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Comparison on Phytochemical Constituents in The Patchouli Oil of *In Vitro* And *Ex Vitro Pogostemon cablin* Leaves

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ABSTRACT

Pogostemon cablin (patchouli) is a medicinal herb well known for its essential oil derived from the leaves. Patchouli oil shows excellent base note in fragrance industries for its fixatives properties and its patchouli alcohol (patchoulol) is used as quality indicator for its oil. However, the P. cablin is the only commercial source of patchoulol and cannot be obtained synthetically in the laboratory. Higher demand in the production of its essential oil gave a significant contribution for in vitro grown P.cablin to meet the market supply for industries. Hence, in this study, the essential oil in both in vitro and ex vitro P.cablin were extracted from its leaves by means of hydrodistillation method and its phytochemical constituents were identified and compared using Gas Chromatography Mass Spectrometry. The yield and quality of its essential oil from both in vitro and ex vitro P.cablin's leaves were investigated. In vitro patchouli essential oil extraction gaves higher yield (40 ml) than the ex vitro patchouli essential oil (26 ml) under similar condition for hydrodistillation. Six major components were identified through GC-MS and was compared between two samples which are β- patchoulene, Caryophyllene, α- guaiene, α- cedrene, α- bulnesene and Patchouli alcohol. The patchoulol, which is the main constituents that is important in fixative had doubled (42.18 %) in the in vitro P.cablin essential oil compared to ex vitro (29.24%). This finding was reflected based on the peak area percentage of each substance through GC-MS. Other constituents in the in vitro P.cablin were found still competitive to the ex vitro in slightly lower values. Overall, in vitro P.cablin showed higher yield and quality compared to the ex vitro grown P.cablin.

Keywords: *Pogostemon cablin;phytochemicals;essential oil;in vitro;ex vitro*

1. INTRODUCTION

Pogostemon cablin, is a small bushy herb belongs to the family of Lamiaceae with fragrant-smelling leaves (Figure 1), from which patchouli oil is extracted. Patchouli oil is widely used as base material in fragrance, food and medicines [1]. The essential oil of several Pogostemon species have been used as natural perfumes and remedies, and were grown in backyards. However, only *P.cablin* is extensively cultivated for its oil. Patchouli oil is a unique substance of oriental notes with strong fixative properties that has been utilized for high value perfumes, cosmetics and flavouring for soaps, detergents, and deodorants [2].

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Figure 1: (a) Patchouli plant (Ex vitro mother plant); (b) Leaves of *Pogostemon cablin*

P.cablin propagates generally through vegetative propagation of stem cuttings. This technique, however, has major disadvantage on its production yield as the propagated plants potentially experiencing various diseases, i.e., generally root knot nematodes, pest and viruses [1]. This traditional approach also reported to be inefficient and slow for large quantities production. Micropropagation through *in vitro* tissue culture technology offers promising rapid, large production of clones for field cultivation. Thus, the *in vitro* raised plants were produced via plant tissue culture techniques (Figure 2) to overcome the problem arise from the traditional propagation of *ex vitro* mother plants [2].

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Figure 2: (a) *In vitro* plantlet; (b) *In vitro* raised plants.

At present, *P.cablin* is the only natural source known for patchouli alcohol that makes it more valuable than ever [3]. Patchouli is basically a native to Southeast Asia and extensive cultivation is practiced in Indonesia, Philiphines and China as well as in Malaysia. The commercial value of patchouli essential oil is directly correlated with its qualitative and quantitative composition which varies according to the cultivation region and extraction technique [4]. Wu *et al*., [5], reported mainly chemical analyses on *P.cablin* grown in China in different localities. Erika & Ermaya, [6] also published on the yield and quality of patchouli essential oil based on several factors including the region of raw material which were obtained from 4 different villages in Indonesia. Factor such as environmental conditions in each region plays a role in affecting the chemical constituents in plants [7]. Malaysia grown patchouli was the subject on chemical analysis in this study.

Hydrodistillation is the most economic and efficient method for essential oil extraction in *P.cablin*. The procedures are the simplest as it only use boiling techniques to obtain the condensed oil utilized from the leaves sample that priorly dried upon usage. The oil and water are separated to obtain pure oil. The oil of patchouli is thick brownish-yellow tinted green in colour with rich musky-sweet herbaceous smell. The freshly distilled oil has slightly harsh aroma but mellows considerably to sweeter and balsamic [3],[8].

