Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(12); 1986-1992

ISSN: 0975-4873

Research Article

Chemical Composition in Different Tissues of *Polygonum minus* by Using GC X GC-TOF MS and Direct Discrimination by Multivariate Analysis of Fourier Transform Infrared Spectroscopy Data

Ahmad R, Bunawan H, Normah M N, Baharum S N*

Metabolomics Research Laboratory, Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

Available Online: 15th December, 2016

ABSTRACT

Volatile compounds in different tissues of *Polygonum minus* (leaves, stems and roots) were isolated using the Solid Phase Microextraction (SPME) technique, and analysed by two-dimensional gas chromatography, GC x GC- TOF MS in the hope of identifying more minor components in the tissues. In total, 55 compounds were identified in the samples, of which 32 were terpene compounds. The overall content of each compound was different in the leaves, stems and roots. The aerial parts (leaves and stems) were relatively rich in terpenoids, whereas the roots were relatively rich in organic acids. The spectral studies of the tissues were done using Fourier transform infrared spectroscopy (FTIR) to further correlate with its chemical constituents of the samples. The principal component analysis (PCA) was used for the discrimination of the different tissues by using their marker bands. With this technique, the different tissues could be well distinguished by this rapid method with the exception of the chromatographic technique and could be further developed as a routine test in food industry.

Keywords: *Polygonum minus*; volatile compound; different tissues; solid-phase microextractions; gas chromatography; Fourier Transform Infrared Spectroscopy (FTIR).

INTRODUCTION

The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Traditional medicine from herbs is getting more attention all over the world due to its pharmacology properties. One of an interesting herb to highlight in Malaysia is *Polygonum minus* (Polygonacea). It is an important aromatic plant in Malaysia and is widely used in Southeast Asia as flavouring ingredient in food and folk medicines. Traditionally, it has been used to treat digestive disorders, reduce dandruff and as a treatment for poor eyesight[1]. Several researchers have reported that *P. minus* Huds contains high levels of antioxidants and phenolic compounds which contain several number of bioactive properties[2,3]. Most recently, extracts from *P.minus* have proved of antimicrobial activity and anticholinesterase activity[4]. In India, *Polygonum* sp., has been used traditionally to treat diuretic, CNS stimulant, diaphoretic, stomachic, styptic, in bleeding and in diarrhea[5]. Other species of the Polygonaceae family have been reported for their effectiveness in cerebral ischemia[6], Parkinson's disease[7] and neuroprotective effects[8]. The volatile compound such as geraniol and farnesene are found responsible for the fragrance and have pharmacological effects as mentioned above. Metabolite profiling is a promising approach that differentiates genotype based on metabolites level that may or may not produce visible phenotypes[9]. Different parts of the plant could be well distinguished by taking into account their specific metabolite. Phytochemical studies of *P.minus* has not much been reported. There are only a few reports available on its chemical constituents. A previous study by Baharum[10], and Yaacob[11] described the composition of the essential oil in *P. minus* leaves. The chemical composition of *P.minus* from other tissues has been reported recently by Ahmad et al.[4] by Gas-Chromatography Mass Spectrometry technique. GC-MS is able to determine the presence of volatile compound, however it has some limitation in separating closely-eluting compound. On the other hand, with comprehensive two dimensional gas chromatography GC x GC, it has become a powerful tool in unravelling the composition of complex samples[12]. Therefore, the aim of this study was to profile the volatile compounds found in the leaves, stems and roots of the widely used medicinal plant, *P. minus*, using a solid phase microextraction (SPME) and analysed by GC x GC-TOF MS. In this study, SPME was combined with GC x GC-TOF MS because of its well-known capability in the analysis of volatile constituents. The SPME technique, which reduces chemical changes in analytes and artefact formation, has been recognised as a powerful, solvent-free method that is suitable for the analysis of the volatile constituents found in plant materials[13,14]. In order to achieve our aim of this studies, FTIR have to be done prior

*Author for Correspondence: nataqain@ukm.edu.my

to metabolite profiling analysis. FTIR studies will give information on the contents of chemical compounds based on the variations of the spectra. Furthermore, by interpreting the characteristic peaks of chemical function groups, the main chemical compound with the highest amount could be revealed[15,16]. That is the main reason why we choose to do FTIR and later focusing on metabolite profiling analysis, to get an idea of the chemical constituents in different tissues. With FTIR results, it could be further relate with its chemical composition later in metabolite profiling analysis. A part from the aforementioned, authentication process of the raw materials (leaf, stem and roots) could be done efficiently with faster and cost effective verification method. Therefore, we also undertake this challenge to develop a rapid quality verification method with the integration of statistical modeling for extracting relevant information base on infrared spectroscopic data. This is very important for future manufacturing process.

