DETECTION OF NON-POLAR CHEMICAL COMPOSITIONS OF *IN-VITRO* CULTURE PRODUCTS OF *Pogostemon cablin* VIA GC/MS

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Abstract

Most of the chemical compound of *P. cablin* was detected after soil plantation rather than during *in-vitro* culture since it is expected to have secondary metabolites due to maturity. Theoretically, culture products may not be able to derive many chemical constituents compared to mature plants. However, it is possible to detect the presence of other beneficial compounds from the culture plants. Therefore, the objective of this study is to provide information for these new findings. The experiment was carried out by yielding two types of *in-vitro* culture products which were callus and microshoot by using plant hormone. After three weeks, high abundances of callus in 0.5 mg/L BAP and 3.0 mg/L NAA were sufficiently acquired by using nodes explant. On the other hand, only a week was required to obtain microshoot from foliar explant by using 0.5 mg/L BAP. Both plant samples were extracted by using hexane solvent for non-polar GC-MS analysis. There are six compounds detected in hexane–callus extraction samples. The highest abundance compound detected is silane (19.04%) and the lowest abundance is tetrasiloxane (8.88%). Only three compounds are found in microshoot which tetrasiloxane is the highest abundance (76.23%) while tetrasiloctane is detected as the lowest (2.37%). Tetrasiloxane which is significantly higher in the microshoot compared to the callus is found to have great beneficial properties such as non-toxic, high compatibility with the lipophilic with extraordinary water repellency and stability that may conclude to play a major role for enhancing plant growth.

Keywords: *Pogostemon cablin*, Callus, Microshoot, Non-Polar, Secondary Metabolite, GC-MS

Introduction

Pogostemon cablin or "Pokok nilam" is known to have various secondary metabolites that are essential not only to treat diseases such as headaches, nausea, vomiting and fever but also is used as fragrance towards mind therapeutic and control therapy. Usually, most secondary metabolites substances such as sesquiterpenoids, phytosterols, flavonoids, organic acids, glycosides, alcohols, and aldehydes (Swarmy et al., 2015) are directly yielded from the leaf of mature plant. The most recent research done on this plant revolved around the potential of patchouli towards pharmacological impacts (Lee et al., 2020) that had remarkably used to fight stomach diseases, where it is used to treat bacteria that are harmful towards our body such as *Helicobacter pylori* (Yu et al.,2015; Juren et al., 2021). All intensive studies about the use of this plant have resulted significant impact on medicinal effects including anti-tumour

(Wei et al., 2018), anti-inflammatory, anti-apoptotic (Wu et al., 2017) and antimicrobial properties (Kumara et al., 2012). These rapid studies have given not only a rise towards another potential of this plant but also the discovery of new possibilities of other beneficial secondary metabolites that may be found in other parts of this plant organ. Therefore, to embark on the new knowledge and more extensive study regarding this plant, the plant tissue culture products were obtained and used as the main data analysis to understand the secondary metabolites present in this plant even at the beginning of the cell development such as callus and microshoot. Although the frequency of the list chemical constituent found may be less in term of quality and quantity which is commonly expected due to explant types, distillation process or extraction process (Chakrapani et al., 2013) especially in non-polar chemical compounds, but this study nevertheless may fill up the gap between the industrial, agriculture and plant tissue culture study regarding this plant.

Materials and Methods

Tissue culture product samples preparation

Nodes of *P. cablin* were used as the main sample for the callus induction process. The explants were cut around 3-5mm^2 sizes and underwent sterilization procedure by using ethanol 70%, 20% sodium hypochlorite (NaOCI) for 5 minutes. Then, the explants were rinsed with distilled water three times before being cultured in petri dish contained Murashige and Skoog (1962) MS media supplement with plant hormone (0.5 mg/L BA and 3.0 mg/L NAA) which were selected from prior study (Nursuria., 2020).

The callus that had optimum morphology which were soft and whitish were collected and underwent callus proliferation by subculturing the potential callus in the same media without the main explant body. Any potential callus that appeared from nodes explants were plucked in small quantities between 1~5 mm of callus diameter and cultured in petri dishes containing the same media selection and maintained in dark condition at room temperature for three weeks. Any petri dish with contamination were discarded or been subculture again until the desired amount was gained for the GC-MS analysis.

In shoot induction, leaf explants were used as samples which were selected from larger and medium leaves and cut in sizes between 0.5 ~ 1.0 cm. Each explant was transferred into each pill box consisting of MS media supplement with plant hormone (0.5 mg/L BA). The final products were obtained based on the good morphology exhibit such as green coloration, lack of necrosis and abundance in a short time.

Non-polar GC-MS analysis

In the GC-MS analysis, about 0.15 g callus in prior dry were soaked and rinsed with 3 mL of distilled water. The solution was stirred for five minutes and filtered using gravity filtration technique three times repeatedly. The product of filtration was collected in a beaker about 2.0 ~ 2.5 mL. The remaining solids were discarded. By using the micropipette, 200 µL was added in each 10-centrifuge tube. Then, five of the centrifuge tubes were added with hexane. All the tubes were shaken vigorously for 10 seconds and the centrifuged for ten minutes at 10000 rpm. Two visible layers were formed and by using micropipettes, the aqueous layer was discarded carefully, and the remaining organic layers were collected for further use in GC/MS analysis.

The 1 mL of each sample was injected in the Agilent columns. GC equipment from Agilent Technologies model 7890A GC system and model 5975 inner XL EI/ CI of MSD system were used. The oven temperature was set up at 50ºC to 250ºC for 7ºC/min. The temperature for the injector and detector were set to 250ºC and 280ºC. The split ratio was 1:9 and helium were used as carrier gas. The ionization current and potential used were 1A and 70 eV,

respectively. The source temperature was set to 150ºC and the 1000 resolutions were used at sec/dec. The retention time and peak of molecular weight show were adjusted, and the chemical compositions detected in the samples were compared by using NIST spectral library. The procedure was repeated with other samples types which were microshoot, leaves, stems, and roots.

