



## **In Vitro Regeneration and Phytochemical Profile of Medicinal Plant *Melastoma decemfidum***

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### **ABSTRACT**

*Melastoma decemfidum* or locally known as 'senduduk putih' is a medicinal plant which has been reported to contain important plant secondary metabolites like phenol, quercetin, kaempferol and naringenin. The study was conducted to determine the *in vitro* plant regeneration of *M. decemfidum* from nodal and leaf explants by using different levels of 6-benzylaminopurine (BAP) plant growth regulators and aims to evaluate the phytochemical constituents of *in vivo* and *in vitro* plants. The node (1 cm) and leaf explants (0.5 cm x 0.5cm) from *in vitro* plantlets were cut and cultured onto MS media supplemented with different levels of BAP hormone. The phytochemical screening was conducted by using Gas Chromatography Mass Spectrometry (GC-MS). For nodal explants, MS media supplemented with 0.25 mg/L of BAP which gave the highest number of shoots ( $7.33 \pm 2.182q$ ) and number of leaves ( $19.00 \pm 0.58$ ). Whereas, MS media treated with 0.5 mg/L BAP was identified to produce the optimum number of shoots by using leaves explants. Phytochemical screening which revealed in *in vivo* were Phenol 2, 4-bis, 1-heptadecene, Hexadecanoic acid, Octadecadienoic acid and Octasiloxane. Meanwhile, only cycloheptasiloxane, dodecanic acid and octadecadienoic were identified in *in vitro* plantlets. Micropropagation of *M. decemfidum* was successfully regenerated using manipulation of BAP hormone and more bioactive compound was found in *in vivo* plant compared to *in vitro* plants of *M. decemfidum*.

**Keywords:** *In vitro*, leaves explants, nodal explants, GC-MS, *Melastoma decemfidum*.

### **INTRODUCTION**

**M***elastoma decemfidum* or locally known as "senduduk putih" belongs to the family of Melastomaceae which is native to Southeast Asia and distributed to other tropical regions. *M. decemfidum* has been regarded as herb due to the effectiveness of this species to cure some disorders. In the previous study, it was reported that *M. decemfidum* could cure toothache, mouth ulcer and act as diuretic.<sup>1</sup> Ethnomedicinal used of the herb is due to its valuable pharmaceutical compounds. A class of phenol called flavonoids was reported to be found in the plant which was identified to be naringenin, and kaempferol.<sup>2</sup> Through the scientific studies, those compounds are well attributed to anti-inflammatory and anti-cancer properties.<sup>3</sup> Due to presence of several bioactive compounds, the micropropagation approach through plant tissue culture technique may be utilized in order to produce plant secondary metabolites in the species. Many studies have been established on Melastomaceae family, especially *Melastoma malabathricum* (purple petal). However, there are limited studies done on *Melastoma decemfidum*, even though the plant has significant medicinal values. In natural habitat, *M. decemfidum* is very difficult to be found as compared to *M. malabathricum* which can be found easily in open area and alongside most of the highways in Malaysia. Furthermore, the study on tissue culture of *M. decemfidum* is also limited. With regard to its phytochemical properties, most of the studies conducted to the extraction of flower and fresh leaves based from

the external plants.<sup>2</sup> So far, there was no study have been reported on phytochemical studies from *in vitro* plantlets of *M. decemfidum*. Therefore, in the present study has focused to determine the optimal concentrations of BAP plant growth regulators on *in vitro* regeneration potential of *M. decemfidum* from nodal and leaves explant. The major secondary metabolites productions from *in vitro* and *in vivo* plants of *M. decemfidum* were also evaluated.

### **MATERIALS AND METHODS**

#### **Surface sterilization**

The seedlings of *M. decemfidum* will be collected from Segamat Nursery, Johor. The seedlings maintained in greenhouse of plant tissue laboratory of Universiti Teknologi MARA, Shah Alam. About 1.5 cm shoot tips were cut and used as explants. The shoot tips were washed thoroughly under running tap water for 30 minutes. Under sterile condition, the explants were immersed in 20% of Clorox with the addition of tween 20 for 15 minutes. Then, the explants were rinsed with sterile distilled water for several times. The explants were cultured onto hormone free MS media.

