Chemometrics-assisted fingerprinting profiling of Mangosteen extracts from Sulawesi island using Fourier Transform Infrared (FTIR) Spectroscopy

Budiman YASIR[1,2] (ID)**, Muhammad AZWAR AR[3]** (ID)**, Asril BURHAN[4]** (ID)**, Khairuddin KHAIRUDDIN[4]** (ID)**, Gemini ALAM[5]** (ID) **, Irnawati IRNAWATI[6]** (ID)**, Abdul ROHMAN[7*]** (ID)

[1] Sekolah Tinggi Ilmu Farmasi Makassar, Makassar 90242, Indonesia.
[2] Faculty of Health Sciences, Almarisah Madani University, Makassar 90245, Indonesia.
[3] Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Tadulako, Palu 94118, Indonesia.
[4] Departement of Biology Pharmacy, Sekolah Tinggi Ilmu Farmasi Makassar, Makassar 90242, Indonesia.
[5] Department of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia.
[6] Faculty of Pharmacy, Universitas Halu Oleo, Kendari 93232, Indonesia.
[7] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia.
* Corresponding Author. E-mail: abdul_kimfar@ugm.ac.id (A.R.); Tel. +62-878-3844 52 16.

ABSTRACT: Applying chemometrics in analyzing the chemical data from natural materials is very important for understanding the complex data of herbal raw materials. This study aims to apply chemometric principal component analysis (PCA) and cluster analysis (CA) techniques to know the fingerprint profiling of 20 types of ethanolic extract of Mangosteen obtained from different area in Sulawesi Island, Indonesia. The extraction uses variations for solvent kinds (70% and 96% ethanol) with maceration techniques and fruit parts (pericarp and seed) representing plant parts from Sulawesi Island. All extracts were analyzed using FTIR spectroscopy at wavenumbers of 4000-400 cm^{-1}, and the obtained FTIR spectra were analyzed using chemometric or multivariate data analysis. The results showed that some peaks were identified as markers for mangosteen extracts, namely at wavenumbers of 3414 cm^{-1} (O-H stretching), 2961 cm^{-1} (CH$_3$ asymmetrical stretching), 2922 cm^{-1} (CH$_2$ asymmetrical stretching), 2859 cm^{-1} (CH$_2$ symmetrical stretching), 1724 cm^{-1} (C=O stretching), 1643 (hydrogen bonded), 1611 cm^{-1} (carboxyl groups), 1582 cm^{-1} (C=C stretching), 1458 cm^{-1} (CH$_2$ bending), and 1283 cm^{-1} (O-H). PCA using the first principle component (PC1) and second principle components accounting of 85.8% variations could differentiate Mangosteen extracts with different area. Furthermore, CA could classify the samples of Mangosteen extracts into 4 clusters with a similarity percentages of 25.65%-99.99%. It can be concluded that chemometric-assisted fingerprinting profiles using variable of FTIR spectral absorbance values can distinguish variations in mangosteen extract according to the type of solvent and the fruit part according to Sulawesi Island origin.

KEYWORDS: Chemometrics; Fingerprinting profiling; Mangosteen; Multivariate data analysis; Spectroscopy.

1. INTRODUCTION

Mangosteen is one of the most delicious and sweet fruits, known as the *Queen of Fruits*. Mangosteen is a fruit naturally found in Southeast Asia, especially in Indonesia. The fruit contains 6-10 aryl segments of various widths. Mangosteen can use as a folk remedy [1]. The mangosteen pericarp contains xanthones, one of the polyphenol compounds. The main constituents of xanthone derivatives in mangosteen pericarp are α-mangosteen, β-mangosteen, γ-mangosteen, gartanin [2, 3], tovophyllin A-B [4, 5]. The compound of α-mangosteen was reported as the main component of the species with several pharmacological activities having the benefit effects to human health [6]. The α-mangosteen exhibits cardio-protective properties [7], anticancer [8, 9], antimicrobial [10], antioxidant potencies [11], anti-inflammatory and antifungal [12], analgesic and inhibition of several diseases including Parkinson's disease [13], antidiabetic and Alzheimer's disease [11]. In addition, gartanin exhibits anticancer [14], anticancer, anti-inflammation, and antioxidant

activities [15]. These biological activities depend on the phytochemicals or active compounds contained in mangosteen in which the active compounds depend on some environmental factors, therefore, the authenticity of geographical origins of mangosteen is very urgent.

