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Rapid detection of synthetic adulterants in Indonesian herbal medicines using ATR-FTIR spectroscopy combined with chemometrics

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ABSTRACT: Herbal medicines have been gaining popularity as alternative medicines in recent years, including in Indonesia. Synthetic drug compounds can be illegally included in herbal medicines to achieve a faster effect. Detection of synthetic drugs in adulterated herbal products is important because of the negative side effects associated with short- and long-term use. Sildenafil, phenylbutazone, and sibutramine HCl are common adulterants in Indonesian herbal products intended to promote sexual arousal and performance, relieve muscle pain, and reduce weight. The aim of this study was the rapid and cost-effective detection of sildenafil citrate, phenylbutazone, and sibutramine HCl in Indonesian herbal products and herbal drug preparations by Attenuated Total Reflectance-Fourier Transmission Infrared Spectrometer (ATR-FTIR) spectroscopy combined with chemometrics. Herbs (aphrodisiac, muscle pain relieving and slimming herbs) and medicinal plants (Pasak Bumi (*Eurycoma longifolia* Jack.) roots, black pepper (Piper nigrum L.) leaves and West Indian Elm (*Guazuma ulmifolia* Lamk.) leaves) were intentionally adulterated with synthetic drug compounds in the range of 0-40% (w/w). ATR-FTIR spectra were recorded for all unadulterated and adulterated samples at 4000 - 650 cm-1. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to analyze the spectra of the unadulterated and adulterated samples. All samples were successfully classified with respect to their synthetic compound content. The presence of sildenafil, phenylbutazone and sibutramine can be rapidly detected with simple and non-destructive sample preparation using ATR-FTIR spectroscopy in combination with chemometrics. In conclusion, this method can be used for screening sildenafil citrate, phenylbutazone and sibutramine HCl adulterants in herbal products and herbal powders.

KEYWORDS: Adulteration; aphrodisiac herb; pain-relieving herb; principal component analysis; hierarchical cluster analysis.

1. INTRODUCTION

To date, herbal medicines have become promising choices for the prevention and treatment of different health problems [1]. The public increasingly prefers to consume herbal medicines due to their natural phytochemicals with beneficial pharmacological effects. In the market, herbal medicine products are promoted to be all-natural, thus claiming to be safe with fewer side effects. Aphrodisiac, pain-relieving, and

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slimming herbal products are by far among those most valued by people who struggle with sexual dysfunctions, muscle pain, and overweight problems. Some medicinal plants have long been proven to produce the aforementioned effects, such as pasak Bumi (*Eurycoma longifolia* Jack) as an aphrodisiac, black pepper (*Piper nigrum* L.) as a pain reliever, and West Indian elm/jati Belanda (*Guazuma ulmifolia* Lamk.) as an anorexiant. However, herbal products often take more time to produce effects compared to synthetic drugs. As a result, synthetic drugs including sildenafil, phenylbutazone, and sibutramine-HCl may be purposely added to the herbal products of, respectively, aphrodisiac, pain relieving, and slimming products in order to ensure claims of effective effects of the products.

Reports of the Indonesian Food and Drug Administration found increasing cases of synthetic drug adulterations including sildenafil citrate, phenylbutazone, and sibutramine-HCl adulterations in herbal products [2]. Adulteration of traditional medicines with synthetic drugs is prohibited by the Indonesian government's statutory regulation [3,4]. The undeclared content of these drug compounds in herbal products can be dangerous and life-threatening. Not only can they cause potential drug interactions, uncontrolled dosages of these synthetic adulterants may also lead to adverse side effects and harmful medical conditions. Thus, there is an urgent need for a rapid and effective technique for the detection of sildenafil, phenylbutazone, and sibutramine in herbal products.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is a fast, inexpensive, and nondestructive analytical tool to obtain chemical fingerprints of molecular structures [5]. This spectroscopy technique allows direct measurement of samples in their solid and liquid states without the need for further sample preparation. Moreover, variations in the particle size of the samples can be ignored [6,7]. To date, ATR-FTIR has been used to identify adulterants in plant material [8]. Recently, studies indicated the potential use of ATR-FTIR for the detection of sibutramine in herbal tea and green coffee products [9]. The disadvantages of ATR-FTIR include difficulties in visual and direct interpretation of spectra due to overlapping absorption spectra of molecules in the sample [10]. Various chemometric techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been used to analyze IR spectra and classify samples to enable the identification of adulterants in products [11].