#### **Media preparation and shoot induction**

Murashige and Scoog's (MS) salt (Duchefa Biochemie, Netherland) media was supplemented with 3.00 % g of sucrose (Duchefa Biochemie, Netherland) and 0.33 % of gelrite (Duchefa Biochemie, Netherland) powder. The pH of the mixed solutions was adjusted to 5.5 - 5.8 and the media were autoclaved for 20 min at 1.06 kg/cm<sup>2</sup>. Different concentration BAP hormones (0.25, 0.50, 1.00



mg/L) were incorporated into the media. A medium without plant growth regulators was used as control. The plant samples were collected from *in vitro* plantlet of *M. decemfidum*. The leaves explants were cut into approximately 0.5 cm x 0.5 cm size.

Then, the leaves explants were cultured in pill boxes containing MS-basal medium containing different concentration of BAP hormone for shoot induction.

For plant regeneration from nodes, nodal segments of approximately 1.0 cm length were cultured onto different concentration of BAP hormone (0.25, 0.50, and 1.00 mg/L).

One explant was cultured for each pill boxes and each treatment was replicates by three.

Then, the culture was then incubated at  $25 \pm 2^{\circ}\text{C}$  for 24 hours photoperiods.

After seven weeks of culture, the mean number (from nodes and leaves) of shoot produced per explant, shoot length, number of leaf, time taken to induce first shoot were accessed.

#### Extraction of plant samples

The fresh leaves of *in vivo* and micro shoots of *in-vitro* *M. decemfidum* were extracted with methanol before injecting into gas chromatography mass spectrometry (GC-MS) for phytochemical analysis.

For *in vivo* plant extraction, the plant sample was also obtained from Segamat Nursery, Johor. The fresh leaves from *in vivo* plants were extracted.

For fresh leaves extraction, 5.0 g of leaves were dried in the oven at  $40^{\circ}\text{C}$  in three days. Then, the dried leaves were crushed using grinding machine into powder. After that, the powders were immersed in methanol for three days. The mixtures were filtered using filter pump to remove the slurry.

The mixtures were evaporated using rotary evaporator to produce crude extracts. The crude extracts were then diluted with methanol until the solution turned yellowish.

Finally, 5  $\mu\text{l}$  of solution were injected into GC-MS system. The method was repeated for *in vitro* plantlets extraction.

#### Gas chromatography mass spectrometry (GC-MS) analysis

GC was conducted by using chromatograph equipment with an HP-5 fused silica capillary column. The temperature was set for  $100\text{-}275^{\circ}\text{C}$  at  $10\text{ min}^{-1}$ . One min was hold for  $100^{\circ}\text{C}$  and 17 min hold at  $275^{\circ}\text{C}$ . The injection temperature was  $275^{\circ}\text{C}$ . The flow rate of the helium gas is  $1\text{ ml}\cdot\text{min}^{-1}$ . 50:1 ratio used for split. Five  $\mu\text{l}$  of extract solution was injected. Agilent Cerity QA/QC software was recorded and processed the chromatographic data. By matching the peaks with computer Wiley MS libraries, extracts components were identified. The component was confirmed by retention

indices relative to (C8-C28) nalkanes with authentic compounds.

#### Statistical analysis

MS media supplemented with different levels of BAP was run for three replicates. The means and standard errors of mean were analyzed statistically using SPSS 18.0 (2009). By using one way ANOVA, the variances of mean were determined with mean comparison  $p = 0.05$ .

