME techniques can be used for analyte extraction of various types of medium as well as for liquid and gas phase (Mitra 2004). Extraction using SPME techniques has been reported effective in metabolite extraction from *in vitro* roots of *Polygonum multiflorum* (Rong-Min et al. 2006) and cell culture of *Cupressus lusitanica* (De Alwis et al. 2009). A total of 60 peaks were detected in this study. Evaluation of compounds was done based on the similarity index (SI) and retention time (RT) by comparing the compounds within the NIST library. Only compounds with SI values higher than 80 were accepted for subsequent analysis (Dalluge et al. 2003). The chromatogram profile for control *P. minus* root is shown in Figure 2.

Analysis presents 26 compounds from 60 peaks with SI value of more than 80 and the compounds were the result of putative identification based on NIST library. These identified compounds are listed in Table 4 according

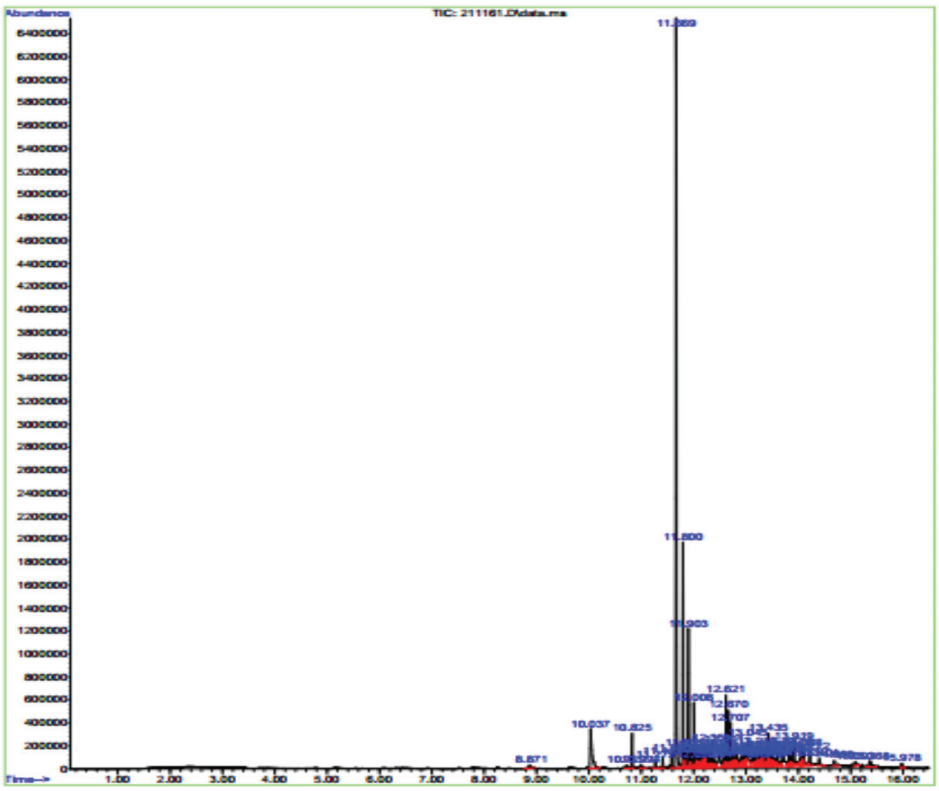


FIGURE 2. Chromatogram profile for volatile compounds from root extract of *Polygonum minus* without elicitation

to their retention time and relative peak area. Among the major compounds in the *P. minus* root extract that have been identified with high relative peak area were dodecanal (22.27%), β-caryophyllene (8.09%), β-farnesene (7.15%), decanal (3.22%) and heneicosane (3.15%) wherein all are fragrance and flavouring agents. Meanwhile, compounds with the lowest number of peaks were undecane (0.22%), 1-decanol (0.37%) and tetradecane (0.47%), which are softener, fragrance and flavour agent.