The aim of this study was to identify chemical adulterants, i.e., sildenafil citrate, phenylbutazone, and sibutramine HCL, in adulterated herbal products and herbal drug preparations using ATR-FTIR in conjunction with chemometric methods such as PCA and HCA.

2. RESULTS AND DISCUSSION

ATR-FTIR combined with chemometric analysis resulted in rapid analysis of synthetic adulterants in herbal products and herbal powders. The use of FTIR spectroscopy required minimal sample preparation, as the herb samples were simply placed in a crystal plate and compressed prior to measurements. The spectra showed unique vibrational bands for each sample, reflecting important information about specific functional groups. However, the IR spectral data are difficult to interpret and use for adulteration analysis in herbal preparations because of overlapping absorption spectra of molecules in the samples. Therefore, chemometric analysis in conjunction with ATR-FTIR was used for adulteration detection.

2.1. ATR-FTIR spectral analysis of sildenafil, sildenafil-adulterated, and non-adulterated samples

The characteristic absorption peaks for sildenafil citrate according to Champagne *et al.* is in the range 1720-1150 cm^{-1} [12]. As seen in Figure 1, the standard sildenafil citrate spectrum shows significant absorption peaks at 1698 cm^{-1} (carbonyl group (C=O stretch)), 1579 cm^{-1}, and 1489 cm^{-1} (aromatic C=C bonds of the benzene ring); saturated C-H strain at 2874 cm^{-1}, unsaturated C-H strain at 3028 cm^{-1}, and secondary N-H strain is at 3294 cm^{-1}. The aromatic C-H out-of-plane deformation occurred at 939 cm^{-1}, which resulted in the addition of new peaks at 1171, 784, 691, and 587 cm^{-1} [13-16].

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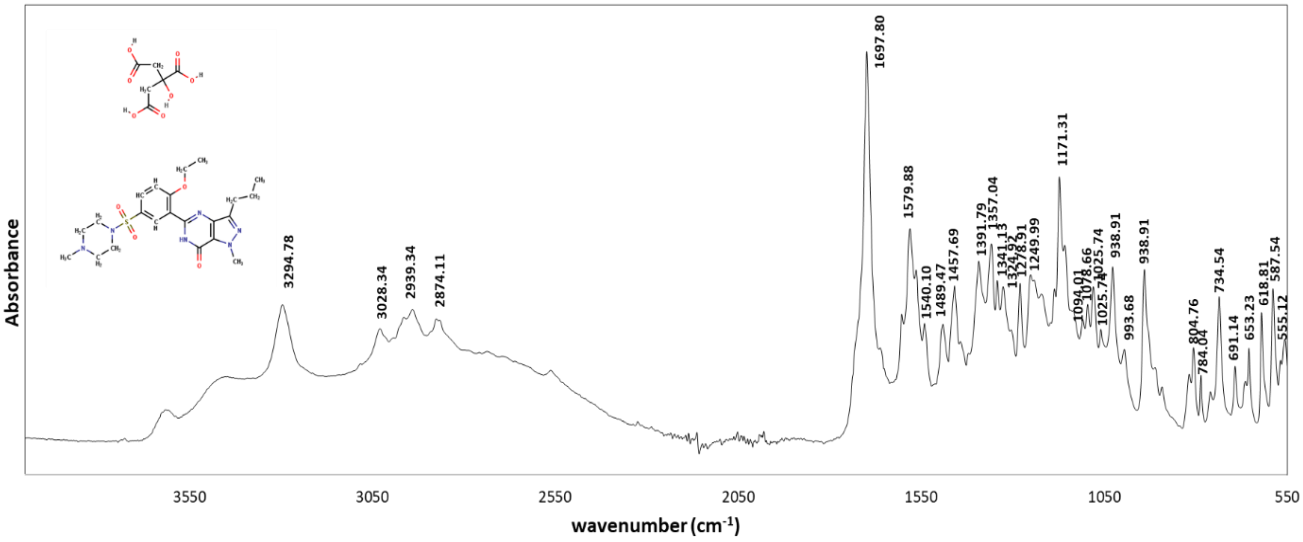

Figure 1. ATR-FTIR spectrum of sildenafil citrate

Figure 2 shows ATR-FTIR spectra of 11 samples of non-adulterated and adulterated aphrodisiac herbs and pasak Bumi (*E. longifolia* Jack.) root powder. Absorption spectra of non-adulterated samples of aphrodisiac herbs and pasak Bumi root powder did not show any strong absorption in 1697 cm^{-1} which indicates the carbonyl group (C=O stretching). Absences of peaks around 938 cm^{-1} were also observed. The presence of sildenafil citrate can be clearly seen by peaks around 1697 and 938 cm^{-1} which were absent in the spectra of non-adulterated pasak Bumi root powder and aphrodisiac herbs samples.