Result and Discussion

The presence of the secondary metabolites were successfully detected in five types of *P.cablin* plant samples which two of them were from tissue culture products (callus and microshoot) and the others were from natural plant. In the experiment, hexane solution was selected due to its less toxicity compared to the other non-polar chemical compound since it is commonly used in clinical study such as anti-diabetic in rats (Okolo et al., 2016). With the total numbers of non-polar chemical compositions found in the samples as summarized in **Table 1**, it was found that natural plant *Pogostemon cablin* had the most non-polar chemical compositions with hexane-root (8 different types) followed by hexane-leaves (7 different compounds), and hexane-stem (6 different compounds). In tissue culture of *Pogostemon cablin,* non-polar chemical compositions of hexane-microshoot were the lowest with only 3 different compounds of chemical compositions detected. The relative percentages abundance of non-polar chemical in all samples summarized also showed varied in both tissue culture and natural plant.

The hexane-microshoot had the lowest number of non-polar chemical compositions but the present of tetrasiloxane was the highest among all the samples with 76.23% while the hexane-stem and hexane callus had the lowest abundance of tetrasiloxane with 5.03% and 8.88% respectively. The tetrasiloxane has beneficial properties which are non-toxic, low surface tension and non-greasy, high compatibility with the lipophilic, high compressibility, high damping action, high dielectric strength, high water repellency and high stability. It also acts as volatile silicone fluids that do not cool the skin when evaporated, therefore it plays role in industrial applications such as personal care products (Nils, 2007). The silane was found to be highly abundance in three types of samples which were hexane-root (15.81%), hexane stem (16.26%), and hexane-callus (19.04%). Silane is well-known to have toxicity properties therefore the high percentages of silane in both natural and cultural plants show that *Pogostemon cablin* is not suitable to be consumed (Fthenakis *et al*, 2003).

The hexane-callus consists of a high abundance of silane and this may relate to plant defenses against infection. This is because callus is an important stage of cell division therefore in order to prevent bacterial infection; high amount of silane may be secreted by the cell as protection. The silane may have similar acts like hydrogen peroxide, however further studies on silane is required. By referring to table 2, there are 17 of non-polar chemical compositions that have been detected, showing that three of non-polar chemical compositions are found in almost all samples which are tetrasiloxane, cyclononasiloxane and silane. However, both cyclononasiloxane and silane are absent in hexane–microshoot. This is because the toxicity properties of silane may become an inhibitor factor for the shoot regeneration. Unfamiliar non-polar chemical compositions which include glaucine and eicosane are both found in hexane-root. While acidic non-polar chemical compounds can only be found in hexane-leaves which are benzoic acid and benzeneacetic acid. Therefore, it can also be said that hexane-leaves of natural plant extract have acid properties compared to other samples in hexane extract.

Table 1 Non-polar chemical compositions abundances found in the tissue cultures and natural plant of *Pogostemon cablin*.

Types of Sample	Total number non-polar chemical been detected	Highest abundance of non-polar chemical (%)	Lowest abundance of non-polar chemical (%)
Hexane-leaves	7	Hexasiloxane 13.89%	Benzeneatic acid 7.75%
Hexane-stem	6	Silane 15.81%	Tetrasiloxane 5.03%
Hexane-root	8	Silane 16.26%	Eicosane 0.39%
Hexane –callus	6	Silane 19.04%	Tetrasiloxane 8.88%
Hexane-microshoot	3	Tetrasiloxane 76.23%	Tetrasilaoctane 2.37%

Tables 2 List of non-polar chemical compositions in tissue cultures and natural plant of *Pogo stemon cablin* detected via GC-MS

List of Non polar chemical composition	Location found in the samples
Tetrasiloxane-1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)	Hexane-microshoot, Hexane-leaves, Hexane-stem, Hexane-root, Hexane-callus
Cyclononasiloxane octadecamethyl-	Hexane-leaves, Hexane-stem, Hexane-root, Hexane-callus
Silane[[4-[1.2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	Hexane-leaves, Hexane-stem, Hexane-root, Hexane-callus
1,3-Xylyl-15-crown-4,2,3-pinaned-ioxyboryl	Hexane-stem, Hexane-callus
Cyclohexasiloxane dodecamethyl-	Hexane-stem, Hexane-callus
Tetradecamethyl-Heptasiloxane	Hexane-microshoot, Hexane-root
Tetrasilaoctane-octamethyl	Hexane-microshoot
1-monolinoleoglycerol-trimetyls ilyl ether	Hexane-root
Benzeneatic acid	Hexane -leaves
Eicosane	Hexane-root
Hexasiloxane-tetradecamethyl	Hexane -leaves

Octasiloxane-hexadecamethyl	Hexane-root
2H-1,4-benzodiazepin-2-one	Hexane-callus
Glaucine	Hexane-root
Dithiocarbonic acid	Hexane-stem
Pentasiloxane-dodecamethyl	Hexane -leaves
Benzoic acid	Hexane -leaves

Conclusion

There are 17 non-polar chemical compositions that can be detected in both in vitro tissue culture and natural plant extracts of Pogostemon cablin in hexane solvent extraction via GC-MS analysis. Three non-polar chemical compositions are found in almost all samples which are tetrasiloxane, cyclononasiloxane and silane. Tetrasiloxane can be found in all tissue cultures (callus and microshoot) and natural plants (leaves, stem, and root) in hexane solvent extraction of Pogostemon cablin with the role and benefits of this compound requiring more extensive study in the future.

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