## RESULTS AND DISCUSSION

### Shoot induction from nodal explant

#### Number of shoots

After seven weeks of culture, the shoots numbers and lengths were measured (Table 1). For nodal explants, the highest number of shoots ( $7.33 \pm 2.19$ ) was obtained from the MS supplemented with 0.25 mg/L of BAP followed by 0.50 mg/L ( $3.33 \pm 1.45$ ), 1.00 mg/L ( $1.67 \pm 0.67$ ) and MSO mg/L ( $1.00 \pm 0.0$ ) for control.

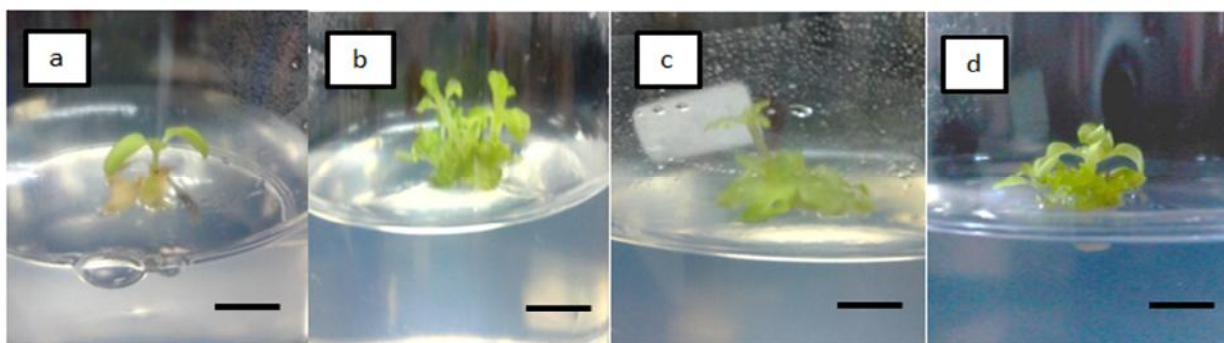
**Table 1:** Influences of different BAP level on shoot induction from nodal explants of *Melastoma decemfidum* after 7 weeks of culture.

BAP mg/l	Number of shoots per explant	
	Nodal explants	Leaf explants
0.0	$1.00 \pm 0.0$	0.0
0.25	$7.33 \pm 2.18$	$1.67 \pm 0.67$
0.50	$3.33 \pm 1.45$	$5.33 \pm 1.86$
1.0	$1.67 \pm 0.67$	$0.67 \pm 0.60$

Based on statistical by ANOVA, there was a significant difference ( $p < 0.05$ ) in the number of shoots among the treatments. The findings from the study revealed that the maximum number of shoots ( $7.33 \pm 2.19$ ) was optimally produced from MS supplemented with 0.25 mg/L BAP. This result was also similar with other finding which showed that BAP hormone can give positive effects on shoot induction.<sup>4-6</sup> Besides, the study also proved that the concentration of BAP hormone had significant effects on number of shoot produced per explants from nodal segments.

In the present study, it was found that as the BAP concentration of media increased (0.25 - 1.0 mg/L), the number of shoots produced per explant were decreased (Figure 1). The result suggests the increasing of BAP above from 0.25 mg/L, gave negative effects on the number of shoots produced. Whereby the increase in BAP concentration can reduce the number of shoots.<sup>7</sup> The reduction in proliferation of *in vitro* propagation is caused by the excess plant growth regulator which caused toxicity and lead to change of morphology, genetic and physiology of plantlets.<sup>5</sup> However, the shoot induction in *M. decemfidum* requires plant growth regulators as manifested in present study whereby multiple shoots were produced from all media except the MS media without BAP.





**Figure 1:** Assessment on shoot induction from nodal explants of *M. decemfidum* after seven weeks of culture. a) MSO (control) b) MS  $\pm$  0.25 mg/L c) MS  $\pm$  0.50 mg/L d) MS  $\pm$  1.00 mg/L. — represent 1 cm

### Shoot length

The effects of different concentration of BAP on the shoot length from nodal explant were also studied. Even though the highest mean of shoot formation was obtained from 0.25 mg/L of BAP hormone, but the highest shoot length ( $1.5 \pm 0.2$  cm) was resulted from the treatment 0.5 mg/L BAP in MS medium. This result indicates that 0.5 mg/L of BAP gave maximum shoot length but low number of shoots. However, there was no significant difference ( $p > 0.05$ ) among the treatments on shoots length.