MATERIALS AND METHODS

Samples

Leaf, stem and root samples from *P. minus* were collected from the INBIOSIS experimental plot on December 2011. Samples were originally collected from Ulu Yam, Malaysia and the voucher specimen was deposited in the UKMB Herbarium, National University of Malaysia. These were identified by taxonomist and further confirmed using ITS sequences[17]. Samples washed and stored at −80 °C.

ATR-FTIR spectral measurements

Freeze- dried leaf, stem and root tissues were milled into a fine powder and placed directly on the germanium piece of the infrared spectrometer with constant pressure applied. The pressure of the ATR-FTIR acquisition was 70 ± 2 psi. The leaves were first freeze-dried to avoid the interfering absorbance in the spectrum caused by water content. The samples were then analysed by attenuated total reflection FTIR (ATR-FTIR). The FTIR spectra were recorded in the mid-IR region (4000–650 cm^{-1}) at resolutions of 4 cm^{-1} with 32 scans using Perkin Elmer FTIR Frontier coupled with DTGS (deuterated tri-glycine sulphate) detector (Perkin-Elmer Inc., Norwalk, CT, USA). Air background spectrum was recorded before each sample and all experiment were performed at least 3 replicates. Prior to data analysis, each spectrum was baseline corrected and the absorbance was normalized so that peak absorbance of the most intense band is set to unity. ATR spectra were statistically analysed by PCA using SIMCA-P+ software (Umetrics, Sweden).

Principle Component Analysis (PCA)

All the peaks and spectra from each sample were combined into a single peak table and transferred into Microsoft Excel 2007. After a filtering step, the table containing the total counts was imported into SIMCA-P+ 12.0 software (Umetrics, Sweden) for multivariate analysis (PCA). Scaling using the square root of the standard deviation (pareto scaling) was performed during the analysis. The PCA output consisted of score plots in order to visualize the contrast between different samples.

Sample preparation for Solid Phase Microextraction (SPME) conditions

Prior to analysis of the volatile compounds, the samples were ground, using a mortar and pestle into a fine powder. The samples were weighted 0.3g and sealed with a 20 ml headspace glass vial (flat bottom, 100pk, Perkin Elmer, USA using a PTFE/silicon septum 200 mm.

Solid Phase Microextraction (SPME) conditions

The Solid Phase Microextraction (Supelco) method was undertaken using 100 μm PDMS (Polydimethylsiloxane) fiber. The fiber and the manual SPME holder were purchased from Supelco (Bellefonte, PA, USA). The fiber was conditioned prior to use, according to the manufacturer's instructions. About 300 mg of fresh leaves were ground with liquid nitrogen and placed in a 20 ml vial. 700 ul of distilled water was added to the ground leaves and the vial was covered tightly using a hole cap with septum to ensure no volatile could escape during the extraction. The fiber was then exposed to the sample headspace by inserting the fiber through the septum and the vial with the exposed fiber was e incubated in a water bath at 45°C for 15 minutes. After 15 minutes, the fiber was thermally desorbed by inserting the fiber into GC injector at 250°C for 10 minutes. At least 3 replicates were used for each analysis.

GC x GC-TOF MS analysis of volatile compounds

The comprehensive two-dimensional gas chromatograph system employed consisted of an Agilent 6890N GC equipped with a flame ionization detector (Agilent) and filled with a cold-jet modulator KT-2007 retrofit prototype (Zoex Corporation, USA). A time-of-flight mass spectrometer (Pegasus 4D, LECO Corporation), equipped with an Agilent 6890N GC, was used to acquire the mass spectra data. The MS parameters included a 70 eV electron impact ionization value and a maximum spectral acquisition rate of 500 spectra per second. Two capillary columns, connected by a universal press-tight connector, were installed in the same oven. The samples were analysed according to Baharum et al. 2010. Features of the GC×GC column sets. Column 1: 30mm x 0.25mm x 0.25μm, Rtx-5MS (Restek Corporation, Bellefonte PA); Column 2: 1mm x 0.25mm x 0.25μm, DB-wax (J&W Scientific, Folsom, CA).

RESULTS AND DISCUSSION

The ATR-FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) is a rapid, simple, high-resolution analytical method that is based on the vibrations of functional groups and highly polar bonds in the components analysed. Thus, FTIR provides biochemical profiles containing overlapping signals from a majority of the compounds that are present in a cell when whole cells are analysed. The biochemical profiles from whole cell samples are extremely high density data sets and, consequently, FTIR data must be analysed by means of multivariate analysis when multiple whole cell samples are compared[18-20]. The results of the FTIR fingerprinting of the different tissues are shown in Figure 2. Identification of the functional groups was based on the FTIR peaks attributed to stretching and binding vibrations (Fig. 2).