Several chemical and biological responses have been applied for the fingerprinting profiling of herbals including mangosteen. Vibrational spectra offering the fingerprinting spectra such as near infrared, mid infrared and Raman spectroscopy as well as nuclear magnetic resonance (NMR) spectra have been used for the authentication of herbal components by identifying the marker peaks characterized by the wavenumbers and peak intensities [16, 17]. In addition, some chromatographic methods including thin layer chromatography, high performance liquid chromatography and liquid chromatography-tandem mass spectrometer (LC-MS/MS) are widely applied for the determination of geographical origins of herbals [18, 19]. Due to the simplicity and rapidity, FTIR spectra are typically applied for quality control of herbal medicines. The data obtained during obtaining FTIR spectra are very large, as a consequence, the use of chemometrics capable of handling big data is inevitable [20].

FTIR spectra combined with pattern recognition chemometrics such as principal component analysis, cluster analysis and discriminant analysis could detect herbal counterfeiting very accurately, flexibly, practically, and cost-effectively. The combination of FTIR spectra and chemometrics have been applied for the fingerprinting profiling of mangosteen pericarp extract and seeds [21, 22]. In addition, this combination is also successful for the classification of mangosteen pericarps from different regions and offer reliable methods for quality assurance of mangosteen pericarp [21]. However, from literature review, the use of FTIR spectra focusing on fingerprinting region combined with chemometrics for geographical origins of Indonesian Mangosteen is very limited. Therefore, the objective of this study was to determine the markers through fingerprint profiling of mangosteen from Sulawesi Island with variations in ethanol solvents and regional basis using the FTIR spectra combined with principal components analysis (PCA) and cluster analysis (CA).

2. RESULT AND DISCUSSION

2.1. Fingerprinting profiling of mangosteen extract

In this study, 20 types of mangosteen samples from 5 different regions in Sulawesi island, Indonesia were used, namely Mamuju (Mm), Masamba (Ma), Morowali (Mo), Palu (Pa), and Poso (Po). These samples were extracted with 70% and 96% ethanol solvents using the maceration technique to obtain the ethanolic extracts of mangosteen. FTIR spectra in combination with chemometric analysis were used for the differentiation of mangosteen extracts. Figure 1 revealed the FTIR spectra of ethanolic extracts of mangosteen obtained from different regions at mid infrared region of 400-4000 cm^{-1}. Furthermore, each peaks and shoulders in Figure 1 corresponds to the functional groups responsible for infrared absorption to provide vibrational transitions in certain wavenumbers (Table 1).

