

## Application of FTIR fingerprints coupled with chemometric for comparison of stingless bee propolis from different extraction methods

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### Abstract

Propolis is a mixture of resin collected by the bees to build their hive. These mixtures contain natural remedy and used to treat variety of health-related problems. Unlike honeybee propolis, the study of stingless bee propolis still lacking. The important part of propolis study is the optimization of extraction procedures. The aim for this study is to employ chemometrics on FTIR data in order to discriminate the chemical fingerprinting of Malaysian stingless bee, *Heterotrigona itama* propolis yielded from different extraction methods, which were maceration, sonication and soxhlet. The chemical fingerprinting was obtained through Fourier Transform Infrared (FTIR). Principle component analysis (PCA) and hierarchical cluster analysis (HCA) were applied as pattern recognition methods for FTIR spectra. PCA of FTIR data for different extraction methods of stingless bee's propolis revealed that variability of PC1 and PC2 is 84.76%. PCA's variation in propolis by different extraction methods was classified based on specific functional groups arisen from the peaks. The FTIR fingerprinting of HCA of stingless bee's propolis were distributed into three clusters based on percentages of ethanol, intensity of peaks and different fingerprint region. FTIR coupled with chemometric analysis showed classification of different extraction methods of propolis from PCA and HCA based on vibration of functional groups presence in the samples. Taken together, these results showed that different extraction methods play important role in detaining of chemical in propolis of stingless bee.

**Keywords:** Propolis, stingless bee, FTIR, PCA, HCA

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## INTRODUCTION

*Heterotrigona itama* is a stingless bee from Apidae family. *H. itama* is one of the popular stingless bee among beekeepers in Malaysia and acts as pollinators to flowering plants. Stingless bee produces honey, propolis and bee bread in their hive. To date, there are more than 300 of chemical compounds have been identified from various honeybee propolis samples, including phenolic acid and their ester, flavonoid, amino acids, sugars or micro- and macroelements (Magdalena *et al.*, 2015). Propolis is a resinous product that collected from various plants by stingless bees and mixed with their beeswax and salivary enzymes,  $\beta$ -glucosidase (Ricardo *et al.*, 2015). Propolis has been used in hive as building materials as well as defensives substances from insects or microorganism (Bankova *et al.*, 2014). The composition of propolis is depended on its botanical sources and geographical origin (Bogdanov *et al.*, 2015) and also depended on season and vegetation (Araújo *et al.*, 2016). Nowadays, propolis is still used in folk medicine, but becoming popular in healthy foods and drinks or in natural cosmetics and therapeutic. Apparently, there are some factors that affect the amount of chemical composition and biological activity in propolis, which are extraction methods (Niken *et al.*, 2014) and

solvents used for extraction (Prashant *et al.*, 2011). At the moment, there are many extraction methods such as maceration (Irene *et al.*, 2012), sonication (Khotai & Jayanthi, 2015), soxhlet (Baldonaso *et al.*, 2015), microwave (Ming *et al.*, 2009) and reflux (Irene *et al.*, 2012) that have been emphasized in order to obtain optimum extraction yield and higher chemical constituents. Generally, several methods have been developed in order to determine chemical composition in propolis such as UltraViolet- Visible (UV-Vis) (Renata *et al.*, 2016), High Performance Thin Layer Chromatography (HPTLC) (Petar *et al.*, 2014) and Gas Chromatography Mass Spectrometry (GC-MS) (Milena *et al.*, 2017). In this study, maceration, sonication and soxhlet methods were used as extraction methods and FTIR was employed in determination of chemical fingerprint. Maceration is simple extraction method whereby the samples were soaked in a closed container and left in room temperature for a period of time (Azwanida, 2015). On the other hand, sonication works by cavitation energy that created the bubbles to release chemical compound in the samples (Khotai & Jayanthi, 2015). Soxhlet is condensation and heating method to evaporate the solvent and concentrate the samples (Khacha-ananda *et al.*, 2013). Fourier transform infrared spectroscopy (FTIR) is one of the simple

