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SPATIAL VARIATION OF *MELALEUCA CAJUPUTI* POWELL ESSENTIAL OILS

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ABSTRACT

Cajuputi essential oil in Indonesia and Malaysia is extracted from the leaves of *Melaleuca cajuputi* Powell. This study identify the variation of *Melaleuca cajuputi* essential oil fingerprinting based on the essential oil. The leaves were collected from 10 different sites in Terengganu undergoes hydro distillation and detailed analysis by FTIR spectroscopy. ATR-FTIR result of *Melaleuca cajuputi* shows that functional group of Gelam are variable between different sites and also gave different fingerprinting characters due to the compound variation in relation to their adaptation to the changing environment. Result shows high correlation between several sites (0.97, 0.98 and 0.976) and also significant difference between different sampling stations (p-value < 0.01). The present study proved a significant spatial variation of *Melaleuca cajuputi* fingerprint in Terengganu from the essential oil collected.

Keywords: essential oils; *Melaleuca cajuputi* Powell; spatial variation; FTIR spectroscopy.

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1. INTRODUCTION

Melaleuca cajuputi Powell, known in Malaysia basically as "Kayu Putih" is a huge perpetual tree of the Myrtaceae family. It can achieve heights of up to 40 meters with matures trees having trunks up to 1.2m in diameter. There are around 250 species from the Melaleuca family [1], 220 of which can be found in Australia and Tasmania [2]. Across the Southeast Asian area its name contrasts, going for "Gelam" in Peninsular Malaysia and also known as "kayu putih”. In different parts of Malaysia and Indonesia, it is often known as the “swamp tea-tree” and also referred to as the “paperback tea-tree” and the “cajuputi” tree. Once in the past, it is known as *Melaleuca leucadendron (L.)* [3].

The family *Melaleuca* includes no less than 100 species, for the most part trees and shrubs. They are appropriated all through Australia, the islands of Southeast Asia and the nations of the Indochinese peninsula. The leaves contain essential oils and serve for their production, as for the example of 'tea tree oil'- for the most part *Melaleuca alternifolia*-or of cajuputi oil (Melaleuca cajuputi Powell). The taxonomy problems issue of the last tree has been quickly examined by [4]. Malaysian articulation 'cajuputi’ is because of the color of the bark of this tree, signifying 'white wood'. *Melaleuca cajuputi* Powell is native to Peninsular Malaysia. [5]

On a more extensive scale, and all through different countries, *Melaleuca cajuputi* leaves are extracted in order to produce cajuputi essential oil. It is fundamental oil utilized range of personal hygiene products as well as shampoo, fragrance and skin analgesic. Cajuputi oil is additionally utilized as a part of herbal medicinal products to relieve symptoms of coughs, colds, as a laxative and general muscle relaxant and sedative [6]. Beyond this, it has been demonstrated that cajuputi oil is a solid disinfectant against micro bacteria [7]. It also and can be utilized as a mosquito and termite deterrent [8]. Cajuputi oil that is produced using *Melaleuca cajuputi* Powell is better quality and achieves a higher price than that separated from eucalyptus leaves [7].

Underlying observation recommend that Gelam can adjust well in various biological systems of Terengganu. Research demonstrated that the histology *Melaleuca cajuputi* Powell diverse in various place of coastal part of Terengganu due to natural regeneration ability in different sites in responses to different ecosystems in Terengganu particularly morphological and anatomical changes. *Melaleuca cajuputi* Powell histology result shows anatomies of Gelam are variable between several sites. Histology of Gelam from two unique destinations is varied. Although, this plant grows across a wide range of sites characterized by soil moisture regime, such as semi-arid and swamp, the current distribution indicates that they have the ability to adapt to a wide range of climates [9]. There are two main interconnected *Melaleuca cajuputi* forest in Terengganu, which is mangrove and wetland. Both of them are fundamental ecosystem that support huge biodiversity for socio-economic activities and play major roles in maintaining ecological function [10-12]. *Melaleuca cajuputi* are lenient and develop well under a series of environmental conditions including those usually related with unfavorable to growth together with acidic soil, saline soil, flooded soil and even dry lands [13]. While it is tolerant to and can exist next to brackish and saline water [6] it flourishes best in peat lands, waterlogged lowlands and other flooded areas, particularly swamps area. While drier surroundings are not an obstacle to growth, plants tend to evolve stunted and adopt curved structures.