The interpretation of each peaks and shoulders present in FTIR spectra of ethanolic Mangosteen extracts is follows: peak at 3414 cm^{-1} related to stretching vibration of O-H-bonded, 2961 cm^{-1} due to C-H asymmetrical stretching vibrations of (CH$_3$), 2922 cm^{-1} due to asymmetrical stretching vibration of CH$_2$, 2859 cm^{-1} due to stretching aliphatic CH$_2$, 2727 cm^{-1} due to stretching vibration of CH aldehyde, 2359 cm^{-1} due to carboxylic acids group, 1776 cm^{-1} due to asymmetric stretching of C=O (ester), 1724 cm^{-1} due to carbonyl group (C=O from acids), 1643 due to hydrogen bonded, 1611 cm^{-1} (C=C stretching), 1582 cm^{-1} stretching (C=C), 1458cm^{-1} due to bending CH$_2$, 1377 cm^{-1} due to bending vibration of CH$_3$, 1283 cm^{-1} due to stretching of carbolic acid (O-H), 1227 cm^{-1} due to stretching vibration of C-O-C, 1190 cm^{-1} bending vibration of (C-O) group, 1155 cm^{-1} due to asymmetric extension (C-O-C), 1074 cm^{-1} (CO from ester carbonyl groups), 1049 cm^{-1} due to stretching vibration (C-O), 978 cm^{-1} (sugar monomers), 899 cm^{-1} due to bending vibrations (O-H), 820 cm^{-1} due to sulfate groups, 777 cm^{-1} due to bending (C-H), 708 cm^{-1} due to C-H$_2$ group, 662 cm^{-1} due to bending N–H, 629 cm^{-1} due to wagging (C-H), 584cm^{-1} due to bending (O-H), 523 cm^{-1} due to stretching vibrations (C–Br), 451 cm^{-1} due to stretching vibration of bending C=C (Tejamukti *et al.*, 2020). The regular peaks with high intensity of functional groups identified as peak markers in mangosteen pericarp and seed extracts were those at 3414 due to stretching (O-H), 2961 cm^{-1}, 2922 cm^{-1}, 2859 cm^{-1}, 1724 cm^{-1}, 1643 cm^{-1}, 1611 cm^{-1}, 1582 cm^{-1}, 1458 cm^{-1}, and 1283 cm^{-1}. The functional groups related to these peaks can be found in the marker compounds of Mangosteen, namely α-mangosteen and gartanin as reported by Rohman *et al.* [21] and Muchtaridi *et al.* [23].
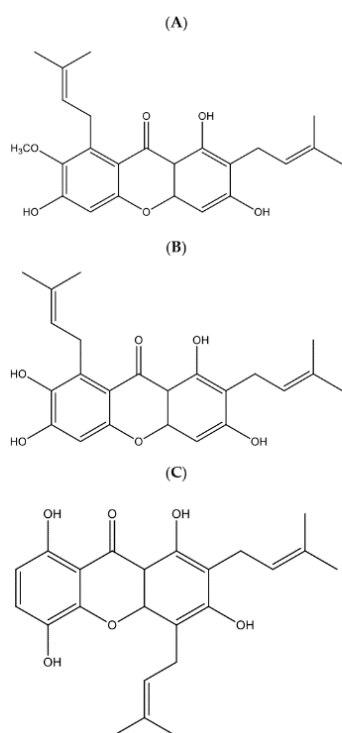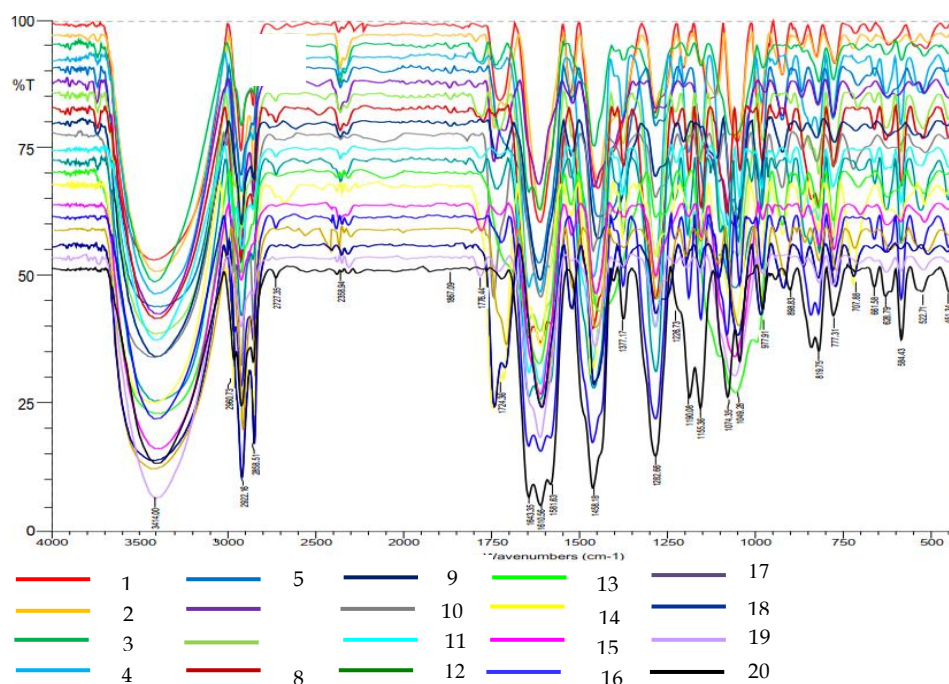
Figure 1. FTIR-Spectra of Mangosteen extracts, extracted by ethanol 70% and 96 along with the chemical structures of active compounds of (A) α-mangostin (AM), (B) γ-mangostin, and (C) gartanin. *see Section of Experimental procedure.*