and rapid tools to determine the functional group in a sample. This instrument produces spectrum occurring between 4000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$  by molecular vibration which are stretching and bending. Typically, the stretching mode has higher energy compared to bending mode. FTIR consists thousands of data sets and overlapping data that cannot be varied by human vision. Thus, chemometric tool is important to classify and discriminate the samples into their factor of interest. Chemometric is a tool that combines both mathematical and statistical methods in order to get optimum results based on chemical measurement obtained (Rohman & Salmah, 2018). The main purpose of chemometric is to investigate dominant modes of variation in data set by discrimination and classification using unsupervised Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). (Rohman & Salmah, 2018). This study was aimed to employ chemometric analysis on FTIR data in order to compare the chemical fingerprints of Malaysian stingless bee, *Heterotrigna itama* propolis yielded from different extraction methods, which were maceration, sonication and soxhlet.

## EXPERIMENTAL

### Materials

All the chemicals and reagents used in this experiment were analytical grade. Ethanol was purchased from Merck Sdn. Bhd, Selangor, Malaysia.

### Sample collection

Propolis of *Heterotrigna itama* was collected from apiary of Universiti Sultan Zainal Abidin, Besut Campus. The samples were ground into powder and kept in  $-80^{\circ}\text{C}$  for further analysis.

### Maceration extraction

In brief, 18 g of powdered propolis were extracted with 60 mL of 70% and 95% ethanol for 1, 3, 5 and 7 days. The ethanolic extracts were filtered using Whatman #41 and concentrated using rotary evaporator (Heidolph Instruments GmbH 5 & Co. KG, German) under vacuum pressure at  $45^{\circ}\text{C}$ . The extracted propolis for maceration were labelled as E70M-1 (Ethanol 70%-Maceration-1 day), E70M-3 (Ethanol 70%-Maceration-3 days), E70M-5 (Ethanol 70%-Maceration-5 days), E70M-7 (Ethanol 70%-Maceration-7 days), E95M-1 (Ethanol 95%-Maceration-1 day), E95M-3 (Ethanol 95%-Maceration-3 days), E95M-5 (Ethanol 95%-Maceration-5 days) and E95M-7 (Ethanol 95%-Maceration-7 days).

### Sonication extraction

Approximately, 18 g of powdered propolis was extracted in 60 mL of 70% and 95% ethanol for 10, 30, 60 and 120 minutes at  $37^{\circ}\text{C}$  in sonicator bath (Jeio Tech UC-10). The ethanolic extracts were filtered using Whatman #41 and concentrated using rotary evaporator (Heidolph Instruments GmbH 5 & Co. KG, German) under vacuum pressure at  $45^{\circ}\text{C}$ . The crude propolis for sonication were labelled as E70S-10 (Ethanol 70%-Sonication-10 minutes), E70S-30 (Ethanol 70%-Sonication-30 minutes), E70S-60 (Ethanol 70%-Sonication-60 minutes), E70S-120 (Ethanol 70%-Sonication-120 minutes), E95S-10 (Ethanol 95%-Sonication-10 minutes), E95S-30 (Ethanol 95%-Sonication-30 minutes), E95S-60 (Ethanol 95%-Sonication-60 minutes) and E95S-120 (Ethanol 95%-Sonication-120 minutes).

### Soxhlet extraction

Approximately 5 g of propolis was extracted in 150 mL of 70% and 95% ethanol for 2, 4, 6 and 8 hours by soxhlet extractor (Soxhlet extractor M-Top, Korean South). The ethanolic extracts were filtered using Whatman #41 and concentrated using rotary evaporator (Heidolph Instruments GmbH 5 & Co. KG, German) under vacuum pressure at  $45^{\circ}\text{C}$ . The crude propolis for soxhlet were labelled as E70SH-2 (70% Ethanol-Soxhlet-2 hours), E70SH-4 (70% Ethanol-Soxhlet-4 hours), E70SH-6 (70% Ethanol-Soxhlet-6 hours), E70SH-8 (70% Ethanol-Soxhlet-8 hours), E95SH-2 (95% Ethanol-Soxhlet-2 hours), E95SH-4 (95% Ethanol-Soxhlet-4 hours), E95SH-6 (95%

Ethanol-Soxhlet-6 hours), and E95SH-8 (95% Ethanol-Soxhlet-8 hours).