2. METHODOLOGY

2.1. Identification of sampling Area.

Gelam (*Melaleuca cajuputi* Powell) is a monospecific, lesser-known commercial timbers mostly occur in swamp forest behind beaches and mangroves. *Melaleuca cajuputi* forest, even though in a small proportion in large areas, it is found abundance fringing behind the mangroves in the north eastern part of Terengganu, a state that resides in the East Coast of Peninsular Malaysia [14]. Determination of sampling point based is based on abundance of the peat swamp forest data using GIS and likelihood of *Melaleuca cajuputi* trees to grow at the peat swamp area in Terengganu Coastal Area. Fig. 1 shows the 10 sampling area were being picked to observe the variation of the essential oil of the species after being extracted.

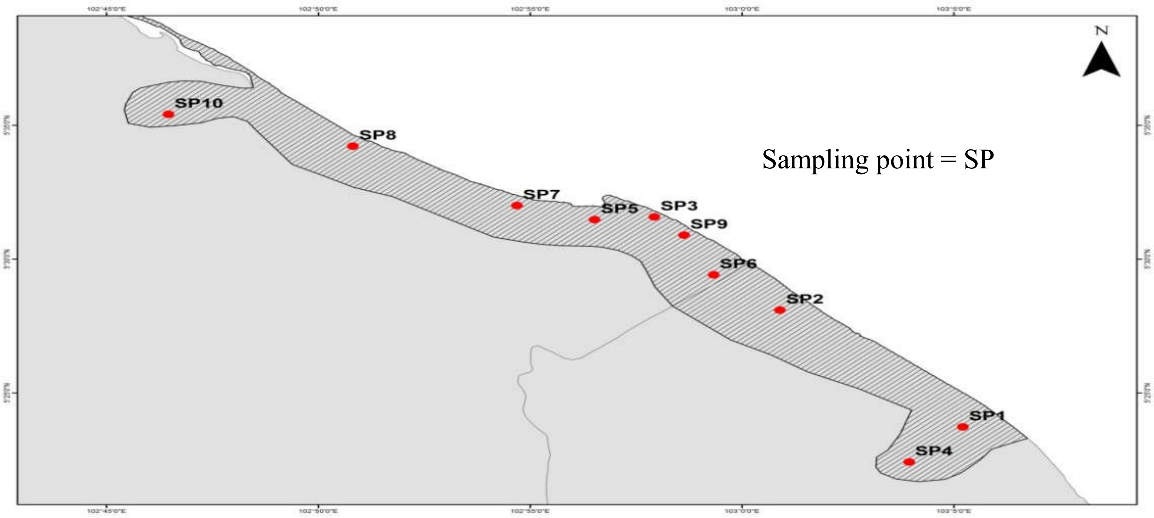

Fig.1. Location of sampling point at coastal Terengganu area

2.2. Steam Distillation

By definition, essential oils are volatile oils, normally odorous which take place in certain plants or specified parts of plants and by the process of extracting it, it clearly specifies that nature and composition of the essential oil must be unchanged. Steam distillation is an appropriate method of extraction for *Melaleuca cajuputi* Powell because of its nature that does not altered or changes the original composition of the essential oils. Furthermore, because steam distillation has been the extraction technique since the oil was first produced, the market accepts steam distilled oil as normal tea tree oil. Oil produce by another technique might be off to some extent have different chemical composition and therefore might not be acknowledged by the market as normal tea tree oil [15]. The yield of the essential oil normally ranges from 0.3% to 0.6% for each extraction. Samples from 10 stations were prepared from dry leaves and small branches by steam distillation for 4 hour for each extraction [16]. This constantly 4 hours extraction has enabled to segregate and generate essential oil from subjected samples, made up with a Clevenger type apparatuses which were comprised with 5000ml round bottomed distillation flask, oil separator tube and condenser. Fresh leaves were left to dry at room temperature and dried leaves were collected and grounded to a small particles to maximize the essential oil production. Samples were then transferred into the distillation flask and weigh were recorded. Samples then covered with distilled water and heated at 100˚ C. The samples allowed boiling slowly until distillation process was completed [17].

2.3. ATR-FTIR Spectroscopic Data

Spectroscopic information was gotten from ATR-FTIR analysis spectra information as it was a basic, fast and precise spectroscopy method that has been connected for multivariate analysis for plant extraction in deciding the vibration functional groups and polar compounds [18]. Using a Perkin Elmer spectrum 400 infrared (IR) spectroscopes, the spectra were recorded and obtained coupled with air-cooled deuterated triglycine sulfate. Instrumental noise region of spectra (400-539 cm^{-1}) was then cut off due to the numerical data generate by the noise. As for the plant studies, only near-IR and mid-IR were been widely used [18]. In this study, FT-MIR (mid-IR) was employed for samples analysis. The spectra were baseline corrected by auto correction option using *Spectrum* (PerkinElmer, Inc.) software in order to minimize the differences between spectra due to baseline shift. Then, the spectra files were saved and exported as a Spectrum.SP file and were imported into *The Unscrambler X* 10.1 (CAMO, Trondheim, Norway) where the wave number of total spectra was transformed into mathematical data in transmittance and reflectance reading. The datasets of numerical were normalized using maximum normalization option in *The Unscrambler X* 10.1 (CAMO, Trondheim, Norway) software, in order to set the normality. The normalized datasets were then rotated using *spectroscopic* option in *The Unscrambler X* software on purpose to alter the transmittance and reflectance to absorbance reading.