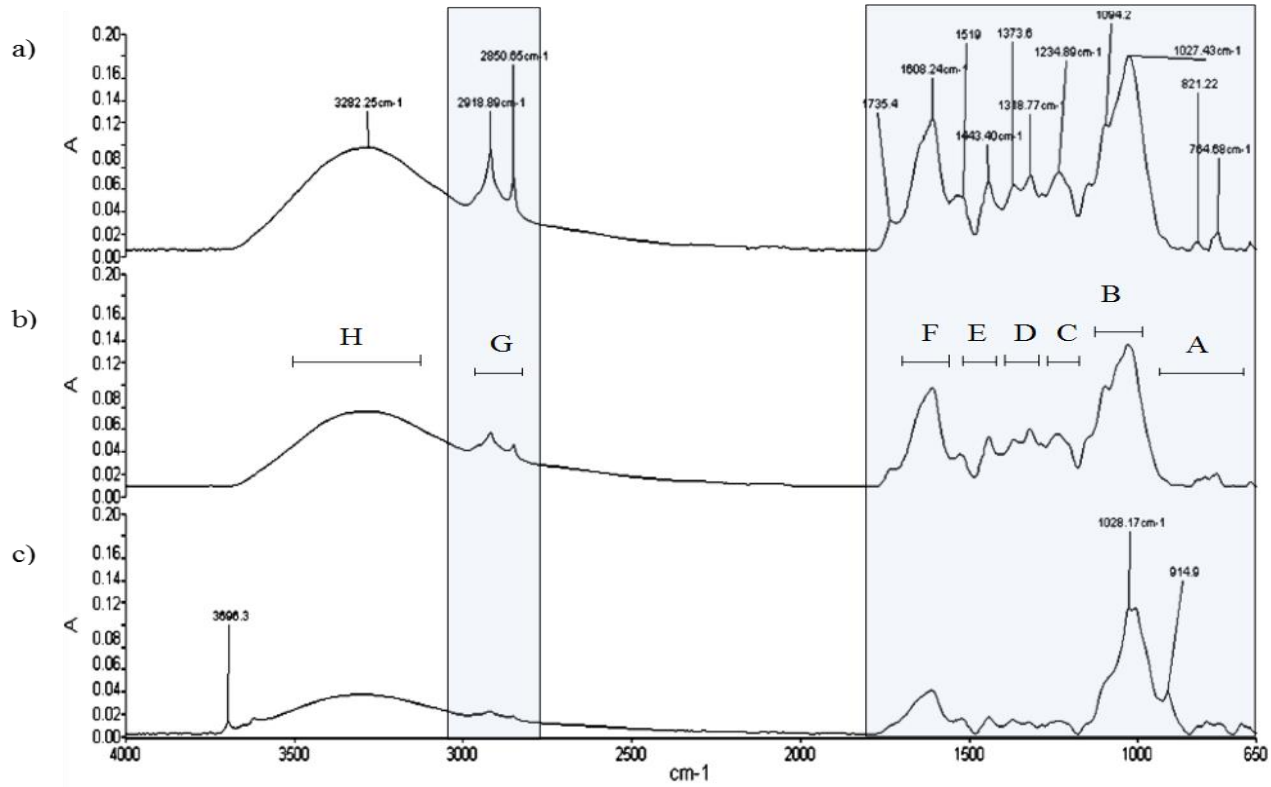

Figure 1: Typical Raw FT-IR Spectra of (a) leaf, (b) stem and (c) root. The highlighted regions showed dissimilarities peak observed in all tissues.