The essential oil from patchouli contains complex chemical composition with distinct compounds which consists of abundant sesquiterpenes [8],[9]. The critical bioactive component in patchouli oil reported mostly by researchers is patchouli alcohol that is responsible for the typical patchouli note [10]. Thus, patchouli alcohol content is regarded as quality assessment of *P.cablin*.

The chemical constituents of patchouli oil obtained from natural plant are widely studied for various applications but *in vitro* raised patchouli has received limited attention. However, Hardjo *et al*., [11] reported on essential oil profiles from cell cultures stage of *P.cablin.* The phytochemical profiles is essential for the quality marker of every plant species especially the highly importance aromatic plants such as of *P.cablin*. Hence, in this study, the comparative

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analysis of patchouli oil phytochemical constituents was employed for both *ex vitro* mother plants and *in vitro* raised plants.

2. MATERIAL AND METHOD

2.1 Preparation of Plant Material

Plant materials of patchouli were collected and cultured from Ayer Keroh, Melaka. Voucher specimen was deposited at the Herbarium Institute of Bioscience, Universiti Putra Malaysia. Leaves from both *ex vitro* mother plant and *in vitro* raised plant of *P.cablin* were harvested randomly. The leaves samples were air dried under direct sunlight for 11 days before being powdered using a mechanical grinder prior to oil extraction process using hydrodistillation.

2.2 Hydrodistillation

Patchouli oil were extracted through hydrodistillation using two different leaves samples obtained from *ex vitro* mother plant and *in vitro* raised plants. Two hundred grams of each powdered sample were weighted. The apparatus were set up as the picture shown in Figure 3. The percentage yield of essential oil (w/w) were calculated according to the formula:

$$\text{Patchouli oil (\%)} = \frac{\text{Total weight of oil collected (ml)}}{\text{Dry mass of leaves sample (g)}} \times 100 \quad (1)$$

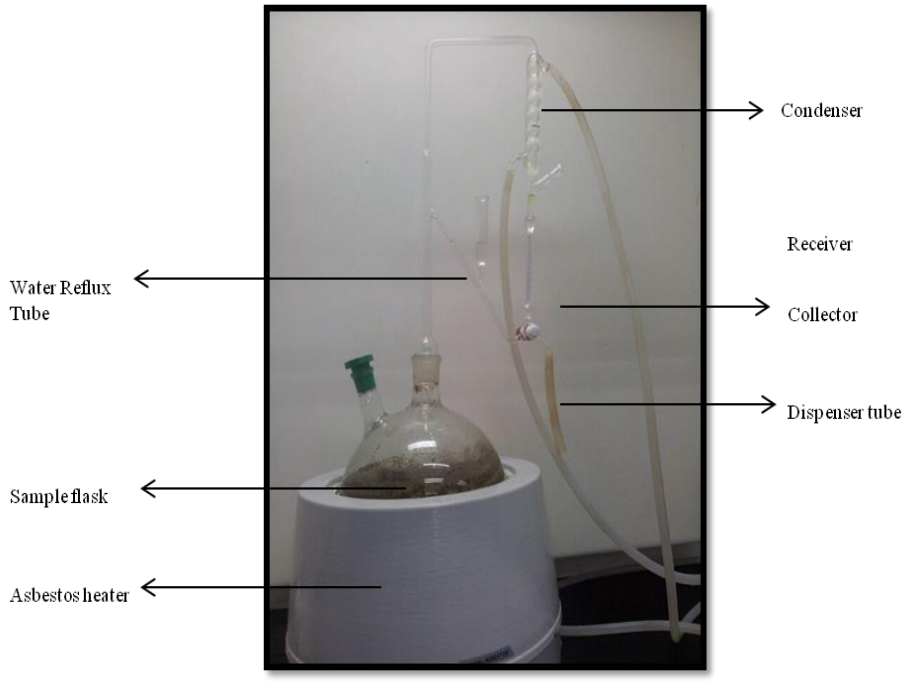

Figure 3: The Clevenger Apparatus Set for Hydrodistillation Process of Patchouli Essential Oil

The powdered sample was transferred into the sample flask and the water was added until the sample fully immersed (2L capacity). The essential oils were periodically dispensed from the

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collector tube into glassware practically along with the distillated water (Figure 4). The oil formed a layer above the water which was then recovered into clean bottles. The essential oils were extracted from *P.cablin* leaves by water distillation for approximately 5 hours until the oil quantity in the extractor did not increase. The obtained essential oil was recovered with non-polar organic n-hexane which is soluble with the patchouli oil. The excess water in the patchouli oil was dried over anhydrous sodium sulfate until the last traces of water were removed and finally stored in glass bottles at 4°C prior to gas chromatographic-mass spectrometric (GC-MS) analysis. Chemical constituents of *in vitro* raised plants and *ex vitro* mother plants were analysed and compared using GC-MS (Agilent 6890).