2.2. Principal component analysis

PCA is exploratory data analysis for considering an unsupervised pattern recognition from chemometrics, typically used for classifying samples. To classify samples using multivariate analysis with a PCA design that statistical data will be produced in the form of a scree plot to find out the data model, a score plot to find out the data distribution and influence between variables, a loading plot to find out the correlation relationship between extract variables and combined with linear regression to find out the positive correlation between extracts and linear equations.
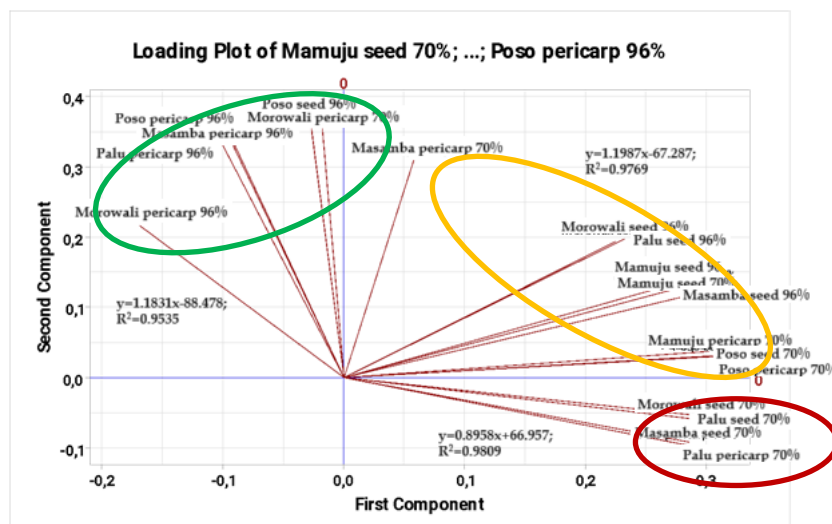

Figure 2. The loading plot describing the contribution of variables during principal component analysis of samples as classified for linear correlation as group 1 (G1) assigned with red line, group 2 (G2) assigned with yellow line and group 3 (G3) assigned with green line