### The FTIR analysis of propolis extracts

The FTIR spectra of propolis extract samples were analyzed using IRPrestige-21 Shimadzu Fourier Transform Infrared Spectrophotometer (Tokyo, Japan) according to method by Azemin *et al.* (2017). FTIR spectrometer was coupled with DLATGS (Deuterated Triglycine Sulfate doped with L-Alanine) and equipped with air-cooled ceramic infrared light source. Propolis extracts were recorded at the middle-IR range 4000-600  $\text{cm}^{-1}$  at resolution 4  $\text{cm}^{-1}$  by co-adding 16 scans. After every scans, the background spectrum was taken. The sticky propolis extract was smeared directly on the diamond prism. The diamond prism was carefully cleaned by rubbing the soft tissue (Kimtech Science, Kimwipes) with 70% ethanol before the next samples were applied. The measurement of FTIR data set was baseline correction to minimize the difference between the spectra during baseline shift, normalized and smoothed to reduce noise in spectral data using Shimadzu IRsolution Version 1.40 (Shimadzu Corporation) software.

### Pre-processing data

The FTIR data sets (1763 x 24 datasets) from Shimadzu IRsolution software for FTIR data from normalized and smoothing were saved in file .txt and copied manually to Microsoft Excel as two data sets (rows: samples; and columns: wavenumber) for extracting their numerical values from spectra files. Then, the noise range 599-400  $\text{cm}^{-1}$  was cut off and the data was aligned in rows for samples and column for wavenumber. All FTIR samples were subjected to unsupervised pattern recognition by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) which conducted by XLSTAT Pro 2014 (Addinsoft, Paris, France), an add-in software program for Microsoft Excel 2010.

### Principal Component Analysis (PCA)

PCA is a mathematical procedure which used to reduce the dimensionality of the large data sets to a small sets by retaining the variability of the data sets in principal component (PCs) (Jolliffe, 2002). Usually, the most two PCs namely as PC1 and PC2 were used because these two PCs contributed to higher variance in a given data sets. If the factors were difficult to interpret after the component have been analysed, the varimax rotation was applied. Varimax rotation is an orthogonal rotation that used to facilitate the interpretation by reducing the variability of the principle component as known as varimax factor (VFs). (Herve & Lynne, 2010). The varimax rotation was applied on the PCs when eigenvalue was more than 1. Eigenvalues would measure the total variation in the total samples in each PCs. The higher the total variation, the more the observation would contribute to that PCs.

### Hierarchical Cluster Analysis (HCA)

The goal of using HCA was to find the best grouping observation which was the cluster dissimilarity to each other and within cluster that similar to each other (Ami *et al.*, 2012). The similarity and dissimilarity were called distance function such as Euclidean distance. (Mohamad-Asri *et al.*, 2018). HCA was performed using the single linkage technique to link the clusters and Euclidean distance. The Hierarchical algorithm was divided into two; agglomerative or divisive. The agglomerative method is many-to-one method, in which the clustering is placed in different clusters and each step of a cluster of observations is merged into another cluster. Meanwhile, the divisive method is one-to-many method, in which is one single cluster is contained all observations and divided to two subclusters. The dissimilarities between the samples increase were observed by the higher relative distance between the samples shown in dendrogram (Azemin *et al.*, 2017).

## RESULTS AND DISCUSSION

Three extraction methods; maceration, sonication and soxhlet were employed in order to obtain bioactive extracts. Maceration is traditional method of extraction for natural product material. Eventhough it is effective, but this method is a time consuming, which required 2-10 days to get higher yield. In comparison, modern method such as sonication and soxhlet extraction have been developed for fast and efficient extraction method. Sonication required short time extraction and lower temperature to extract and thus, it can avoid thermal damages and preserve the structural and molecular properties of propolis (Tian *et al.*, 2013). On the other hand, soxhlet required small amount of solvent and can produce higher extraction yield because of the continuous process during extraction. Extraction method is important in order to produce higher extraction yield besides to extract bioactive compound in propolis.