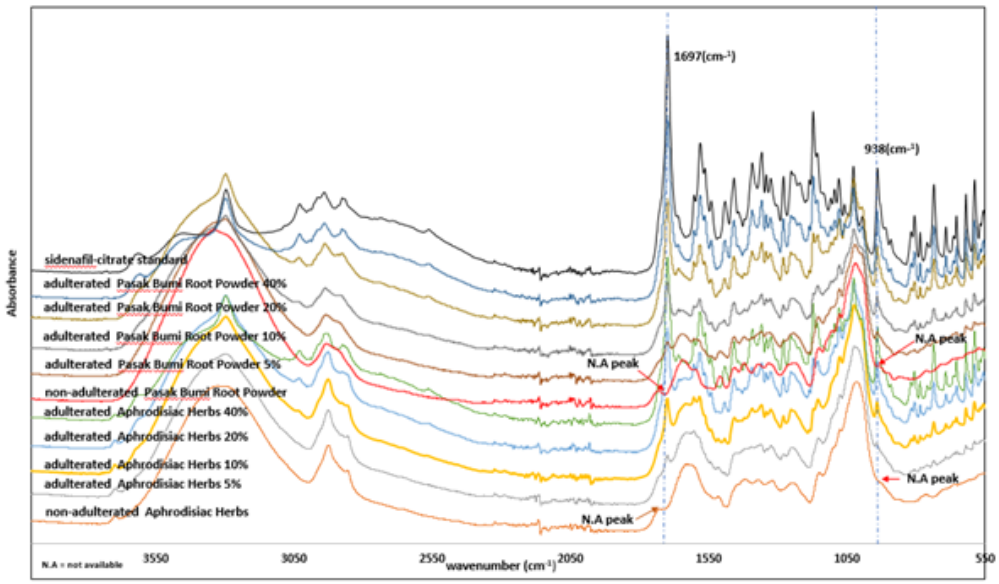

Figure 2. ATR-FTIR spectra of non-adulterated and adulterated pasak Bumi (*E. longifolia* Jack.) root powder and aphrodisiac herbs samples.

2.2. ATR-FTIR spectra analysis of phenylbutazone, phenylbutazone-adulterated, and non-adulterated samples

As seen in Figure 3, the standard phenylbutazone spectrum showed significant absorption peaks at 1712 cm^{-1} and 1750 cm^{-1} (carbonyl group (C=O stretching)); C-H strain at 751 cm^{-1} and 1486 cm^{-1}; and C-N strain at 1292 cm^{-1}. The spectrum corresponds well to the phenylbutazone spectrum tested by Waters *et al* in their study [17].

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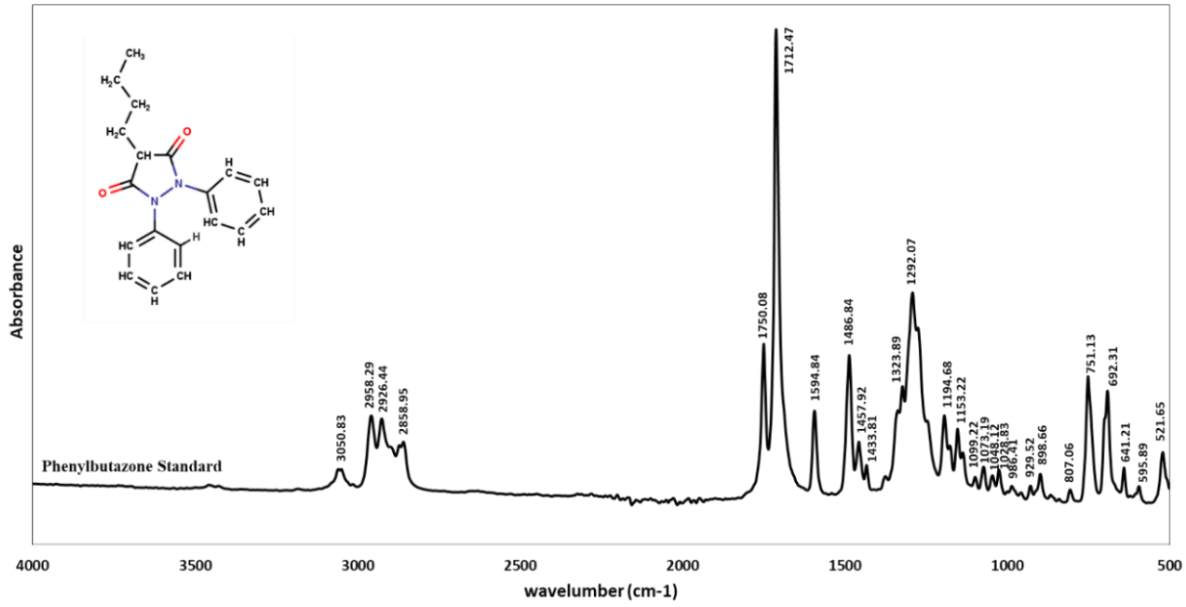