Table 4 shows that the volatile extract of *P. minus* root consisting 50.11% aliphatic (27.59% aldehyde, 9.17% alkane and 13.35% others) and 19.39% sesquiterpene (β-caryophyllene, α-bergamotene, β-farnesene, α-caryophyllene and β-curcumene). This result was different from that of previous study in which Ismail et al. (2011) reported that the volatile extract of *kesum* root comprised 8.65% aliphatic (5.58% alkane and 3.07% aldehyde) and 32.85% sesquiterpene (β-caryophyllene, trans-α-Bergamotene, β-farnesene, α-caryophyllene and α-Panasinsene) where the compound with the highest relative peak area was β-caryophyllene. There are two possibilities that might cause this difference. First, the parameters used in different SPME methods produce different types of metabolites. For example, terpene extraction through the SPME method depends on parameters such as sampling time and temperature condition (Rohloff 1999). For the SPME method used in this study, compounds with high evaporation character were evaporated first compared to less volatile compounds. Second, the different apparatus and the extraction method produce different types of secondary metabolites. Types and diameter of fibre used in the SPME method affect the type of secondary metabolite produced (Schafer et al. 1995). The volatile compounds such as terpene and aldehyde are neither polar nor semi-polar having very different preference on fibre types (Rohloff 2002).

However, the results of GC-MS in this study were quite similar to that by Yaacob (1987), which reported that the essential oils of leaf and stem of *kesum* contained 76.59% and 56.17% aliphatic compounds where two major aliphatic compounds namely decanal (24.36 %) and dodecanal (48.18%) have been found to be the major contributors in *P. minus* as well as side compounds such as 1-decanol, 1-dodecanol, undecanal, tetradekanal, 1-undekanol, nonanal, 1-nonanol and β-caryophyllene.

EFFECTS OF YEAST EXTRACT ON PRODUCTION OF SECONDARY METABOLITES OF *POLYGONUM MINUS IN VITRO* ROOT

The parameters manipulated in this elicitation study were the concentration of yeast extract and duration of treatment. Two yeast extract concentrations (100 and 250 mg/L) and 2 treatment periods (1 and 3 days) were studied. These parameters were monitored to ensure that the yeast extract concentration was not too high or the duration of the treatment was not too long, thus resulting in the death of *P.*

TABLE 4. Chemical composition of volatile compound extracts from the root of *Polygonum minus* without elicitation

Retention time	Compound	Relative peak area (%)	Group
8.26	Undecane	0.22	Aliphatic (Alkane)
10.04	Decanal	3.22	Aliphatic (Aldehyde)
10.72	1-Decanol	0.37	Aliphatic (Other)
11.58	Tetradecane	0.47	Aliphatic (Alkane)
11.67	Dodecanal	22.27	Aliphatic (Aldehyde)
11.80	β-caryophyllene	8.09	Sesquiterpene
11.83	α-bergamotene	0.61	Sesquiterpene
11.90	β-farnesene	7.15	Sesquiterpene
12.01	α-caryophyllene	2.40	Sesquiterpene
12.11	Naphthalene	0.71	Aliphatic (Other)
12.14	Pentadecane	1.22	Aliphatic (Alkane)
12.21	Phenol	1.74	Aliphatic (Other)
12.24	β-curcumene	1.15	Sesquiterpene
12.30	Naphthalene	0.87	Aliphatic (Other)
12.62	Hexadecane	1.46	Aliphatic (Alkane)
12.71	Tetradecanal	2.10	Aliphatic (Aldehyde)
13.00	Sabinene hydrate	0.84	Aliphatic (Other)
13.05	Heneicosane	3.15	Aliphatic (Alkane)
13.22	Eicosane	1.11	Aliphatic (Alkane)
13.44	Octadecane	0.99	Aliphatic (Alkane)
13.61	Hexahydrofarnesyl acetone	1.93	Aliphatic (Other)
13.71	Phthalic acid	1.37	Aliphatic (Other)
13.92	Methyl palmitate	1.86	Aliphatic (Other)
14.10	Dibutyl phthalate	1.27	Aliphatic (Other)
14.22	Eicosane	0.55	Aliphatic (Alkane)
14.76	Phytol	2.39	Aliphatic (Other)

minus sample tree. In addition, the optimum yeast extract concentration and optimum duration of treatment were determined to ensure that volatile compounds can be induced to the maximum yield level compared to control sample.