The results of the scree plot data analysis showed that the eigenvalue value must be above one and be able to explain >80% of the data if the data model is excellent and optimal. From this result, there were three eigenvalue values >1, namely PC3 1.75 explained 85.8% of the data, PC2 6.78 explained 77% of the data, and PC1 8.63 explained 43.2% of the data. The loading plot follows the normal distribution; if no outlier is present, each variable will be randomly distributed around zero [24]. It shows a randomly distributed extract sample and showed a positive correlation (Figure 2), namely Group 1 (G1) mangosteen extract sample Ma mangosteen seed extract 70%, Pa mangosteen seed extract 70%, Mo mangosteen seed extract 70%, and Pa mangosteen pericarp extract 70% with an influence on PC1 of 0.285-0.286 and has a positive correlation with the equation (y=0.8958x+66.957; R^2 = 0.9809).
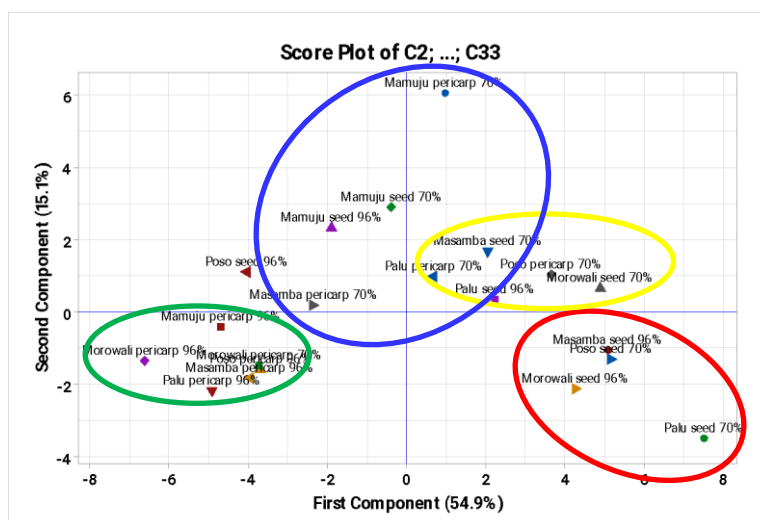

Figure 3. The sore plot for classification of samples using principal component analysis as classified 4 groups (red, yellow, blue, and green line)

Group 2 (G2) Ma mangosteen pericarp extract 70% and seed extract 96%, Mo mangosteen seed extract 96%, Pa mangosteen seed extract 96%, Po mangosteen seed and pericarp extract 70%, Mm mangosteen seed and pericarp 70% and seed extract 96%, with an influence of PC1 of 0.058-0.307 and has a positive correlation with the equation (y= 1.1987x-67.287; R² = 0.9769). Group 3 (G3) Po mangosteen seed and pericarp extract 96%, Mo mangosteen pericarp extract 70% and 96%, Ma mangosteen pericarp extract 96%, and Pa mangosteen pericarp extract 96% with pc2 influence of 0.216-0.354 and have equation (y=1.1831x-88.478; R² = 0.9535).
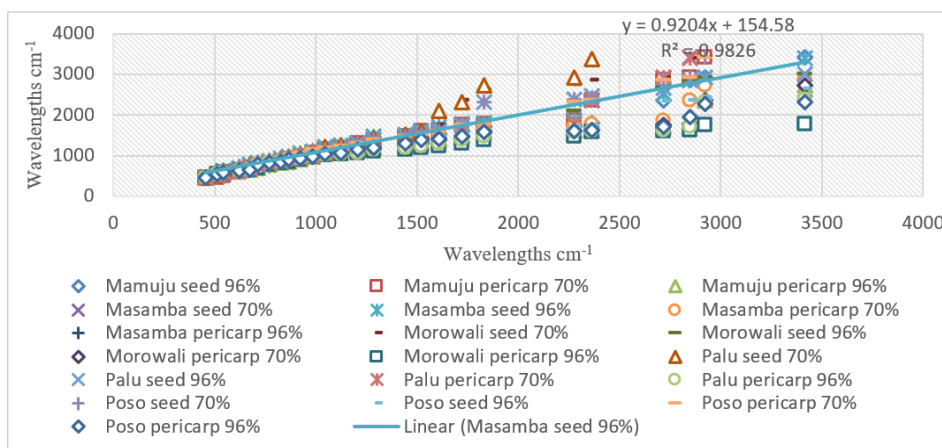