Figure 3. ATR-FTIR spectrum of phenylbutazone standard.

Figure 4 shows the overlaid ATR-FTIR spectra of non-adulterated and adulterated samples of black pepper powder and muscle pain herbs in comparison with the standard phenylbutazone. Non-adulterated samples of black pepper powder and muscle pain herbs did not show any strong absorption in the range of 1712– 1750 cm^{-1} which are related to the carbonyl group (C=O stretching). Peaks around 692, 751, and 1292 cm^{-1} were also absent. In contrast, spectra of phenylbutazone-adulterated samples of black pepper powder and muscle pain herbs identified the appearance of peaks around 1712 – 1750 cm^{-1}, 692 cm^{-1}, 751 cm^{-1}, and 1292 cm^{-1}.

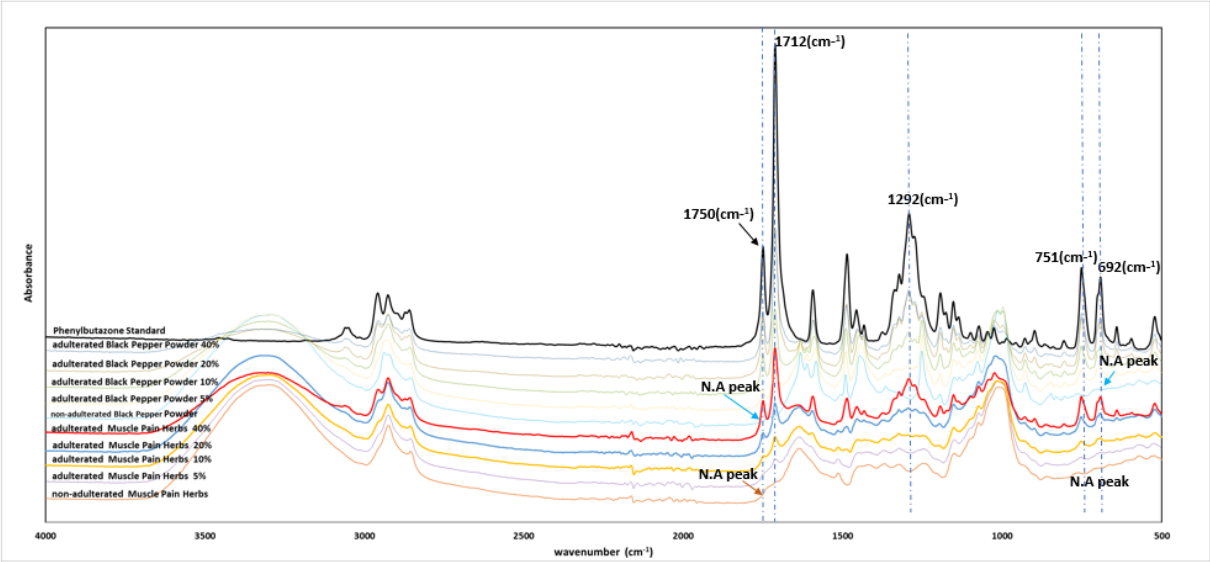

Figure 4. ATR-FTIR spectra of non-adulterated and adulterated black pepper powder and muscle pain herbs samples.