From the observation conducted, the shoots lengths were retarded as the concentration of BAP increased from 0.5 up to 1.0 mg/L. The increasing of BAP concentration can give negative effects on shoot length.<sup>8</sup> This negative effect also can be related to the toxicity effects of excessive BAP hormone supplemented on MS media.

### Number of leaves

For nodal explants, the effect of BAP treatments on the number of leaves per explants was studied. Plantlets grown on MS basal medium was observed which gave the lowest number of leaf ( $3.67 \pm 1.55$ ). The highest number of leaves ( $19.0 \pm 0.58$ ) was observed in the plantlets derived from the treatment of 0.25 mg/L of BAP followed by 0.5 mg/L ( $15.0 \pm 1.0$ ) and 1.0 mg/L ( $6.3 \pm 3.0$ ) of BAP. The ANOVA showed that the supplementation of BAP significantly ( $p < 0.05$ ) affected the mean number of leaves per explants.

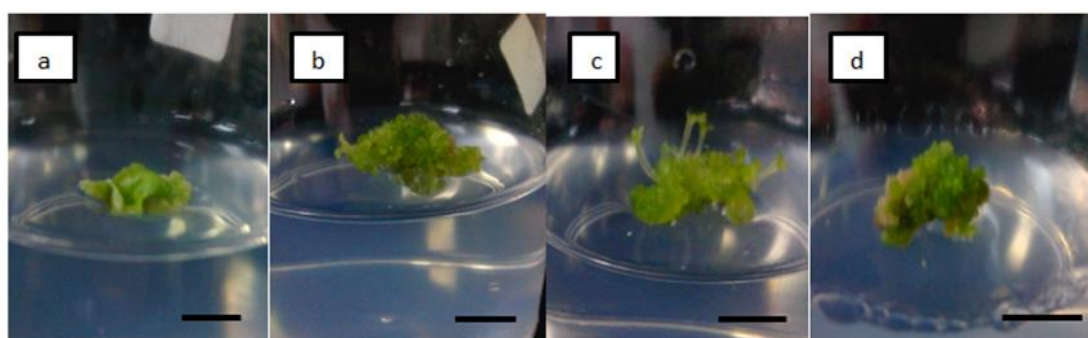
The formation of new leaves is related to the apical dominance suppression which is resulted from lateral

branch, hence resulting in increasing number of leaves.<sup>9</sup> The optimum concentration of BAP (0.25 mg/L) gave the optimum production of leaves in the *in vitro* regenerated plantlets. Similar response was observed on *Jatropha curcas* L where the area and number of leaves in the MS media supplemented with cytokinin hormone was higher than hormone-free media.<sup>10</sup> However, the addition of BAP above 0.25 mg/L showed the decreasing number of leaves. This result was in contrast to previous study on *Dendrobium* orchid whereby the supplementation of BAP at higher concentration would increase the number of leaves.<sup>11</sup> In addition, a study was reported that higher BAP concentration can inhibit the regeneration of multiple numbers of shoots and leaves.<sup>12</sup>

### Shoot induction from leaves explants

#### Number of shoots

For direct regeneration of shoot from leaves explants, the maximum number of shoots was successfully produced from MS media supplemented with 0.5 mg/L BAP ( $5.33 \pm 1.86$ ) followed by 0.25 mg/L ( $1.67 \pm 0.67$ ), 1.00 mg/L ( $0.67 \pm 0.60$ ) and MSO (0.0) (Table 1). Statistically, different concentration of BAP used in the regeneration media provided a significant difference ( $p < 0.05$ ) toward the number of shoots produced from the leaf explants. This study revealed that BAP concentration gave significant influences on the direct regeneration of shoots from leaves explants. This result was supported by previous study which multiple adventitious shoots formation can also be induced from the leaf explants by utilizing BAP hormone.<sup>13</sup>