Area A (< 1000 cm^{-1}) corresponded to C-H bending vibrations from isoprenoids; area B (1130–1027 cm^{-1}) to stretching vibrations by polysaccharides and area C (1270–1150 cm^{-1}) corresponded to stretching vibrations due to carbonyl C-O or O-H bending. Area D (1450–1300 cm^{-1}) corresponded to the amide region and C-C stretching by the phenyl group. Area E (1600–1500 cm^{-1}) was assigned to the aromatic domain and N-H bending vibrations (region of proteins). Area F (1760–1600 cm^{-1}) vibrations were assigned to protein groups and C=O stretching (aldehydes, ketones and esters). Area G (2900–2800 cm^{-1}) corresponded to C-H stretching vibrations that were specific to CH3 and CH2, methoxy derivatives and C-H (aldehydes) and Area H (3600–3200 cm^{-1}) vibrations were assigned to stretching of the OH groups (water, alcohols, phenols and carbohydrates)[21]. Differences between tissue biochemical fingerprints are shown in specific absorbance at 2918 cm^{-1}, 2850 cm^{-1}, 1735 cm^{-1}, between 1500 cm^{-1} to 1000 cm^{-1} and between 1000 cm^{-1} to 650 cm^{-1}. Comparison of the two IR spectra of the leaf and stem samples (Fig. 2a and b) revealed that the peaks positions and shapes of the main specific bands in the spectra were quite similar to each other, except for the differences in the intensity and positions of the IR spectra. Such spectra, essentially invisible to the naked eye, illustrate the need to employ multivariate statistical techniques for their analysis. To develop the discrimination model to distinguish between tissues, all mid-FTIR spectra were subjected to SIMCA analysis. Figure 2 shown a scatter plot of the FTIR data generated from SIMCA model. PCA of the FTIR data is displayed in a two-dimensional plot using the first two principal components. At least three replicate samples of each tissue were grouped in discrete clusters, which indicated that PCA was able to discriminate between the different tissues. Figure 2 showed clear separation between tissues. The results of PCAs provided a plot of principal component (PC) scores for the most important PCs (PC1 vs PC2). The first two PCs account for 89.52% of the total variation. The R2 and Q2 values are 0.895 and 0.856. The leaves were separated from the roots by PC1 (76.2%), whereas the roots and leaves were separated from the stems by PC2 (13.2%), as shown in Fig. 3. In future, the different types of tissues will be rapidly monitored by ATR-FTIR/PCA in order to improve the quality control of the *P. minus* samples. These results have shown that FTIR is an effective method for differentiating *P. minus* tissues. The band of interest is the strong band at 2918 cm^{-1} and 2850 cm^{-1}, which were assigned to the C-H and C-H (methoxy compounds) stretching vibrations, respectively, in aliphatic compounds[21]. The C=O stretching mode occurred in the range: 1760 cm^{-1} to 1600 cm^{-1} and was due to carbonyl compounds, such as esters, ketones and aldehydes. The peak occurring at 1610 cm^{-1} to 1653 cm^{-1} represented the C=O stretching mode of the aldehyde group[22]. The bands mentioned above (2918 cm^{-1}, 2850 cm^{-1} and 1610cm^{-1} to 1653 cm^{-1}) all indicated the presence of an aldehyde compound in the samples. The leaves produced the most intense and sharp peaks in the respective bands, followed by the stems and roots. Most of the aqueous carboxylic acids, including hexadecanoic acid and tetradecanoic acid, showed low-intensity absorption in the 3000–1800 cm^{-1} range, which many researchers call a

Table 1: Volatile Compounds identified in leaves, stems and roots of *P.minus* by SPME using two dimensional gas chromatography time-of-flight mass spectrometry GC x GC-TOF MS.

No	Metabolite	RI[f]	t^1R^e (S)	t^2R^e)	% Peak Area[b]		
					Leave	Stems	Roots
	Terpenes (Monoterpene)						
1	α-Pinene[a]	939	576.000	1.280	0.317	0.19270	0.5230
2	Limonene	1030	801.000	1.520	0.041	0.05510	-[d]
3	Linalool	1099	991.002	1.704	0.003	-	-
4	Myrtenal	1233	1256.000	2.392	-	0.00940	0.0493
5	-(z)-Myrtanol	1288	1416.000	2.232	-	0.00710	0.6509
6	Beta-Ocimene	1044	856.002	1.544	0.071	-	-
7	α-Terpinolene	1086	956.000	1.696	-	0.01610	-
8	Borneol	1162	1166.000	2.056	0.098	0.00190	-
9	cis-geraniol	1227	1336.000	1.968	-	0.03000	0.0128
10	Citronellol	1223	1341.000	1.848	-	0.00940	-
11	Geraniol	1249	1406.000	2.016	-	0.07090	-
12	Geranyl acetone	1453	1900.998	2.160	0.037	0.03930	-
	Terpenes (Sesquiterpene)						
13	Copaene	1377	1696.002	1.760	0.138	0.09070	-
14	Germacrene D	1484	1761.000	1.840	0.012	0.40310	-
15	α-Zingiberene	1494	1735.998	1.728	0.286	0.64680	0.1226
16	δ-Elemene	1335	1741.002	1.800	0.131	0.07300	0.1051
17	Isocaryophyllene	1438	1776.000	1.880	0.089	0.18840	0.1609
18	α-Cedrene	1410	1786.002	1.872	0.289	0.01230	-
19	β-Caryophyllene	1467	1825.998	2.032	8.904	16.3200	5.6925
20	Alpha-Bergamotene	1431	1866.000	1.864	1.984	0.52300	1.1401
21	Beta-Farnesene	1445	1906.002	1.872	0.296	1.29780	0.1473
22	cis-α-Bisabolene	1506	1951.002	2.040	0.389	0.90990	-
23	α-Himachalene	1449	2020.998	2.120	0.127	0.13020	-
24	α-Panasinsen	1381	2041.002	2.168	0.461	0.44530	0.0659
25	β-Sesquiphellandrene	1560	2056.002	1.920	0.088	-	0.0358
26	Nerolidol	1539	2146.002	1.976	0.049	0.12230	-
27	Caryophyllene oxide	1573	2185.998	2.400	0.188	0.75840	0.2365
28	Humulene epoxide	1642	2245.998	2.424	0.062	0.36750	0.1089
	Organic Acids						
29	Dodecanoic acid	1564	2166.000	1.928	0.032	0.03290	0.1320
30	Pentanoic acid, decyl ester	933	2305.998	1.728	0.004	0.00160	0.1300
31	Tetradecanoic acid	1720	2575.998	1.984	0.030	0.03070	1.2500
32	Undecanoic acid	1550	2770.998	1.960	0.012	0.01280	0.1127
33	n-Hexadecanoic acid	1946	2961.000	2.040	0.070	0.05010	2.6355
34	Oleic Acid	2141	3265.998	2.240	0.006	0.02640	0.8920
	Aliphatic compounds						
35	Undecane	1101	985.998	1.264	0.142	0.04780	0.0837
36	Nonanal	1104	1006.002	1.728	0.059	0.03680	0.1219
37	Dodecane	1200	1251.000	1.288	0.006	0.00120	
38	Decanal	1209	1296.000	2.096	18.53	1.98480	0.5131
39	1-Decanol	1274	1465.998	1.992	3.157	1.44770	-
40	Pentanal	732	1501.000	1.792	-	0.00210	0.0668
41	Tridecane	1300	1510.998	1.328	0.031	0.01670	0.0135
42	Undecanal	1308	1536.000	1.848	0.280	0.21740	0.0362
43	1-Nonanol	1154	1591.000	1.696	-	0.00140	-
44	Dodecanal	1413	1846.002	2.088	32.34	22.3463	2.2587
45	1-Dodecanol	1469	1960.998	1.944	0.701	0.75920	-
46	Tetradecane	1400	2001.000	1.376	0.019	-	-