The analysed data discovered that the yeast extract elicitation was able to induce or inhibit the production of certain secondary metabolites. This has been evidenced by the study of Chen and Chen (2000) on the *Salvia miltohirzia* cell culture where high concentration of yeast extract was able to inhibit the production of rosmarinic acid while low concentration of yeast extract increased the production of cryptotanshinone. The compounds in Table 5 was the result of putative identification based on NIST library. The result shows that the volatile compounds produced in *in vitro* root of *P. minus* was either increased, decreased, existed or absent after elicited with the yeast extract. The results were depending on the yeast extract concentrations and elicitation period.

Table 5 presents that there were 12 compounds with the highest relative peak area on the elicitation of 100 mg/L of yeast extract for 1 day including all compounds from the sesquiterpene group representing almost half of the volatile compounds produced in the experiment. It can be concluded that to produce a high sesquiterpene compound, the *in vitro* root of *kesum* elicited with 100 mg/L of yeast extract for 1 day is particularly suitable, whereas for aldehyde production, elicitation using 100 mg/L of yeast extract for 3 days is the best.

Figure 3 shows that the highest relative peak area was recorded by dodecanal compound (23.92%) obtained from root of *P. minus* elicited with 100 mg/L of yeast extract for 3 days. On the other hand, β-farnesene (9.43%), β-caryophyllene (8.74%) and α- caryophyllene (3.96%) showed the highest relative peak area when elicited with 100 mg/L of yeast extract for 1 day. Several compounds were seen with a lower relative peak area of less than 1% including tetradecane, α-bergamotene, octadecane and eicosane. Further, compounds such as tetradecane and phthalic acid did not show any difference between control and other treatments. There was also a compound produced only on certain treatments such as palmitic acid (2.77%) and myristic acid (1.00%), which only existed in the treatment of 250 and 100 mg/L yeast extract, respectively, for 3 days. Inhibitory production of 1-decanol compound was not affected by elicitation period but the concentration of yeast extract.

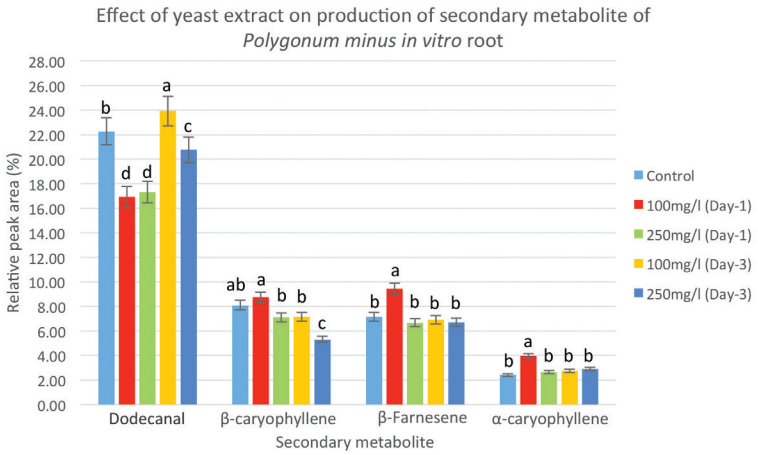
CONCLUSION

The fresh weight of *P. minus in vitro* root for nodal explant in shaking MS liquid media supplemented with 0.5 mg/L NAA had the highest production (0.38±0.08 g) compared to other treatments including the control. This proved that the fresh weight of induced root produced from the explant is influenced by the type and concentration of plant growth regulator used together with the form and position

TABLE 5. Relative peak area of volatile compounds of *Polygonum minus* root after elicitation by yeast extract