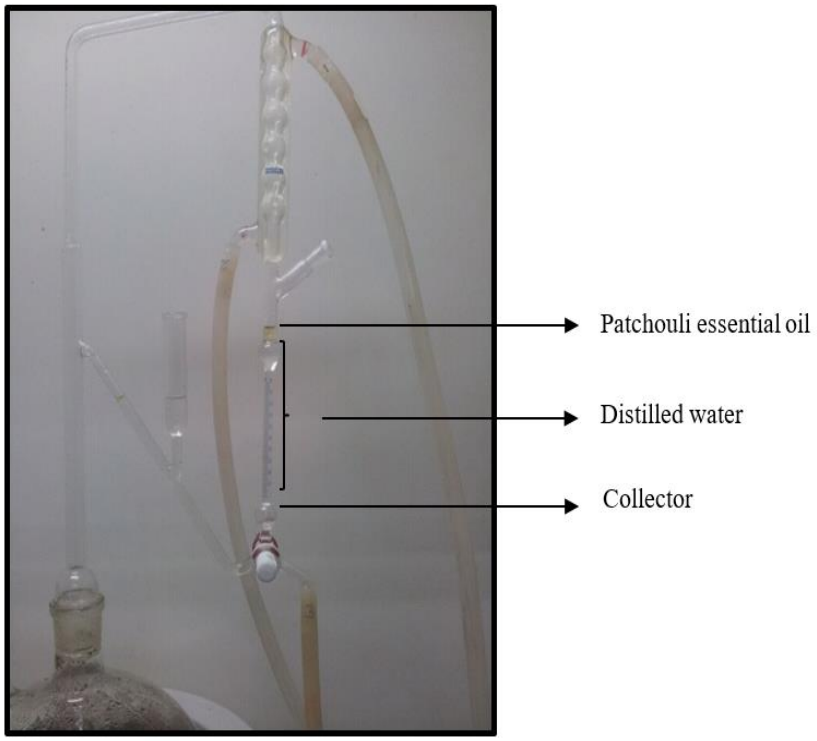

Figure 4: The Oil And Distillated Water Were Dispensed And Collected Through The Collector Tube

2.3 Chemical Composition Analysis in GC-MS

The GC-MS analysis was carried out using Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer inert. Compounds were separated on capillary column HP-5MS (5% phenyl / 95% methylpolysiloxane, 30m x 0.25 mm i.d., 0.25 µm film thickness). The instrument was initiated at initial column temperature of 50 °C and then progressively heated up at the rate of 2 °C min-1 to 280° C. Helium was used as the carrier gas with flow rate of 1 ml/min with splitless injection. The ionization and inlet source temperature was 280 °C and 120 °C, respectively. Both samples were analysed three times.

2.4 Identification of phytochemical constituents in the Essential oil

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Mass spectral data were analysed by the database and compared with standard published data. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The calculation formula is as follows:

$$\text{Relative amount (\%)} = \frac{\text{Area under peak}}{\text{Total area}} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1 Oil Extraction

Patchouli oils were extracted with 200g samples and the oil were trapped in the receiver within the first 30 minutes after the samples were boiled. The yields of patchouli oils obtained from hydrodistillation of both samples were calculated as shown in Table 1.

Table 1: Extraction of patchouli oils from *ex vitro* mother plant and *in vitro* raised plant leaves samples

Sample	Sample weight (g)	Amount of Patchouli oil (ml)	Patchouli oil /dry weight of sample (%)
Ex vitro mother plant	200	26	13.0
In vitro raised plant	200	40	20.0

Patchouli oils obtained from *in vitro* raised plant (Figure 5 (a)) using the same constant amount to the *ex vitro* mother plant sample (Figure 5 (b)) gave higher oil yield that is 7% higher volume per sample dry weight. The oils were then injected to GC/MS for further phytochemical analysis.

The necessity to produce the most reliable sample preparation has led to this extraction method. Hydrodistillation was able to extract the essential oil from *P.cablin* leaves that provided enough sample stocks for further utilization in Gas chromatography analysis for its chemical constituents [3]. Furthermore, the procedures involved were very practical as the beginner in any extraction method could possibly handle this equipment with low operation cost.