[a]As identified by GC-MS Software; names according to NIST mass spectral library.

[b]Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100.

[c]The results are the mean atleast 3 experiments and the data mentioned in parenthess are the corresponding relative standard deviations of the readings,

[d]Not detected or percentage of the component is lower than 0.001%.

[e]t^1R and t^2R is the retention time of peaks on first and second dimension respectively.

[f] Retention index on DB-5

continuum of absorption[23]. It is easily recognized as an acid as it produces a very broad peak between the range: 3300 to 2500 cm^{-1}. The presence of carboxylic acid produced two broad, low intensity bands, which were located near the 2900 and 2580cm^{-1} peaks[24]. In Figure 2, two sharp peaks (2850 cm^{-1} and 2900 cm^{-1}) were observed in the leaf and stem tissues, but almost no peaks (both broad and low intensity peaks) were observed for the root tissues. Root tissues showed low-intensity absorption in the 3000–1800 cm^{-1} range, especially near to 2900 cm^{-1}, which indicated that the concentration of carboxylic acid was high. The main differences between the leaves and stems were observed in the region between 900 cm^{-1} and 700 cm^{-1}. According to Naumann et al.[25], the spectrum between 900 cm^{-1} to 700 cm^{-1} is the true fingerprint, showing some remarkably specific spectral patterns that have yet to be assigned to cellular components or to functional groups. In addition, biomarkers were also commonly found in the carbohydrate fingerprint region (1200–800 cm^{-1}) where differences in cell-wall composition and structure are reflected[26]. Two tiny, but obviously well separated peaks, were observed at 821.22 cm^{-1} and 784.56 cm^{-1} in the leaf samples. However, an overlap peak spectrum was observed in the fingerprint region (900 cm^{-1} to 700 cm^{-1}) for the stem and root samples. These two peaks could be biomarkers for leaf samples. The spectral differences in this region showed the polysaccharide differences in these tissues. The region below 1000 cm^{-1} could also be assigned to the C-H bending vibrations in isoprenoids, which indicated the existence of terpenoids[21]. The IR spectra of the root samples were different to the IR spectra produced by the leaves and stems (Fig. 2). The obvious peak at 914.9 cm^{-1} in the root spectra was a unique peak because the leaves and stems showed no noticeable peaks at 914.9 cm^{-1}. This could be a fingerprint for root samples. The peaks at 2918 cm^{-1}, 2850 cm^{-1}, 1735.4 cm^{-1} and 1094.2 cm^{-1} that were observed in the leaves and stems could not be seen clearly in the root IR spectra. Overall, the root samples displayed distinct peak intensities and positions compared to the leaves and stems.