2.3. ATR-FTIR spectra analysis of sibutramine, sibutramine-adulterated, and non-adulterated samples

The ATR-FTIR spectrum of sibutramine is shown in Figure 5. Significant vibrational bands were observed at 3418, 2945, 2866, 2698, 1490, 1472, 1370, 1091, 1010, and 822 cm^{-1}, corresponding to functional

groups in sibutramine structure. The strongest peak was observed at 3418 cm^{-1}, which corresponded to the asymmetric and symmetric stretching vibrations of O-H groups of water molecules [18-20]. The spectrum of sibutramine exhibited strong bands in the region between 3000 and 2500 cm^{-1}. Peak points at 2963 and 2865 cm^{-1} corresponded with asymmetric and symmetric stretching vibrations of CH_3, CH_2, and CH functional groups, whereas the peak at 2698 cm^{-1} was associated with stretching vibrations of N-H groups [21]. Important peaks resulted from the aromatic ring of the sibutramine structure were also observed. The peak at 1490 cm^{-1} was due to C=C stretching vibrations of the aromatic ring, whereas the peak at 1407 cm^{-1} was associated with the NCH_3 bending vibrations. The peak observed at 1370 cm^{-1} was related to isobutyl CH_3 bending vibration, and the peak at 1010 cm^{-1} correlated with *p*-substituted aromatic stretching. Peaks in the range of 1225-950 cm^{-1} were related to C-H in-plane bending in the aromatic ring, and at 833 cm^{-1} and 822 cm^{-1} resulted from aromatic C-H out-of-plane bending vibrations [18-19][21].

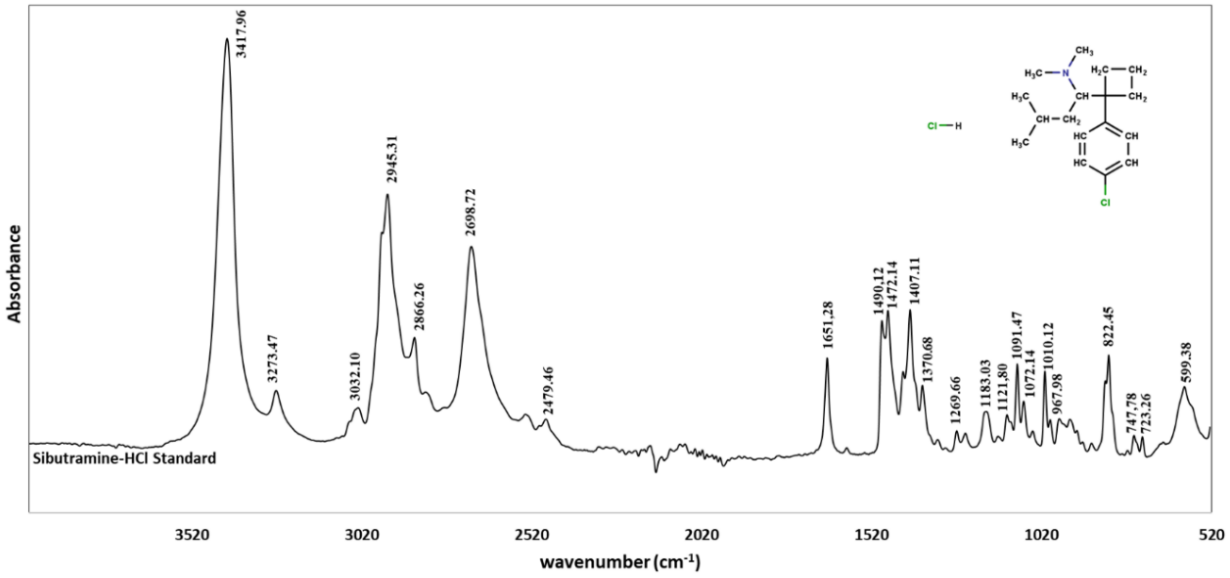

Figure 5. ATR-FTIR spectrum of sibutramine-HCl standard

Figure 6 shows ATR-FTIR spectra of non-adulterated and adulterated samples of west Indian elm/jati Belanda (*G. ulmifolia* Lamk.) and slimming herbs samples, in comparison with sibutramine-HCl standard. All spectra of non-adulterated and adulterated samples showed fairly similar-looking peaks, therefore it was difficult to identify the differences. However, there were minor changes with regard to the broadness and shifting of the peaks when spectra of non-adulterated samples were compared with adulterated samples, which can be noted at 3418 cm^{-1}.