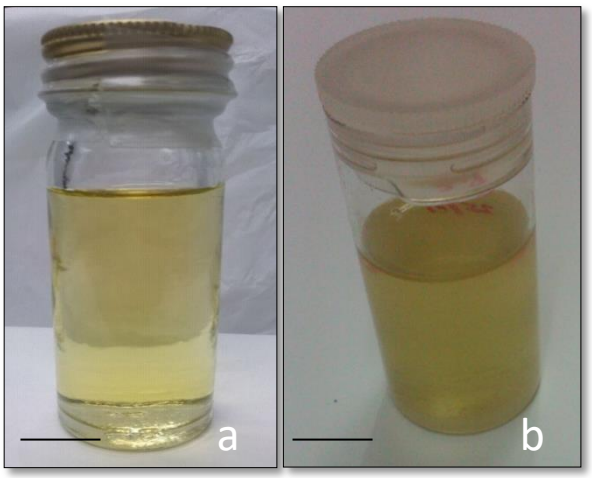

Figure 5: The oil obtained from hydrodistillation process from *in vitro* and *ex vitro P.cablin* leaves (a) Patchouli oil (*in vitro* raised plants) (b) Patchouli oil (*ex vitro* mother plant); Bar = 5cm.

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The oil yield was better from *in vitro* raised plants gave advantages to the practitioners to overcome the supply constraint. The drying method and temperature are directly linked with the quality of the essential oil and yield of essential oil . Basically, leaves that are dried through air without any heat pressure contributes to high oil yield. Based on the findings of this study (Table 1), the *in vitro* raised plants gave higher yield (40 ml) than the *ex vitro* mother plant (26 ml). This resulted in 2:1 yield ratio in the *in vitro* an *ex vitro* patchouli essential oil. This technical was also reported by Kongkathip *et al*., [4] in the study on patchouli extraction by which the naturally dried leaves sample of patchouli under direct sunlight yielded higher essential oil than those in fresh leaves and heat shocked drying leaves samples. However, Pandey et al., [5] reported that increasing the temperature up to 40°C will increase the yield of essential oil but the yield of essential oil will decrease if the temperature is higher than 40°C. The quality of the essential oil were found to be higher in the drying under direct sunlight as compared to the other methods that involve heat shocked such as using oven to regulate the temperature. The drying process contributes to the evaporation of highly volatile compounds such as the monoterpenes and hence, resulting in increasing of sesquiterpenes amounts particularly Patchoulol content [5]. This patchouli alcohol content is parallel to the quality of the essential oil in Patchouli oil.

3.2 GC-MS

The study on the phytochemical in the *P.cablin* for both *ex vitro* mother plant and *in vitro* plant extracted through hydrodistillation method by gas chromatography- mass spectrometry (GC-MS) analysis showed the presence of six interest compounds. The list of interest components identified in both sample of *P.cablin* is presented in Table 2. The retention time and the concentration indicated by its peak area for each component were also listed down in the same table.

Table 2: Chemical constituents identified in the *ex vitro* mother plant and *in vitro* raised plant of *P.cablin* using GC-MS

Chemical components	Retention time (RT)	*Ex vitro P.cablin* peak area in %	Quality	Retention time (RT)	*In vitro P.cablin* peak area in %	Quality
β- patchoulene	30.82	4.54	98	32.30	3.15	97
Caryophyllene	33.22	4.03	99	34.74	3.45	99
α- guaiene	34.86	14.74	99	36.05	10.10	99
α- cedrene	35.44	8.57	94	37.08	6.23	99
α- bulnesene	38.85	18.64	99	40.63	18.83	98
Patchouli alcohol	47.14	29.24	95	49.18	42.18	99

The mass chromatography for both samples (Figure 6 and Figure 7) showed parallel similarity in their components based on the peak corresponding to the retention time (RT). The chemical compositions for both samples of *ex vitro* mother plant and *in vitro* raised plant of *P.cablin* were identified with the matches of the components with the National Institute of Standards of Technology (NIST) library (Table 3).