Volatile compounds detected by SPME, GC x GC-TOF MS

SPME extracts of the volatile compounds present in the leaves, stems and roots were identified by GC x GC-TOF MS as shown in Table 1. SPME with PDMS fiber was choose based on its capability to absorb a wide range of volatile compound with high reproducibility[27]. It is well known that most of flavor analyses are performed with 100µm PDMS fiber. Moreover, PDMS was found to present good sensitivity and stability among the other fiber such as PDMS/DVB and DVB/CAR/PDMS[28]. Due to the complexity of the aroma profiles, rapid analysis using a detector with fast mass spectral acquisition, such as time-of-flight mass spectrometry, is recommended, based on the results of this study. Furthermore, flavor compounds present at minute level concentrations can still play a significant role in the key aroma profile. With the new GC x GC technique, more metabolites could be detected because of its sensitivity due to the combination of two GC columns and re-focusing by a modulator[12]. GC x GC-TOF MS has been used in analysis of volatile compounds from various plants and herbs[29]. Thus, we further analysed the volatile compounds in different tissues with SPME technique combined with GC x GC TOF-MS. In total, 106

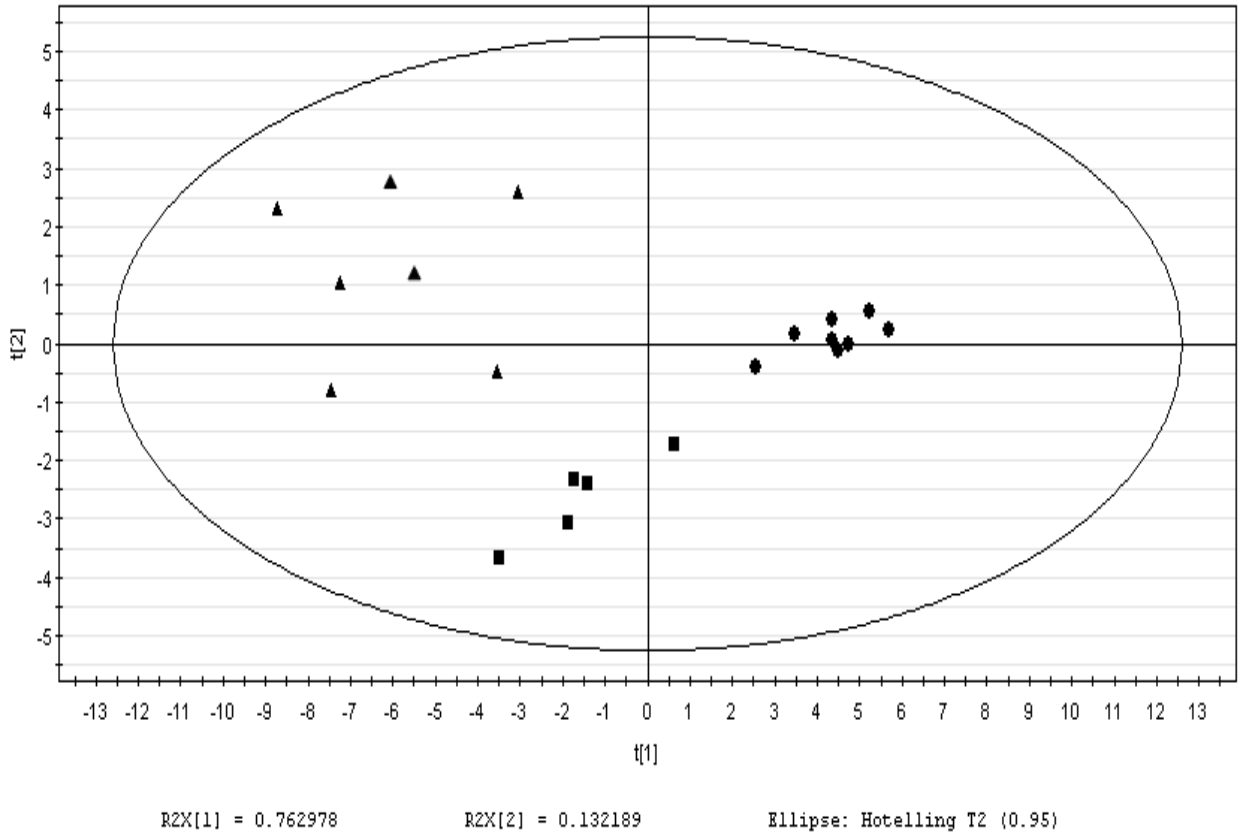

Figure 2: Scatter Plot of FTIR spectra in different tissues of *P.minus.* Leaf (•), stem (■) and root (▲).