### Fingerprinting of propolis by Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR is a rapid, efficient and accurate analytical method that measured the vibrational of functional group in a wavelength middle-IR range 4000-600  $\text{cm}^{-1}$  based on bending and stretching vibrations. The typical FTIR spectra for propolis extracts of different extraction methods were shown in Fig. 1. From Fig. 1, the patterns of FTIR peaks were appeared similar but mostly occurred in different intensities. FTIR of stingless bee's propolis by different extraction methods showed complex chemical composition. It could be observed that peak at 3550-3200  $\text{cm}^{-1}$  was assigned to intermolecular hydrogen bonding (O-H stretching vibrations). This peak was presence in all extraction methods but with different intensities. The intensity of this peak range was found to be broad and strong peak for 70% ethanol extracts as compared to 95% ethanol extracts in all extraction methods. The peak at 2968  $\text{cm}^{-1}$  and 2855  $\text{cm}^{-1}$  were attributed to asymmetrical stretching of methyl group ( $\text{V}_{\text{as}}\text{CH}_3$ ) and symmetrical stretching vibration of methylene group ( $\text{V}_{\text{s}}\text{CH}_2$ ) respectively. These peaks were presence in maceration (E70M-1, E70M-3, E70M-5 and E70M-7) and in sonication (E70S-30, E70S-60 and E70S-120). While the 2923  $\text{cm}^{-1}$  peak was due to symmetrical stretching of ( $\text{V}_{\text{s}}\text{CH}_3$ ) of methyl group presence in all extraction methods.

In the range of 1730-1726  $\text{cm}^{-1}$ , peaks could be seen probably related to C=O stretching vibrations of aliphatic aldehydes was found in all extraction methods. However, 95% ethanol produced intense peak as compared to 75% ethanol. Furthermore, peak at 1715-1711  $\text{cm}^{-1}$  (normal C=O stretching vibration of saturated aliphatic ketone) was presence in maceration methods (all samples), sonication (all samples in 95% ethanol and E70S-30, E70S-60 and E70S-120) and soxhlet (95% ethanol). The peaks at 1697-1692  $\text{cm}^{-1}$  (C=O stretching vibration of unsaturated and aryl conjugated carboxylic acid), 1666-1665  $\text{cm}^{-1}$  (C=C stretching vibration of disubstituted trans alkene, tri- and tetraalkyl-substituted alkenes) and 1659  $\text{cm}^{-1}$  (C=C stretching vibration of cis-alkene) were found in 70% ethanol by soxhlet and 70S-10.

Peak at range 1645-1641  $\text{cm}^{-1}$  was presence in all extraction methods that contributed to C=C stretching vibration of vinyl group. Besides that, peak at range 1636-1632  $\text{cm}^{-1}$  was attributed to C=C stretching vibration and only found in maceration (E70M-1, E70M-3, E70M-5 and E70M-7) and in sonication (E70S-30, E70S-60 and E70S-120). On the other hand, conjugated C=C stretching vibration was found in all extraction methods approximately at 1600  $\text{cm}^{-1}$ . The peaks at 1587-1582  $\text{cm}^{-1}$ , 1574-1572  $\text{cm}^{-1}$ , 1564  $\text{cm}^{-1}$ , 1557-1553  $\text{cm}^{-1}$  and 1549-1547  $\text{cm}^{-1}$  were assigned as C=C stretching vibration. The peak in the ranges of 1517-1515  $\text{cm}^{-1}$  was assigned as C=C-C aromatic stretching and occurred in all extraction methods. was only stable until 6 hours extraction. On the other hand, there were two distinct peaks occurring in all extraction methods approximately in the range 1439-1433  $\text{cm}^{-1}$  and 1375  $\text{cm}^{-1}$ , which attributed to asymmetrical bending vibration ( $\delta_{\text{as}}\text{CH}_3$ ) and symmetrical bending vibration ( $\delta_{\text{s}}\text{CH}_3$ ). The O-H bending vibration at 1295-1285  $\text{cm}^{-1}$  found in all extraction methods. The C-O stretching vibration

occurred at range 1237-1235  $\text{cm}^{-1}$  and 1196-1192  $\text{cm}^{-1}$  were only found in all extraction methods