Figure 4. All the mangosteen extracts showing a positive correlation

Correlation between all extracts (y =0.9204x + 154.58; R² = 0.9826) indicates a positive correlation between compound functional groups found between extracts and Ma mangosteen seed extract 96% as the standard linear line in (Figure 4), in which all functional groups were found in all sections. The data score plot (Figure 3) illustrates the contribution of each variable to PC1 and PC2. The score contributes to each variable used for sample classification—the higher variable's contribution to the PCA model. The plot score in the result of this study obtained that Po mangosteen seed extract 70% had a significant effect of 0.307 on PC1 and Po mangosteen seed extract 96% with Ma mangosteen pericarp extract 96% 0.354 on PC2, as for the influence of all variables on PC1 54.9% and PC2 15.1%.
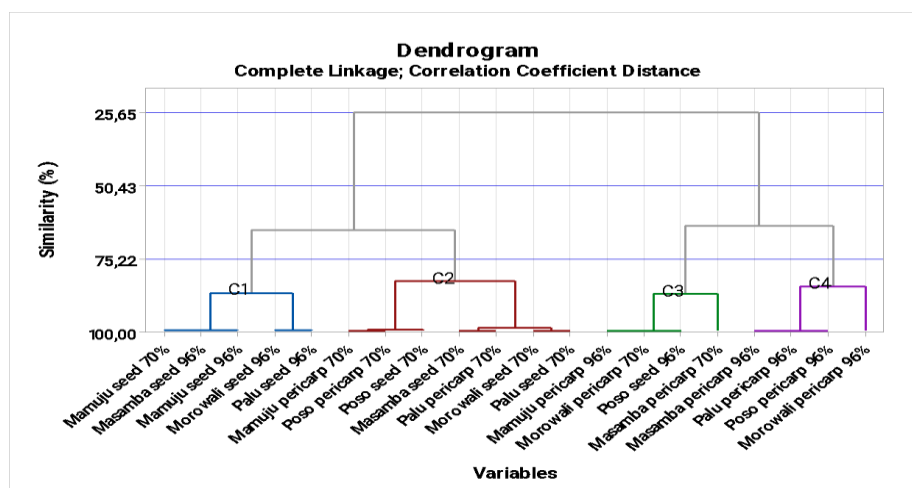

Figure 5. The dendrogram for classification of samples using cluster analysis. For members of each cluster C1, C2, …C14 are assigned with the same-colored line

2.3. Cluster analysis

Cluster analysis is one of the unsupervised pattern recognition systems in which variables and samples can be grouped based on Euclidean distance. Figure 5 reveals the dendogram obtained during the grouping of variables, i.e., the value of the peak area detected at a wavenumbers of 4000 - 400 cm⁻¹. The variations in Mm, Ma, Mo, Po, and Pa regions from Sulawesi Island with different extraction solvents using

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ethanol 96% and ethanol 70% extracts of 20 samples were classified into 4 clusters with the percentage of similarity between the 4 clusters of > 80%. The overall cluster had a similar rate of 25.65% - 99.99%. Cluster 1 (C1) Mm mangosteen seed extract 70% and 96%, Ma mangosteen seed extract 96%, Mo mangosteen seed extract 96%, and Pa mangosteen seed extract 96% similarity of 87.05%, cluster 2 (C2) Mm mangosteen pericarp extract 70%, Ma mangosteen seed extract 70%, Mo mangosteen seed extract 70%, Pa mangosteen seed and pericarp extract 70%, Po mangosteen seed and pericarp extract 70% similarity of 82.87%, cluster 3 (C3) Mm mangosteen pericarp extract 96%, Ma mangosteen pericarp extract 70%, Mo mangosteen pericarp extract 70%, and Po mangosteen seed extract 96% similarity of 87.24%, and cluster 4 (C4) Ma mangosteen pericarp extract 96%, Mo mangosteen pericarp extract 96%, Pa mangosteen pericarp extract 96%, and Po mangosteen pericarp extract 96% similarity of 84.62%.