2.4 Statistical Analysis

Spectroscopy procedures have been connected for phytochemical recognition and give the significant data with respect to the qualitative and quantitative essential oil chemical compound on top of their pattern recognition by chemometric analysis [19-20]. Multiple Linear Regressions (MLR) is used in modeling data [21]. This technique was been used for investigating the relationship among various independent and dependent variables by fitting a linear equation to observed data [22-23]. In this study, it was used to justify the relationship between the fingerprinting of the essential oil based on the spatial variations. Multiple linear regressions are statistical technique that allows us to predict the variability between the independent variable and the dependent variable [24-25]. The quantity R called the linear correlation coefficient measures the strength and the direction of a linear relationship between two variables. The linear correlation coefficient it sometimes referred to as the Pearson product moment correlation coefficient in honour of its developer Karl Pearson. The mathematical equation for computing R is (Equation (1)):

$$R = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}} \quad (1)$$

The value of R is ranges between $-1 \leq R \leq +1$. The positive and negative signs used for positive linear correlations and negative linear correlations respectively. If x and y have a strong positive linear correlation, R is close to +1. An R value of exactly +1 indicates a perfect positive fit. Positive values indicate a relationship between x and y variables such as values for x increases, values for y also increase. While, if x and y have a strong negative linear correlation, R is close to -1. An R value of exactly -1 indicates a perfect negative fit. Negative values indicate a relationship between x and y such that as values for x increases, value for y also decrease [26].

3. RESULTS AND DISCUSSION

3.1. Assignments and Comparison of the ATR-FTIR Spectra

ATR-FTIR Fingerprinting ATR-FTIR is a simple, rapid, high resolution, reliable and non-destructive spectroscopy analytical techniques which also do not require sample pre-treatment prior analysis [27]. FTIR analysis was based on the analysis of the vibrations of functional groups and highly polar bonds of the components or polar compounds [18]. According to [28], FTIR is a physico-chemical analytical technique which provides a capture of the metabolite composition of a tissue at a given time, instead of determining the concentrations of individual metabolites [29]. This mean that, the overlapping signal of vibrations of bonds within chemical functional groups from a majority or mixtures of compounds produces a spectrum so-called as biochemical profile or metabolic fingerprint of the sample [8, 29]. In herbal analysis, FTIR has been used as a requisite method to identify medicines in Pharmacopoeia of many countries [30].

As for *Melaleuca cajuputi* essential oils, the descriptive statistics of FTIR analysis for 10 different sampling stations are summarized in Table 1 and Box and whisker plots of 10 stations of *Melaleuca cajuputi* essential oils are shown in Fig. 2. From the box and whisker plot shown in Fig. 2, there are variations of fingerprinting between the sampling stations in

Terengganu even though all the samples have similar physical characteristic and similar essential oils produce. Sampling point 7 (99.1) and sampling point 8 (97.9) have higher average value of transmittance percentage compare to the sampling point 1 (89.9) and sampling point 10 (89.2). The highest value for percentage transmittance recorded is from sampling point 7 with 115.1. Sampling point 3, 4, 5 and 6 have smaller variation of percentage transmittance for the essential oil with (97.4, 96.7, 94.0) respectively. Through the general observation, the value of percentage transmittance towards essential oils of *Melaleuca cajuputi* have several variations towards the sampling location of *Melaleuca cajuputi* species based on the descriptive statistic and illustrated by the box and whisker plot shown on Fig. 2.