A peak at 1121-1117  $\text{cm}^{-1}$  was seen only in 95% of ethanol due to C-O stretching vibration of saturated secondary alcohol. The presence of peak at 1034-1032  $\text{cm}^{-1}$  was related to C-O stretching vibration of primary alcohol, which was found in all extraction methods. However, 70% ethanol by maceration and sonication showed strong and intense absorption as compared to 95% ethanol and both in soxhlet method. Peak at 883-880  $\text{cm}^{-1}$  was presented in all extraction methods and attributed to aromatic C-H out-of-plane bending vibration of 1,3-disubstitution (meta) while peak at 826-813  $\text{cm}^{-1}$  was contributed to aromatic C-H out-of-plane bending vibration of 1,4-disubstitution (para). This peak was degraded after propolis was extracted more than 60 minutes by sonication. Peaks in the range of 773-768  $\text{cm}^{-1}$  and 745-743  $\text{cm}^{-1}$  were attributed to the monosubstitution (phenyl) or 1,2-disubstitution (ortho) and occurred in all extraction.

In addition, peak at 719-718  $\text{cm}^{-1}$  was assigned to aromatic C-H out-of-plane bending vibration was only presented in 95% ethanol in all extraction methods. Peaks in the ranges of 634-630  $\text{cm}^{-1}$ , 590-586  $\text{cm}^{-1}$  and 565-550  $\text{cm}^{-1}$  were attributed to O-H out-of-plane bending vibration of alcohol. These peaks presence in all extraction methods. The summary of the FTIR assignments was illustrated in Table 1. Typically, the stretching mode has higher energy as compared to bending mode. Stretching mode could be divided into two; symmetrical and asymmetrical, which the asymmetrical usually has higher energy.

The energy of stretching mode was decreased as the mass of atom was increased such as the C-H stretching vibration has higher energy compared to C-C stretching vibration. Furthermore, the stronger the bond ( $\text{C}\equiv\text{C} > \text{C}=\text{C} > \text{C}-\text{C}$ ) the higher the energy to vibrate. The higher energy, the higher wavenumber. Overall, the assignment of peaks from FTIR spectra of propolis extracts by three different methods of extraction for both 70% and 95% ethanol was found that, the extraction methods and their durations played significant roles in extracting different types of compounds which resulted in giving out different vibrationals of functional groups at different periods of time.

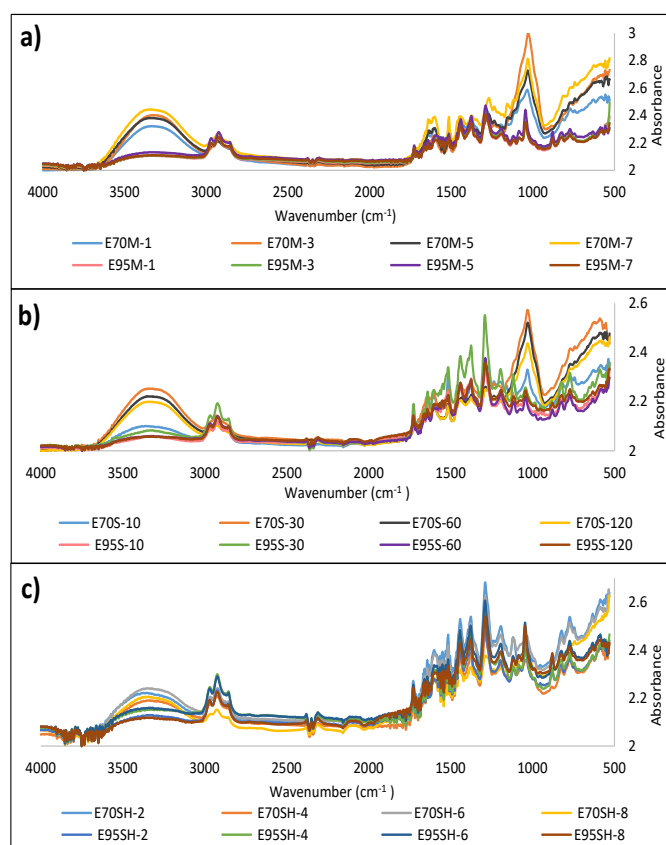


Fig. 1 FTIR spectra of propolis extraction of stingless bee by different extraction methods (a) maceration, (b) sonication and (c) soxhlet.