Retention time	Compound	Relative peak area (%)				
		Control	Day 1		Day 3	
			100 mg/L	250 mg/L	100 mg/L	250 mg/L
8.26	Undecane	0.22*	-	-	-	-
10.04	Decanal	3.22	2.90	2.47	3.44*	2.15
10.72	1-Decanol	0.37	0.46*	-	0.28	-
10.99	Undecanal	-	-	-	0.38*	-
11.58	Tetradecane	0.47	0.56*	0.41	0.46	0.42
11.67	Dodecanal	22.27	16.92	17.32	23.92*	20.77
11.76	α-santalene	-	-	-	-	0.11*
11.80	β-caryophyllene	8.09	8.74*	7.09	7.15	5.31
11.83	α-bergamotene	0.61	0.67*	0.52	0.56	0.41
11.90	β-farnesene	7.15	9.43*	6.66	6.90	6.69
12.01	α-caryophyllene	2.40	3.96*	2.65	2.74	2.89
12.11	Naphthalene	0.71*	0.70	0.61	0.57	0.39
12.14	Pentadecane	1.22	1.72	-	2.91	4.84*
12.21	Phenol	1.74	2.01	2.04*	1.68	1.50
12.24	β-curcumene	1.15	1.60*	1.17	1.48	1.31
12.30	Naphthalene	0.87	0.85	1.08	1.09	1.74*
12.62	Hexadecane	1.46	1.56*	1.23	1.55	2.02
12.71	Tetradecanal	2.10*	2.04	1.77	2.08	1.90
12.80	Butylated hydroxytoluene	-	-	0.86*	-	-
13.00	Sabinene hydrate	0.84	1.53*	-	1.08	1.27
13.05	Heneicosane	3.15*	-	-	-	3.08
13.22	Eicosane	1.11*	-	0.87	0.58	-
13.30	Myristic acid	-	-	-	1.00*	-
13.44	Octadecane	0.99	0.96	0.97	1.30	1.44*
13.52	Isopropyl myristate	-	1.77*	1.61	-	0.92
13.61	Hexahydrofarnesyl acetone	1.93	2.31	2.44*	1.88	1.17
13.71	Phthalic acid	1.37	-	1.63*	1.26	1.40
13.92	Methyl palmitate	1.86	3.25*	2.13	1.45	2.38
14.07	Palmitic acid	-	-	-	-	2.77*
14.10	Dibutyl phthalate	1.27	1.85	1.96*	1.31	1.06
14.22	Eicosane	0.55	0.91*	0.74	0.64	0.83
14.76	Phytol	2.39*	1.05	1.20	1.13	-

*Compound with the highest relative peak area compare with all treatments



Means within a secondary metabolite with the same letters were not significant at $p \leq 0.05$ according to DMRT

FIGURE 3. Graph on effects of yeast extract on production of four secondary metabolites in *Polygonum minus* root

of media. The results from metabolite profile showed that the volatile compound of *P. minus* root produced without any elicitation contained of 50.11% aliphatic (27.59% aldehide, 9.17% alkane and 13.35% others) and 19.39% sesquiterpene (β-caryophyllene, α-bergamotene, β-farnesene, α-caryophyllene and β-curcumene) in which the dodecanal compound (22.27%) and β-caryophyllene (8.09 %) had the highest percentage value for aliphatic and sesquiterpene groups, respectively. Moreover, elicitation of *P. minus* root culture using yeast extract at 100 mg/L concentration for 1 day showed the ability to increase the production of secondary metabolites in many volatile compounds of *kesum* root including the sesquiterpene compounds compared to control and other yeast extract elicitation treatments (β-caryophyllene: 8.09% to 8.74%; β-farnesene: 7.15% to 9.43%; α-caryophyllene: 2.40% to 3.96%). Elicitation using 100 mg/L of yeast extract for 3 days was recorded the best treatment for aldehyde production (Dodecanal: 22.27% to 23.92%).

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Mohd Azhar Hassan*, Ahmad Hafiz Baharom, Muhammad Shafie Md Sah & Mohamad Zulkiffely A. Rahman
MARDI Headquarters
Persiaran MARDI-UPM
43400 Serdang, Selangor Darul Ehsan
Malaysia

Mariatulqabtiah Abdul Razak
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: mazhar@mardi.gov.my

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