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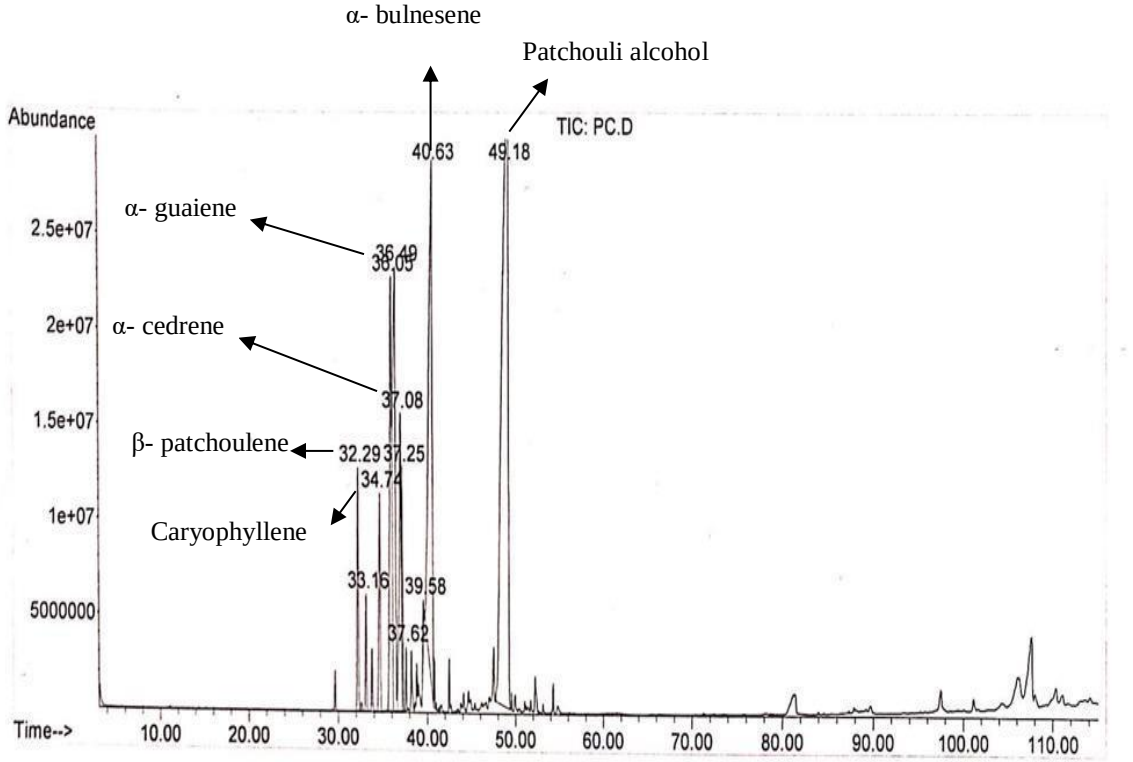
Several previous studies were reported in *P.cablin* essential oil worldwide especially in China. For example Hu *et al*., [6] reported on GC-MS fingerprinting among the *P.cablin* in China particularly for quality assessment. On the other hand, Sundaresan *et al*., [7] identified the composition of *P.cablin* essential oil with respect to *Pogostemon travancoricus* Bedd. Var. *travancoricus* by which the report confirmed that patchouli alcohol only present in *P.cablin* plant. Another study by Murugan and Mallavarapu [8] also found that there was no trace of patchouli alcohol in Pogostemon travancoricus var. travancoricus. According to Paul *et al*., [9], the chemical constituents from *in vitro* plants derived from leaf explants and *ex vitro* plants were similar to each other but differ significantly in their contents, in which patchouli alcohol percent content was found higher in the *in vitro* derived plants compared to *ex vitro* mother plants. Several studies by [2], [10] claimed that the supplementation of plant growth regulators played a role for the increment in the *P.cablin* essential oil contents.

Table 3: Chemical components identified in the *ex vitro* mother plant and *in vitro* raised plant of *P.cablin* using GC-MS (Source: NIST 02 MS Library)

Chemical components	Library ID	CAS #
β- patchoulene	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1α,4α,7α)]-	000514-51-2
Caryophyllene	Caryophyllene	000087-44-5
α- guaiene	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,4α,7α)]-	003691-12-1
α- cedrene	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3α,3aβ,7β,8aα)]-	000560-32-7
α- bulnesene	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	003691-11-0



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Patchouli alcohol	Patchouli alcohol	005986-55-0