**Table 1** Summary of the FTIR assignments.

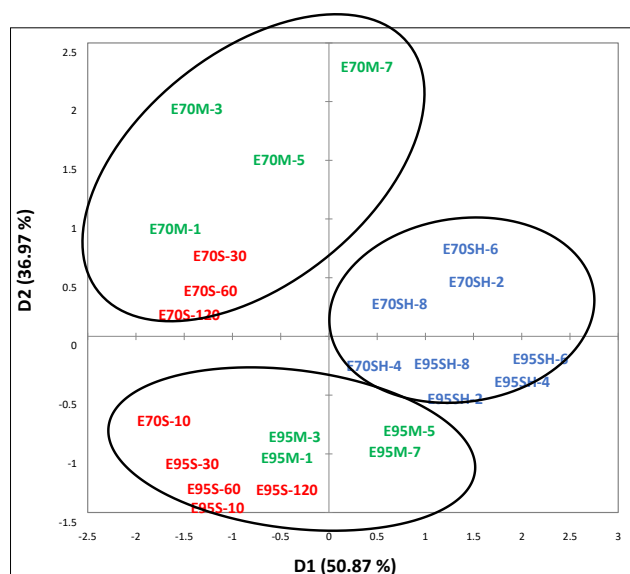
Peak ranges	Functional groups
3500-3200	Intermolecular hydrogen bonding (O-H stretching vibrations)
2968	Asymmetrical stretching (VasCH3) of methyl group
2926	Asymmetrical stretching (VasCH2) of methylene group
2855	Symmetrical stretching of (VsCH2) of methylene group
2382-2350	False positive
1728-1726	C=O stretching vibration of aliphatic aldehydes
1715-1711	Normal C=O stretching vibration of saturated aliphatic ketone
1697-1692	C=O stretching vibration of ab unsaturated and aryl conjugated carboxylic acid
1666-1665	C=C stretching vibration of disubstituted trans alkene, tri- and tetraalkyl-substituted alkenes
1659	C=C stretching vibration of cis- alkene
1644-1641	C=C stretching vibration of vinyl group
1636-1632	C=C stretching vibration
1604-1600	Conjugated C=C of stretching vibration
1587-1582	C=C -C aromatic ring stretching
1574-1572	C=C stretching vibration
1564	C=C stretching vibration
1557-1553	C=C stretching vibration
1549-1547	C=C stretching vibration
1517-1515	C=C-C aromatic stretching vibration
1439-1433	C-H asymmetrical bending vibration CH3
1377-1374	C-H symmetrical bending vibration CH3
1295-1285	O- H bending vibration
1237-1235	phenols C-O stretching
1196-1192	Phenol, C-O stretching vibration
1121-1117	C-O stretching saturated secondary alcohol
1034-1032	C-O stretching primary alcohol
883-880	1,3- disubstitution (meta) of aromatic C-H out-of-plane bending vibration
826-824	1,4- disubstitution (para) of aromatic C-H out-of-plane bending vibration
773-768	1,2- disubstitution (ortho) of aromatic C-H out-of-plane bending vibration
745-743	
719-718	Alcohol, O-H out-of-plane bend of aromatic C-H out-of-plane bending vibration
634-630	Alcohol, O-H out-of-plane bending vibration
590-586	Alcohol, O-H out-of-plane bending vibration