compounds were identified and classified into the following groups: 56 terpenes, 11 organic acids and 39 hydrocarbon compounds (data not shown) with similarity index of more than 80%. However, we listed down compound with more than 0.0001% in table 1. The number of terpenes identified with this technique was greater than the number identified using GC-MS. Indeed, the number of terpenes found using this technique was far more than that reported by Baharum et al.[10] and Yaacob[11] both on the essential oil of *P.minus*. This proved that the SPME technique, combined with GC x GC-TOF MS, is a powerful separation and identification tool that can identify a much larger number of complex volatile compounds of the 46 compounds listed, 38 compounds were detected in leaf, 42 compounds in stem and 27 compound in root. As expected, the leaves and stems were rich in terpenoids, whereas the roots contained more organic acids compared to the other tissues. This results correlated well with FTIR findings earlier where from IR spectra, root showed high concentration of organic acid as shown in Table 1. Similar findings were recorded for the most abundant terpenoid compounds using GC-MS[4]. However, -(z)-myrtanol, which could only be detected in the roots by GC-MS, was also found in the stems using GC x GC-TOF MS at low levels. This may be due to the less sensitivity of GC-MS compared to GC x GC-TOF MS. In all compound detected, decanal and dodecanal are the major compound found in leaf (18.53 % and 32.34%) and stem (1.98 % and 22.35 % respectively), similar to previous findings by Baharum[10] and Yaacob[11]. Results from FTIR spectra showed that, the peak intensity for aldehyde group is high in leaves and stems compared to root. Figure 1 showed 2D-GC chromatogram of leaf, stem and root where dodecanal is the major compound in all tissues except root. α-pinene is the highest monoterpene compound detected in leaf and stem (0.317% and 0.1927 % respectively). On the other hand, β-Carryophyllene is the highest sesquiterpene compound detected in leaf, stem and root (8.9 %, 16.32% and 5.69% respectively). By using GC x GC TOF-MS, we are able to detect organic acids in all tissues but at very low level compared to roots. Hexadecanoic and tetradecanoic acid were the highest organic acid found in root (2.64 and 1.25 % respectively). Regarding the differences of compound in different tissues, it is interesting to point out that several environmental related factors have great influence in its chemical compositions. For example, terpenoid was found high amount in leaf and stem due to its photosynthetic activity[32] and exposure to high temperature[33]. Organic acid was found high in root in response to soil stress[34].

CONCLUSION

The first analysis of SPME extracts of volatile compounds for different tissues analysed by GC x GC-TOF MS was successfully done in this study. The SPME technique, combined with GC x GC-TOF MS, identified more metabolites compared to previous studies. PCA analysis of the FTIR spectra, succeeded in differentiating the tissue profiles in *P. minus*. This study has shown that FTIR is a rapid method that could be used to differentiate tissues, based on IR spectra. We believed that, with these findings, it will aid the researcher to understand more on the metabolites in different tissues which might have different compounds that should not be neglected.

ACKNOWLEDGMENTS

This research was supported by Universiti Kebangsaan Malaysia grants (UKM-AP-BPB-14-2009) and Genomics and Molecular Biology Initiative of the Malaysia Genome Institute, Ministry of Science, Technology and Innovation, Malaysia (07-05-MGI-GMB 004). Authors would like to thank Khairunisa Khairudin and Nur Afiqah Sukiran for analytical help.

REFERENCES

1. Zakaria M and Mohad M A. Traditional Malay Medicinal Plants. ITNM 2010.
2. Maizura M, Aminah A and Aida WMW. Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract. International Food Research Journal 2011;18:529-534.
3. Huda-Faujan N, Noriham A, Norrakiah A and Babji A. Antioxidant activity of plants methanolic extracts containing phenolic compounds. African Journal of Biotechnology. 2009;8:484-489.
4. Ahmad R, Baharum S, Bunawan H, Lee M, Normah MN, Rohani E, Ilias N and Zin N. Volatile Profiling of Aromatic Traditional Medicinal Plant, *Polygonum minus* in Different Tissues and Its Biological Activities. Molecules 2014; 19:19220-19242.
5. Sharma R. Medicinal plants of India-An encyclopedia. Daya Publishing House, 110035 Delhi, India. 2013.
6. Chan YC, Wang MF, Chen YC, Yang DY, Lee MS and Cheng FC. Long-term administration of *Polygonum multiflorum Thunb*. reduces cerebral ischemia-induced infarct volume in gerbils. Am. J. Chin. Med. 2013; 31: 71-7.
7. Chen L, Wang Y, Wei L, Shi M and Chan Y. Chinese herbs and herbal extracts for neuroprotection of dopaminergic neurons and potential therapeutic treatment of parkinsons disease. CNS Neurol Disord. Drug Targets 2007;6;273 - 281.
8. Li X, Matsumoto K, Murakami Y, Tezuka Y, Wu Y and Kadota S. Neuroprotective effects of Polygonum multiflorum on nigrostriatal dopaminergic degeneration induced by paraquat and maneb in mice. Pharmacol. Biochem. Behav. 2005;82:345 - 352.
9. Fiehn O. Metabolomics--the link between genotypes and phenotypes. *Plant Mol. Biol.* 2002;48:155-171.
10. Baharum SN, Bunawan H, Ghani MA, Aida WMW and Normah MN. Analysis of the chemical composition of the essential oil of polygonum minus huds. Using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC-TOF MS). Molecules 2010; 15:7006-7015.
11. Yaacob KB. Kesom oil- a natural source of aliphatic aldehyde. Perfum. Flavor 1987;12: 27-30.