Table 1. Descriptive statistic of FTIR essential oil

Statistic	SP 1	SP 2	SP 3	SP 4	SP 5	SP 6	SP 7	SP 8	SP 9	SP 10
No. of observations	1795	1795	1795	1795	1795	1795	1795	1795	1795	1795
Minimum	74.1	77.3	84.7	84.7	77.8	85.1	84.5	83.9	79.6	74.8
Maximum	103.8	99.7	102.8	102.0	99.2	99.9	115.1	110.0	102.8	99.3
Mean	89.9	91.5	97.4	96.7	94.0	95.4	99.1	97.9	95.1	89.2
Variance (n-1)	40.0	17.1	12.8	13.7	17.0	7.3	47.3	31.3	23.1	28.6
Standard deviation (n-1)	6.3	4.1	3.6	3.7	4.1	2.7	6.9	5.6	4.8	5.3

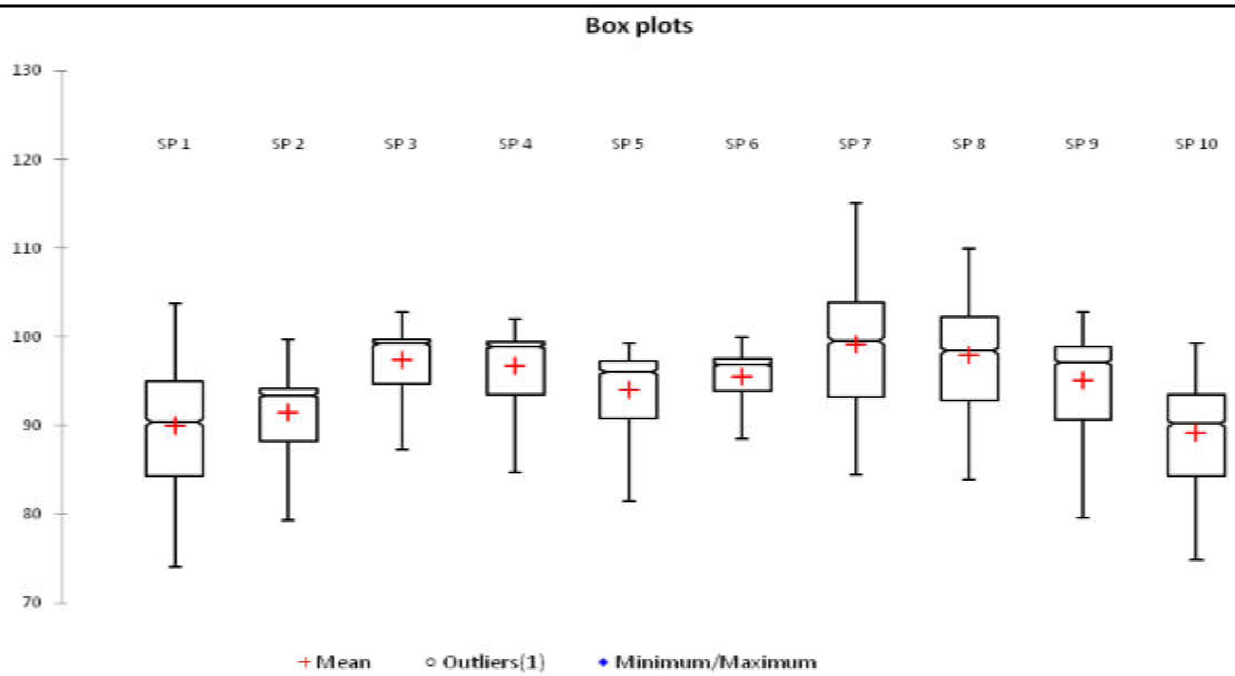

Fig.2. Essential oil fingerprint according to sampling station

The Fourier infrared spectra of 10 distinctive variations of *Melaleuca cajuputi* essential oils were specifically gathered by ATR-FTIR and closely look towards the patterns of the spectra due to the variations of percentage transmittance from the Fig. 2. The ordinary FTIR spectra for the essential oil extracts of various areas of *Melaleuca cajuputi* are appeared in Fig. 3. Through the general observation, the spectra of all samples appear an impression of being fundamentally similar. Between the variations, the quantity of the number of peaks was generally more or less similar as well as for their intensity of the peaks. Nevertheless, despite the resemblance pattern of the peaks, there were a few peaks that are slightly significance with other variations. On top of that, the intensity varied greatly within varieties which the most significant peaks can be viewed in both spectrum types. It was discovered that *Melaleuca cajuputi* essential oils extracts showed slightly significant fingerprints FTIR peaks that are attributed to stretching and bending vibrations that characterize the functional groups.