### Chemometric analysis of FTIR fingerprint

FTIR spectrum revealed certain functional groups based on x-axis (wavelength) and y-axis (transmittance) that coming from the vibration of molecules in the propolis samples. It formed a complex and overlapping spectrum in each extraction methods. Hence, chemometric analysis is important to differentiate each of them. The Principal Component analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed in chemometric analysis. Based on Fig. 2, the PCA of FTIR data for different extraction methods of stingless bee's propolis revealed the variability of PC1 and PC2 after varimax rotation (VFs) was 87.84%. The result from factor score showed that maceration (70M-1), sonication (70S-10, 70S-30, 70S-60, 70S-120 and 95S-30) and soxhlet (all samples) were classified into VF1. Meanwhile maceration (70M-3, 70M-5, 70M-7, 95M-1, 95M-3, 95M-5 and 95M-7) and sonication (95S-10, 95S-60 and 95S-120) were classified into VF1. There were fourteen variables that classified the extraction methods of propolis into PC1 and nine variables in PC2 as shown in Table 2. The loading factor (Table 2) showed PCA's variation in propolis by different extraction methods was classified based on specific functional groups arise from the peaks. From PCA score plot, the propolis extraction methods were classified into three groups which were group 1 (all samples by maceration in 70% ethanol and sonication in 70 % ethanol for 30, 60 and 120 minutes), group 2

(all soxhlet methods) and group 3 (all samples by maceration in 95 % ethanol and all sample by sonication in 95 % ethanol and 70% ethanol for 10 minutes extraction).

The results of these three groups from PCA score plot (Fig 2) were related to HCA of dendrogram results (Fig 3). The extraction methods that classified in group 1 were distributed in Cluster I, while group 2 and 3 were classified into cluster II and III. Samples were classified within cluster and found to be similar to each other, meanwhile, samples that classified into different clusters were different with each other in some senses. The dot lines in dendograms indicated that the automatic truncation was cut off to determine the significant clusters. The HCA revealed the extraction methods of propolis were classified based on percentages of ethanol and peak intensities and different fingerprint region. The propolis extracted with 70% of ethanol was classified into cluster I and III, while propolis extracted with 95% of ethanol classified into cluster II. Cluster I was classified based on C=C stretching vibration and have highest intensities spectra of C-O stretching of primary alcohol. Cluster II was characterized based on lowest intensities spectra of O-H intermolecular hydrogen bonding stretching vibration, C-O stretching vibration and aromatic C-H out-of-plane bending vibration. Cluster III was classified based on highest intensities spectra of O- H bending vibration and C-O stretching saturated secondary alcohol.



**Fig. 2** PCA score plots of propolis extraction of stingless bee by different extraction methods (a) maceration, (b) sonication and (c) soxhlet.

**Table 2** Summary of the FTIR assignment based on varimax factors.

VFs	Peaks range	Assignment for vibrational of functional groups for specific peaks
VF1	2855	Symmetrical stretching of (VsCH <sub>2</sub> ) of methylene group
	2382-2350	False positive (due to atmospheric intrusion such as humidity (Chalmers, 2006))
	1728-1726	C=O stretching vibration of aliphatic aldehydes
	1697-1692	C=O stretching vibration of unsaturated and aryl conjugated carboxylic acid
	1666-1665	C=C stretching vibration of disubstituted trans alkene, tri- and tetraalkyl-substituted alkenes
	1659	C=C stretching vibration of cis- alkene
	1587-1582	C=C -C aromatic ring stretching
	1574-1572	C=C stretching vibration
	1564	C=C stretching vibration
	1557-1553	C=C stretching vibration
	1549-1547	C=C stretching vibration
	1439-1433	C-H asymmetrical bending vibration CH <sub>3</sub>
	1295-1285	O-H bending vibration
	1196-1192	C-O stretching vibration
VF2	3500-3200	Intermolecular hydrogen bonding (O-H stretching vibrations)
	1636-1632	C=C stretching vibration
	1121-1117	C-O stretching saturated secondary alcohol
	1034-1032	C-O stretching primary alcohol
	883-880	Aromatic C-H out-of-plane bending vibration
	826-824	
	773-768	
	745-743	
719-718		

## CONCLUSION

From this study, different extraction methods played important role in determination of chemical composition in propolis of stingless bee. FTIR coupled with chemometric analysis showed classification of different extraction methods of propolis from PCA and HCA based on vibration of functional groups presented in the samples. Hence, PCA and HCA were considered to be very popular tool to summarize a dataset into a few factors which highlighted the most important information to optimize method of extractions.

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