Figure 6: The chromatogram of *in vitro P.cablin* using GC-MS

Similar situation was found in this study as the findings for both patchouli oil samples consist of the same six interest component but vary in the relative amount. Patchouli alcohol, the active compounds of patchouli essential oil [11] was identified as the major component that consists of the most abundant with highest peak area contributed to the highest relative amount. However, the *in vitro* raised plant sample of *P.cablin* was analysed and found to have higher patchouli alcohol content (42.18%) in its essential oil when compared to the *ex vitro* mother plant sample (29.24%). The quality of patchouli oil is determined by its patchoulol concentration [12], [11]. This finding approved that *in vitro* raised plant could provide high quality patchouli oil. α- bulnesene was the second abundant compound identified in both *ex vitro* and *in vitro P.cablin* plant with 18.64% and 18.83%, respectively. Minor components such as Caryophyllene, α- guaiene, α- cedrene and α- bulnesene were also traced in both samples with higher relative amount in oil constituents of *ex vitro* mother plant of *P.cablin*. The mass chromatogram for *in vitro* and *ex vitro P.cablin* essential oil samples were shown in Figure 6 and 7, respectively.

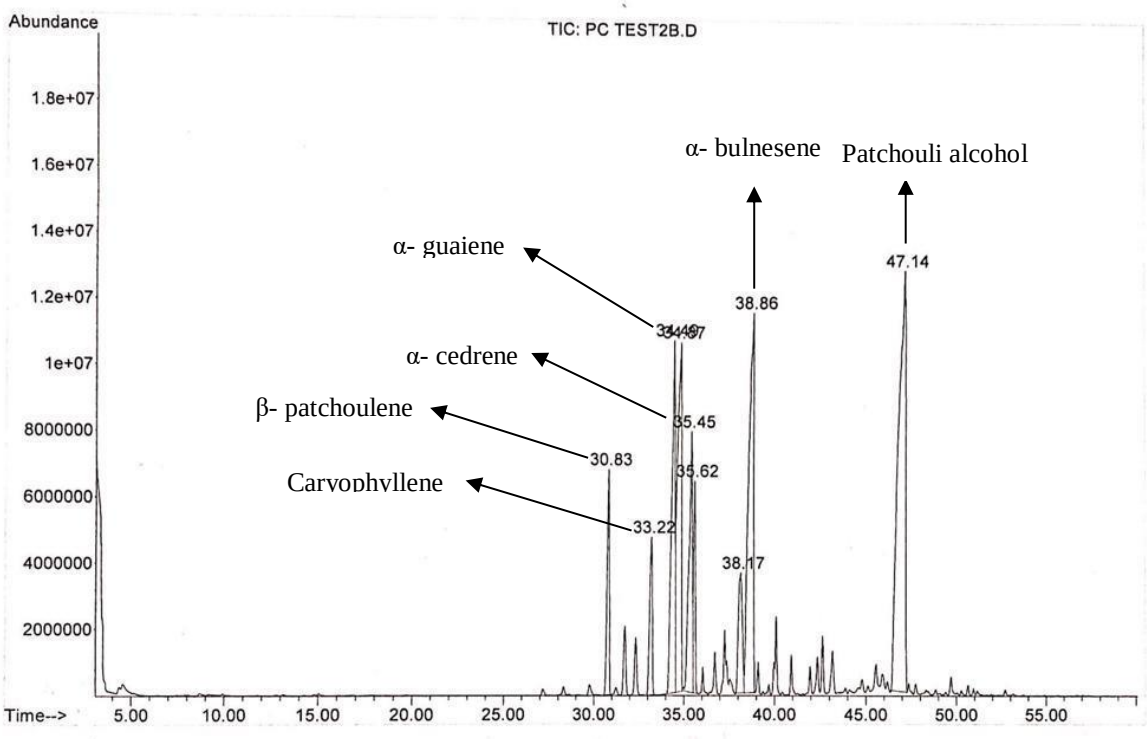

Figure 7: The chromatogram of *ex vitro P.cablin* using GC-MS

4. CONCLUSION

The chemical constituents in *P.cablin* plant involving both *ex vitro* and *in vitro* plant were identified and compared using gas chromatography mass spectrophotometer (GC-MS). *In vitro* sample yielded higher oil in respect to constant dry weight of dried leaves (20 % oil yield) when

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compared to *ex vitro* that produced only 13% of oil yield. Patchouli alcohol is the major compound for patchouli oil, in which it was identified in this study in both *ex vitro* mother plant and *in vitro* raised plant sample. The relative amount of patchouli alcohol was higher in the *in vitro P.cablin* raised plant sample with 42.18% to only 29.24% in *ex vitro* mother plant sample. In economic view, the *in vitro* raised plants of *P.cablin* yielded a promising alternative techniques to ensure the market demand for the patchouli oil can be fulfil and also provide higher oil quality as well as the production yield.

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