FTIR peaks are attributed to stretching and bending vibrations that characterize the functional groups. As shown in Fig. 3. FTIR spectra of the essential oil extractions, there are 11 major peaks exist and it was similar for all of the variations. The most stable peaks are the peaks at 3400-3200 cm^{-1} are assigned to alcohol and hydroxyl stretch. Specific peaks such as 3412 cm^{-1} shows normal "polymeric" OH stretching, the peaks at 2970-2950 cm^{-1} are assigned to the peak at approximately 2964 cm^{-1} is the asymmetric stretching of methyl and the peak approximately at 2918 cm^{-1} assigned to the asymmetric stretching of methylene. In addition, the peaks at 2865- 2845 cm^{-1} are assigned to the peak at approximately 2852 cm^{-1} is the symmetric stretching of methylene (V_{as} CH_2) [31-32]. The peak at 1882- 1660 cm^{-1} in FTIR fingerprint attributes shows bands of aromatic combinations. The peaks at 1750- 1735 cm^{-1} are assigned to the C=C stretching vibration. Furthermore, the peaks at range 1410-1310 cm^{-1} at specific peaks of 1367 is assigned to phenol or tertiary, OH bending. Peaks at 1380-1350 cm^{-1} attribute to asymmetric stretching of aliphatic nitro compounds at specific peaks 1383 cm^{-1}. Meanwhile, peaks assigned from 1100- 1150 cm^{-1} at specific peaks 1130 is assigned for secondary alcohol C-O stretching and specific peaks of 1124 cm^{-1} also for secondary alcohol stretching. As for assignments peaks from 1225- 950 cm^{-1}, its attribute is for aromatic ring stretching as significant peak at 1205 cm^{-1} assign for aromatic C-H in plane bending vibrations in aromatic rings. Besides that, peaks at 915-890 cm^{-1} are assigned to absorption of polynuclear aromatic results from out-of-plane C-H bending vibrations of a ring hydrogen atom which strongly coupled to adjacent hydrogen atoms as well as the vinyl C-H out of plane bending vibrations at approximately peaks of 916 cm^{-1}. There are many other spatially related scenarios that tend to follow well-orchestrated patterns, examples being in-plane and out-of-plane vibrations, the differences between *cis* and *trans* spatial relationships and a variety of multicenter vibrations that are defined as twisting or rocking modes [32].