**Figure 2:** Assessment on shoot induction from leaves explants of *M. decemfidum* after seven weeks of culture. a) MSO (control) b) MS  $\pm$  0.25 mg/L c) MS  $\pm$  0.50 mg/L d) MS  $\pm$  1.00 mg/L. — represent 1 cm

### Shoot length

Morphological observation (Figure 2) showed the majority of leaf explants produced micro shoots where the sizes of shoots were very small as compared to the shoots produced from nodal explants. Thus, the mean length for leaves could not be measured.

### Number of leaves

As for leaf explants, the leaves formed were found in clumps (Figure 2). Thus, the number of leaves from leaf explants was not taken into account as the size of the leaves was very small to measure.

### Effects of explant types on shoot induction

The nodal explants gave higher number of shoots ( $7.33 \pm 2.19$ ) as compared to the number of shoots produced from the leaves explants ( $5.33 \pm 1.86$ ) after seven weeks of culture. From the observation, more number of shoots was produced from the nodal explants compared to leaf explants (Table 1). The phenomenon is due to endogenous hormonal balance inside different types of tissue explants that give different responses on the number of shoots and their morphology. The expression of genes encoding hormone receptor and the level of endogenous hormone explains the different responses in different explants.<sup>14</sup>

### Time taken for shoots induction from nodal and leaf explants

The time taken for the first shoots induced from nodal and leaf explants were investigated. For nodal explants, the plantlets treated with 0.25 mg/L of BAP showed the most rapid shoot initiation two weeks of culture ( $0.23 \pm 0.33$ ) followed by 0.5 mg/L ( $2.6 \pm 0.33$ ) and 1.0 mg/L ( $3.3 \pm 0.33$ ) of BAP. The control group from leaves explants took the longest duration to induce the first shoot which was at week 6.

Meanwhile, the most rapid shoot initiation was obtained from treatment of 0.5 mg/L BAP which at weeks three ( $3.3 \pm 0.33$ ) followed by 0.25mg/L ( $5.33 \pm 0.33$ ) and 1.0 mg/L ( $5.33 \pm 0.33$ ) in leaf explants. However, there was still no shoot induction obtained from the control group after seven weeks of culture. For both explants, there was significant difference ( $p < 0.05$ ) of time taken for shoot induction. The result indicates that leaves explants depend on plant growth regulator for shoot initiation. This is in accordance with the study conducted on *Dendrothema grandiflora*, in which shoot initiation and proliferation required appropriate level of cytokinin (BAP) for nodal and leaves explants.<sup>15</sup>

### Evaluation of bioactive compounds from *in vivo* and *in vitro* plantlets of *Melastoma decemfidum*.

The quality, percentage constituents (area) and retention time of phytochemicals in methanolic leaves extract of *M. decemfidum* are shown in table 2. Five phyto-constituents were identified from *in vivo* plants whereas three phyto-constituents were determined from *in vitro* plantlets. The

result indicated that more major bioactive compounds were found in the *in vivo* plants than *in vitro*. The intact plant was found to exhibit more bioactive constituents than *in vitro* plantlets and this was regarded to be different due to high intoxication in soil.<sup>16</sup> The main compounds present in the *in vivo* plants are Phenol 2, 4-bis, 1-heptadecene, Hexadecanoic acid, Octadecadienoic acid and Octasiloxane. In contrast, for *in vitro* plantlets, cycloheptasiloxane, dodecanic acid and octadecadienoic acid were determined as main compounds.