12. Dallüge J, Beens J and Brinkman UAT. Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool. Journal of Chromatography A 2013; 1000: 69-108.
13. Romeo V, Ziino M, Giuffrida D, Condurso C and Verzera A. Flavour profile of capers (*Capparis spinosa L*.) from the Eolian Archipelago by HS-SPME/GC–MS. Food Chemistry 2007;101:1272-1278.
14. Siquera EP, Alves TMA and Zani CL. Fingerprint of volatiles from plant extracts based on SPME GC-MS. Brazilian Journal of Pharmacognosy 2007;17:565-571.
15. Sun SQ, Zhou Q and Chen JB. Infrared Spectroscopy For Complex mixtures : Application in Food and Traditional Medicine. Chemical Industry Press: Beijing, China 2011.
16. Lu GH, Zhou Q, Sun SQ, K. Leung SY, Zhang H and Zhao ZZ. Differentiation of Asian ginseng, American ginseng and Notoginseng by Fourier transform infrared spectroscopy combined with two-dimensional correlation infrared spectroscopy. Journal of Molecular Structure 2008; 883–884: 91-98.
17. Bunawan H, Choong C, Md-Zain B, Baharum SN and Normah MN. Molecular systematics of Polygonum minus Huds. based on ITS sequences. Int. J. Mol. Sci. 2011;12: 7626 - 7634.
18. Freeman R, Goodacre R, Sisson PR, Magee JG, Ward AC and Lightfoot NF. Rapid identification of species within the Mycobacterium tuberculosis complex by artificial neural network analysis of pyrolysis mass spectra. Journal of Medical Microbiology 1994; 40: 170-173.
19. Goodacre R, Timmins EM , Burton R, Kaderbhai RN, Woodward AM, Kell DB and Rooney PJ. Rapid identification of urinary tract infection bacteria using hyperspectral whole-organism fingerprinting and artificial neural networks. Microbiology 1998; 144:1157-1170.
20. Wenning M, Seiler H and Scherer S. Fourier-Transform Infrared Microspectroscopy, a Novel and Rapid Tool for Identification of Yeasts. Applied and Environmental Microbiology. 2002; 68: 4717-4721.
21. Zavoi S, Fetea F, Ranga F, Pop RM, Baciu A and Socaciu C. Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, as Determined by UV-Vis and FT-MIR Spectroscopy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39, 82-89.
22. Konwar M and Baruah GD. On the nature of vibrational bands in the FTIR spectra of medicinal plant leaves. Archives of Applied Science Research 2011;3: 214-221.
23. Maiorov VD, Burdin VV, Voloshenko GI and Librovich NB. Ions with a strong symmetric H-bond in solutions of sodium acetate in acetic acid. Russ. Chem. Bull. 1996;45:1766-1769.
24. Max JJ and Chapados C. Infrared Spectroscopy of Aqueous Carboxylic Acids: Comparison between Different Acids and Their Salts. The Journal of Physical Chemistry A 2004; 108: 3324-3337.
25. Naumann D, Helm D and Labischinski H. Microbiological characterizations by FT-IR spectroscopy. Nature 1991; 351: 81-82.
26. Kim SW, Ban SH, Chung H, Cho S, Chung HJ, Choi PS, Yoo OJ and Liu JR. Taxonomic discrimination of flowering plants by multivariate analysis of Fourier transform infrared spectroscopy data. Plant Cell Rep. 2004; 23: 246-250.
27. Vas G and Vekey K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. J. Mass Spectrom. 2004; 39:233-54.
28. Rodrigues CII, Hanson CM and Nogueira JMF. Coffees and Industrial Blends Aroma Profile Discrimination According to the Chromatic Value Coffee Science 2012; 7:167-176.
29. Gao X, Yang X, Mitrevski BS and Marriott PJ. Headspace solid-phase microextraction combined with GC×GC-TOFMS for the analysis of volatile compounds of Coptis species rhizomes. Journal of Separation Science 2011;34:1157-1166.
30. Opitz S, Kunert G and Gershenzon J. Increased Terpenoid Accumulation in Cotton (*Gossypium hirsutum*) Foliage is a General Wound Response. J. Chem. Ecol. 2008;34:08-522.
31. Tingey DT, Turner DP and Weber JA. Factors controlling the emission of monoterpenes and other volatiles organic compounds. Academic press: San Diego, 1991.
32. Loreto F, Ciccioli P, Cecinato A, Brancaleoni E, Frattoni M, Fabozzi C and Tricoli D. Evidence of the Photosynthetic Origin of Monoterpenes Emitted by *Quercus ilex L*. Leaves by 13^C Labeling. Plant Physiology 1996; 110: 1317-1322.
33. Peñuelas J and Llusià J. The Complexity of Factors Driving Volatile Organic Compound Emissions by Plants. Biologia Plantarum 2001;44: 481-487.
34. Herrera-Estrella L, Guevara-Garci'a A and Lo'pez-Bucio J. Heavy Metal Adaptation. Macmillan Publishers, 1999.