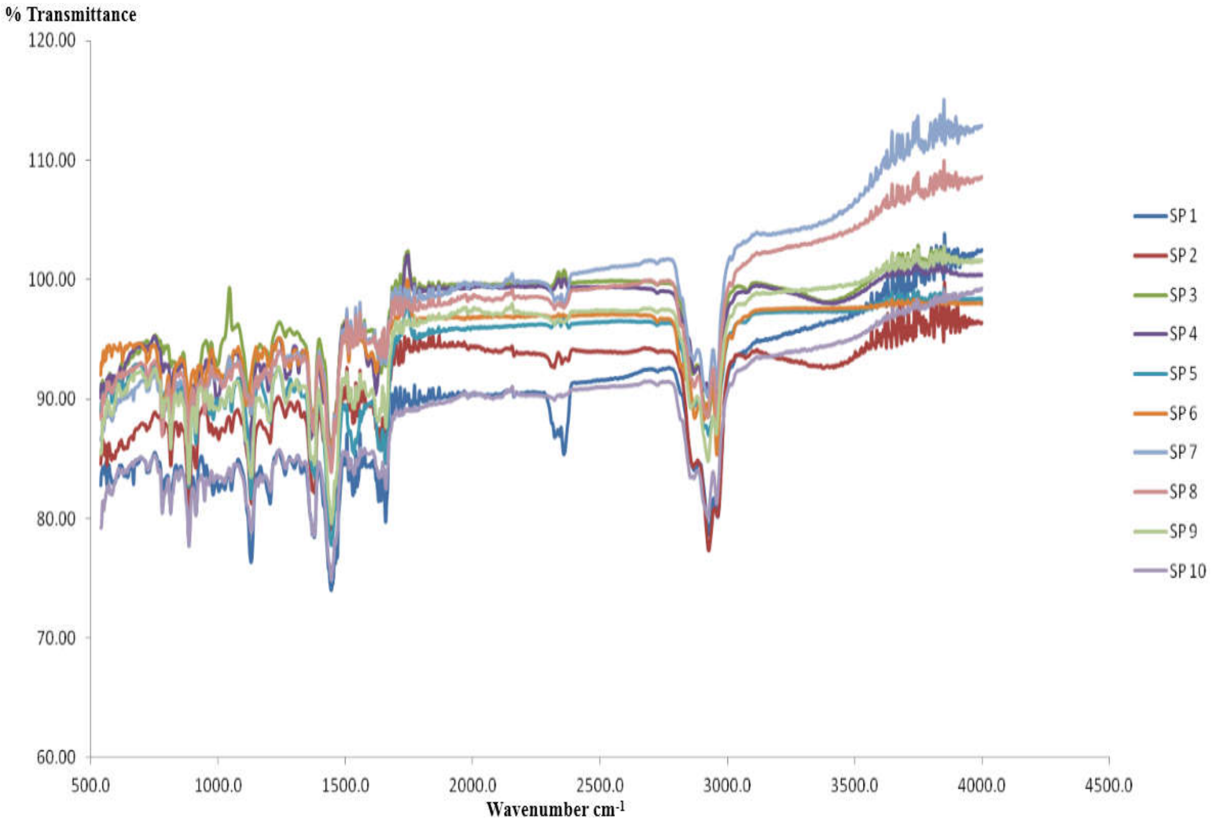

Fig.3. Essential oil FTIR spectra

In addition, Table 2 shows that the correlation between all the 10 sampling point of the *Melaleuca cajuputi* essential oils. From the correlation table below, there is strong correlation fingerprinting relationship between *Melaleuca cajuputi* essential oil produce in sampling point 1, 7 and 8 with (R = 0.976 and 0.982). Fig. 4 illustrated the relationship graph of *Melaleuca cajuputi* essential oil fingerprint between the sampling point of 1, 7 and 8. Table also shows week correlations between sampling point 1 to sampling point 2, 3, 4 and 6 with R-value (0.871, 0.861, 0.857 and 0.834) respectively. As for *Melaleuca cajuputi* from sampling point 3, 4 and 9, there is also strong correlation between the fingerprinting of the essential oil with (R = 0.962 and 0.941) close to the 1 value and illustrated by the Fig. 5. The pattern of the graph shows similar pattern with strong relationship between *Melaleuca cajuputi* in sampling point 3 towards sampling point 4 and 9, even though the place of the sampling are difference area.

As for Fig. 6, graphs shows the similar pattern of fingerprinting of *Melaleuca cajuputi* essential oil, due to the strong correlation and relationship between the sampling point 1, 8 and 10 with R-value (0.982 and 0.987) respectively. This spatial variation and strong correlation between different sampling station in Terengganu for same species of *Melaleuca* may be factorize due to the different location and variation of ecosystem at the sampling point of *Melaleuca* species.

This variation of essential oil fingerprint for *Melaleuca cajuputi* species is support by [33], that shows histological observation of Gelam *(Melaleuca cajuputi* Powell*)* was varied in different ecosystems in Terengganu, especially morphological and anatomical change that contribute towards the variation of essential oil fingerprint in *Melaleuca cajuputi* Powell. Current distribution also indicates that *Melaleuca cajuputi* have the ability to adapt to a wide range of climates [9].

Table 2. Correlation of essential oils between sampling station

Variables	**SP 2**	**SP 3**	**SP 4**	**SP 5**	**SP 6**	**SP 7**	**SP 8**	**SP 9**	**SP 10**	**SP 1**
SP 2	1									
SP 3	0.976	1								
SP 4	0.945	0.962	1							
SP 5	0.91	0.922	0.928	1						
SP 6	0.915	0.906	0.924	0.898	1					
SP 7	0.848	0.858	0.847	0.849	0.808	1				
SP 8	0.872	0.873	0.868	0.876	0.861	0.992	1			
SP 9	0.939	0.941	0.951	0.969	0.915	0.935	0.955	1		
SP 10	0.891	0.897	0.894	0.92	0.871	0.984	0.993	0.978	1	
SP 1	0.871	0.861	0.857	0.903	0.834	0.976	0.982	0.959	0.987	1