3. CONCLUSION

FTIR spectroscopy combined with pattern recognition chemometrics of principal component analysis (PCA) and cluster analysis has been successfully applied for the fingerprinting profiling of mangosteen extracts from different origins in Sulawesi Island, Republic of Indonesia. PCA is also capable for the classification of mangosteen extracts according to the type of solvents and the fruit part based on the score plot of the first principle component (PC1) and second component (PC2). The combination of FTIR spectra and chemometrics is effective tools for the quality control of herbals, especially for the authentication of geographical origins.

4. MATERIALS AND METHODS

4.1. Materials

Mangosteen fruit was taken from the Mamuju (Mm), Masamba (Ma), Morowali (Mo), Palu (Pa), and Poso (Po) areas of Island Sulawesi. The reagents and solvents used were of pro-analytical grade with purity ≥ 98%. The part of the mangosteen taken was the pericarps and the seeds. The wet sorting was done to remove moldy and the damaged parts of the pericarps to avoid unwanted impurities. Dry sorting was carried out to separate contamination and damage due to mold. Then continued to cut into small pieces and carried out drying with the help of an oven with a temperature range of 40-50 C for 4-7 days. The samples were packed in plastic cefic simplicia and added silica gel, blended coarsely, and performed the extraction process [2].

4.2. Preparation extraction

Simplicia in the form of 20 g of seeds and 200 g of mangosteen pericarps from Mm, Ma, Mo, Pa, and Po, which have been dried, were blended and extracted by the maceration method with 96% and 70% ethanol solvent with a ratio of simplicia to solvents 1: 10 [22], for 3 x 24 hours every day stirred once a day. Then a liquid extract was obtained, and a filtering process was carried out with Whatman paper assisted by a vacuum pump. Evaporation was carried out with the help of a rotavapor (Buchi, Germany) with a rotation speed of 60-80 rpm, a temperature of 40-60 ºC, and a water bath of 2-3 hours. Obtained (1) Mm mangosteen seed extract 70%, (2) Mm mangosteen seed extract 96%, (3) Mm mangosteen pericarp extract 70%, (4) Mm mangosteen pericarp extract 96%, (5) Ma mangosteen seed extract 70%, (6) Ma mangosteen seed extract 96%, (7) Ma mangosteen pericarp extract 70%, (8) Ma mangosteen pericarp extract 96%, (9) Mo mangosteen seed extract 70%, (10) Mo mangosteen seed extract 96%, (11) Mo mangosteen pericarp extract 70%, (12) Mo mangosteen pericarp extract 96%, (13) Pa mangosteen seed extract 70%, (14) Pa mangosteen seed extract 96%, (15) Pa mangosteen pericarp extract 70%, (16) Pa mangosteen pericarp extract 96%, (17) Po mangosteen seed extract 70%, (18) Po mangosteen seed extract 96%, (19) Po mangosteen pericarp extract 70%, (20) Po mangosteen pericarp extract 96%.

4.3. Analysis of fingerprinting profiling extracts

FTIR spectra was obtained by scanning 20 samples of mangosteen pericarp and seed extracts. The ethanolic extract (5 mg) was mixed with 50 mg KBr and scanned with Perkin Elmer Spectrophotometer GX, with 16 scannings and 4 cm-1 resolution. The absorption data of FTIR spectra was scanned in the mid infrared region at wavenumbers of 4000 - 400 cm-1 [23].

4.4. Chemometrics analysis

The value of peak area data from 20 extracts of mangosteen pericarp and seed obtained from spectra FTIR were statistically analyzed using chemometric analysis. The software used was Minitab® version 18 (Minitab Incorporation, USA), performing a multivariate principal components analysis (PCA) and cluster analysis (CA). The absorbance values of FTIR spectra of Mangosteen extracts from different regions, estracted by maceration using ethanol 70% and 96% were used as variable.

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