For *in vivo* plant, phenol-2, 4-bis (1, 1-dimethylethyl) was identified to present at retention time of 8.87 min (Table 2). This compound has molecular formula of  $C_{22}H_{22}O$  and comprised 6.42 % of the methanolic leaves extract of *M. decemfidum*. Besides, phenol-2,4-bis (1, 1-dimethylethyl) was reported to have antifungal effects which is important for defense mechanism.<sup>17</sup> However, this important compound was detected only in *in vivo* plants. The absence of phenolic compound in plant extract could be related to the missing enzyme or catalyst that is responsible for phenolic pathway.<sup>18</sup>

The second compound which comprised 1.57 % of methanolic leaves extract was identified as 1-heptadecene with molecular formula of  $C_{17}H_{34}$  at retention times of 12.00 min (Table 2). The previous studies revealed that 1-hexadecene from *Cirsium japonicum* has anti-inflammatory and antibacterial effects.<sup>19</sup>

The third compound that was identified namely Hexadecanoic acid or palmitic acid ( $C_{18}H_{36}O_2$ ) which was determined at retention time of 13.36 min (Table 2). This commercial compound can be obtained by saponification of palm oils and bring value to the manufacturing industry as it can be used in producing products like cosmetics. Palmitic acid could alter the secretion of insulin and suppress leptin (key hormone of weight regulation).<sup>20</sup>

Octadecadienoic acid or oleic acid was found from both *in vivo* and *in vitro* *M. decemfidum* (Table 2). This unsaturated fatty acid ( $C_2OH_36O_2$ ) amounted as much as 15.08 % in leaves extract from *in vivo* whereas 12.6 % of micro shoot for *in vivo*. This bioactive compound also give benefit to human as unsaturated acid which plays important function to every cell in the body for normal growth and healthy skin.<sup>21</sup>

Octasiloxane is one of the main compounds that were identified by GC-MS from leaves extract of *M. decemfidum*. This compound was identified at 18.74 min and contributes 0.81% of methanolic leaves extract (Table 2). The Octasiloxane is a valuable compound as it has been identified to cause lysis of fungal activity hence may effectively function as anti-fungal agent.<sup>22</sup>

Cycloheptasiloxane is also one of the chemical constituent that was identified from *in vitro* plantlets. This compound comprised 2.55 % of micro shoots extracts (Table 2). The compound is known to have antimicrobial activities. Upon antimicrobial assay,



*Argemon ochroleuca* extract which contained cycloheptasiloxane was found to be effectively inhibited the growth of *Altenaria sp* fungi.<sup>23</sup>

Dodecanoic or lauric acid was found at retention time of 10.57 min which comprised 2.7 % of *in vitro* extract. Lauric acid also plays main role in inhibiting microbial

growth as it was proven to inhibit the growth of *Paenibacillus* larvae (spore-forming bacteria) that is found at plant pollen.<sup>24</sup>

Generally, most compounds were identified in both *in vivo* and *in vitro* plant samples are fatty acid.

**Table 2:** Analysis of secondary metabolites from *in vivo* and *in vitro* plantlets of *M. decemfidum*.

<i>In vivo</i>				<i>In vitro</i>			
Compound	Retention time	Area	Quality	Compound	Retention time	Area	Quality
Phenol,2,4-Bis	8.87	6.42	96	Cycloheptasiloxane	5.99	2.55	93
1-heptadecene	12.00	1.57	91	Dodecanoic acid	6.60	2.74	96
Hexadecanoic acid	13.36	6.16	97	Octadecadienoic acid	10.57	12.67	99
Octadecadienoic acid	15.08	5.73	98				
Octasiloxane acid	18.74	0.81	91				

## CONCLUSION

The micropropagation system by utilizing nodal and leaves explants of *M. decemfidum* was successfully established. Low concentrations of BAP were the most appropriate for shoot induction of *M. decemfidum*. Nodal explants produced better plant regeneration and took shorter time to produce first shoot than leaves explants. *In vivo* plants have higher number of phytochemical compounds than *in vitro* plantlet and both consisted of phenol and fatty acid.

The present protocol could be used for the conservation of this endangered medicinal plant and can be a starting point for production of secondary metabolites by biotechnological approaches.

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