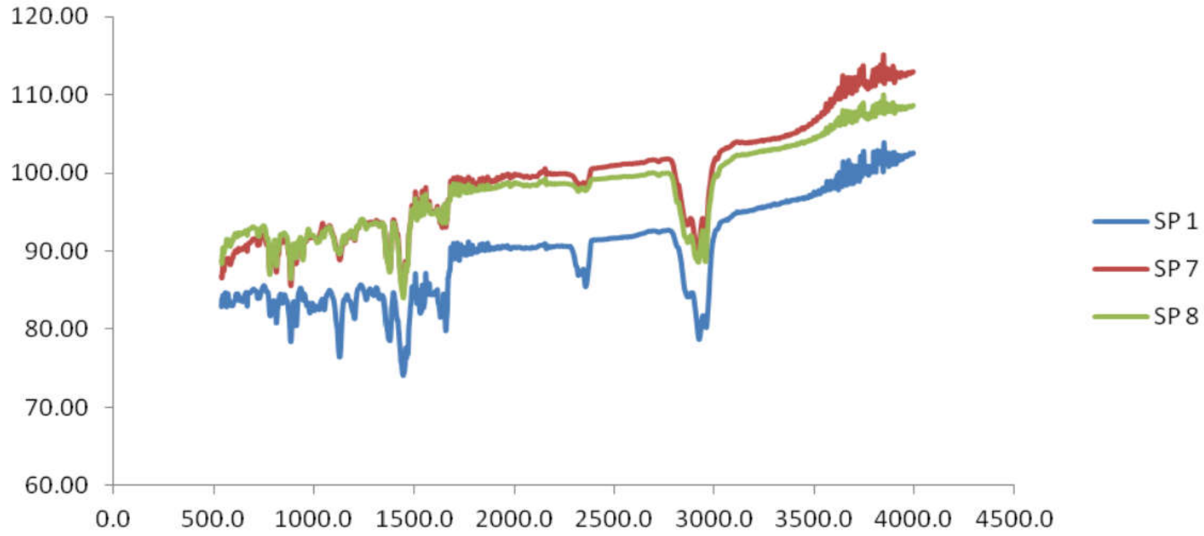

Fig.4. The relationship graph of *Melaleuca cajuputi* essential oil fingerprint between the sampling point of 1, 7 and 8

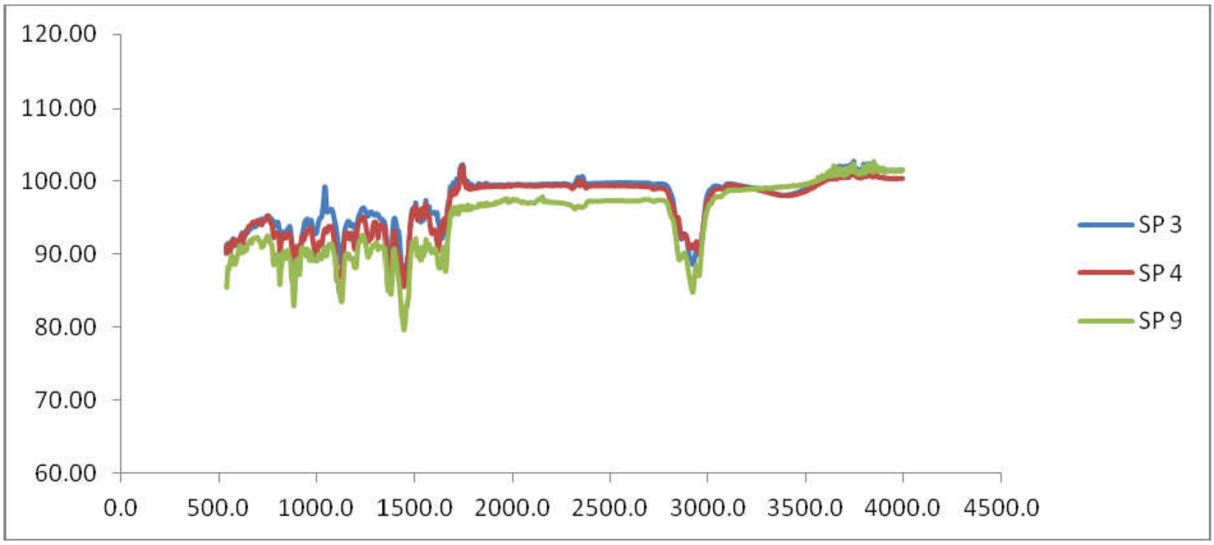

Fig.5. *Melaleuca cajuputi* from sampling point 3, 4 and 9

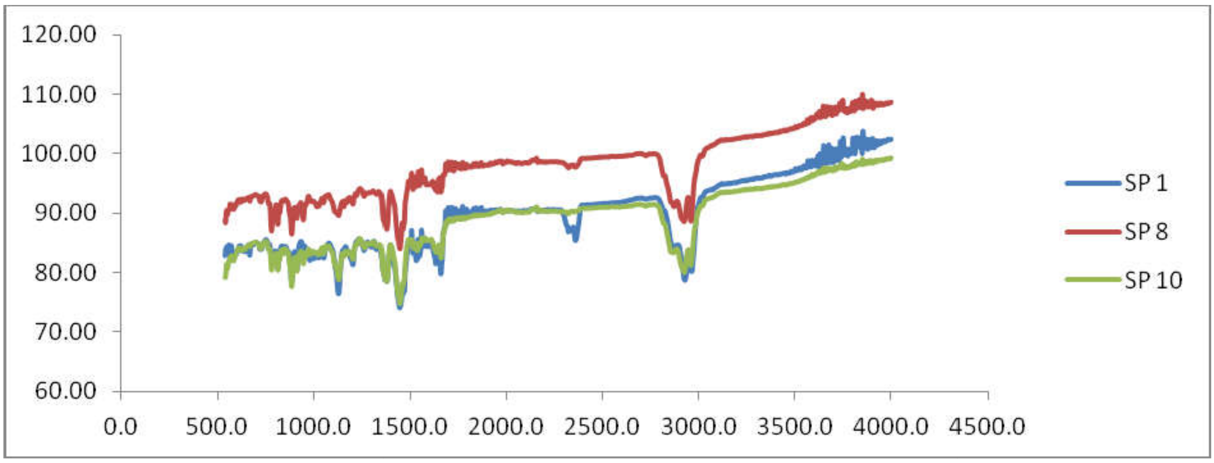

Fig.6. pattern of fingerprinting of *Melaleuca cajuputi* essential oil due to the strong correlation and relationship between the sampling point 1, 8 and 10

However, even the *Melaleuca cajuputi* essential oil fingerprint have strong correlation relationship between them because of the same species and almost similar pattern of the fingerprint, there are still significant difference of characteristic of the essential oils based on the spatial and places of the sampling point. Table 3 shows the p-value of the essential oil fingerprint compare towards the different locations of the sampling point. From Table 3, essential oil fingerprint from sampling point 1, have 7 significant different of essential oil fingerprint with p-value < 0.01, sampling point 2 have 8 different location with p-value < 0.01, essential oil from sampling point 3 have only 6 location that are significant different with p-value is less than 0.01, whereas sampling point 4 only have 2 essential oil fingerprint that have significant different from the sample which is fingerprint form sampling point 2 and 3. As for essential oil fingerprint for sampling location 5, there are 8 sampling locations that have p-value less than 0.01 showing significant different of the fingerprint. In addition, sampling point 6 essential oil fingerprint shows it has 7 different location showing significant different with its fingerprint with sample from sampling location 7 have 6 significant difference of location in its characteristic of the fingerprint. Furthermore, sampling point 8 fingerprints have 7 different location showing p-value less than 0.01 and as for sampling point 9, it had almost all significant different of fingerprint print except from sampling point 4 and lastly for the sampling point 10, it have 6 different locations with p-value less than 0.01 with 99% confidence interval. Even though fingerprint from all the sampling locations showing similar pattern and have strong correlation between the location, the behaviour of the carbon in the essential oil gives the significant edges of on fingerprint from one location to another. From Fig. 3, different location of the peak represents the different behaviour and characteristic of the functional group and carbon inside the essential oils of the *Melaleuca cajuputi* species. This specific behaviour of the carbon are the main factor that contribute towards the significant different of the essential oil based on different locations. Hence, from this study, it shows that essential oils of *Melaleuca cajuputi* from various locations have spatially variations and significant difference from each other.

Table 3. p-value of essential oil between sampling stations

Station/ y-dependent	SP 1	SP 2	SP 3	SP 4	SP 5	SP 6	SP 7	SP 8	SP 9	SP 10
SP 1										
SP 2	< 0.0001									
SP 3	< 0.0001	< 0.0001								
SP 4	0.029	< 0.0001	< 0.0001							
SP 5	< 0.0001	< 0.0001	< 0.0001	-0.506						
SP 6	< 0.0001	< 0.0001	0.000	0.684	< 0.0001					
SP 7	0.097	0.001	< 0.0001	0.617	< 0.0001	< 0.0001				
SP 8	< 0.0001	< 0.0001	0.742	-1.412	< 0.0001	< 0.0001	< 0.0001			
SP 9	< 0.0001	< 0.0001	< 0.0001	1.180	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
SP 10	< 0.0001	< 0.0001	0.035	-0.322	< 0.0001	< 0.0001	0.000	< 0.0001	< 0.0001	

4. CONCLUSION

The research showed that the essential oil extract from *Melaleuca cajuputi* Powell species in different sites of coastal part of Terengganu have spatially variations and significantly difference from one location of the sample to another. ATR-FTIR result shows the characteristic and the pattern of the *Melaleuca cajuputi* fingerprint have strong correlation with one location to another location but the different behavior of functional groups and carbons contain in the essential oil extract from the *Melaleuca cajuputi*. It gives the significant edge of spatially differences of the *Melaleuca cajuputi* Powell. This observation may suggest that Gelam can adapt well in different micro-ecosystems of Terengganu with different characters of the functional group behaviour will produce variations of essential oil from the *Melaleuca cajuputi* Powell. The tree has the ability to response well toward Terengganu's different micro-climates and coastal environmental changes and the finding is essential to increase baseline knowledge of flora presence at the study area.

In conclusion, *Melaleuca cajuputi* Powell species have tendency to produce spatially variations of essential oils depending on the micro-ecosystem of the sample locations being extracted.

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