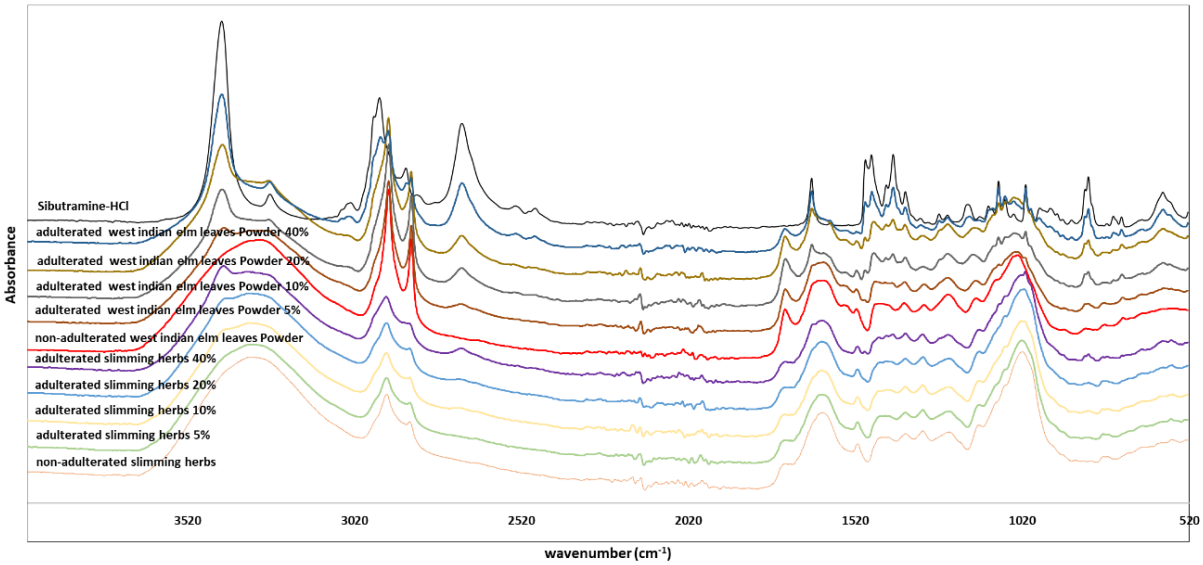

Figure 6. ATR-FTIR spectrum of non-adulterated and adulterated West Indian elm/jati Belanda (*G. ulmifolia* Lamk.), slimming herbs samples, and sibutramine-HCl standard.

2.4. PCA and clusterization of non-adulterated and adulterated samples

The present study provides assessments of adulteration of sildenafil, phenylbutazone, and sibutramine in herbal products and plant medicine preparations. For this purpose, PCA and HCA were employed to process ATR-FTIR spectral data for discrimination between non-adulterated and adulterated samples.

Chemometrics analysis was first performed for non-adulterated and sildenafil-adulterated aphrodisiac herb and pasak Bumi root powder. Over-layed spectral profiles (Figure 2) showed clear differences among sildenafil, non-adulterated, and sildenafil-adulterated samples in the spectral region of 3294 – 555 cm^{-1}. This spectral region provides information on vibrations related to chemical data in the samples. In the previous section, characteristic vibrational bands of sildenafil were described (Figure 1), in particular at around 1697 and 938 cm^{-1}, which were not seen in non-adulterated aphrodisiac herb and pasak Bumi root powder.

Principal components analysis (PCA) of non-adulterated and adulterated samples was built based on sildenafil vibrational bands in the region of 3294–555 cm^{-1} (Figure 1). The PCA plot presented in Figure 7 represents the score plots of the first (F1) and the second (F2) components. The F1 component represented 88.33% of the total data variance, whereas F2 accounted for 10.73% of the total data variance. Thus, the total variance explained by these two components accounted for 99.07% of the total variance and only 0.94% variance was not explained with the remaining components. This indicates that by the score plots of F1 and F2 components, non-adulterated and adulterated samples of aphrodisiac herbs and pasak Bumi (*E. longifolia* Jack.) root powder are well discriminated.

A clear separation was observed for the score plots of F1 and F2 of non-adulterated aphrodisiac herbs and adulterated aphrodisiac herbs at concentrations 5, 10, 20, and 40% (Figure 7). Likewise, plots for non-adulterated pasak Bumi root powder and adulterated pasak Bumi root powder at concentrations of 5, 10, 20, and 40% gave diverse values, and are well separated.

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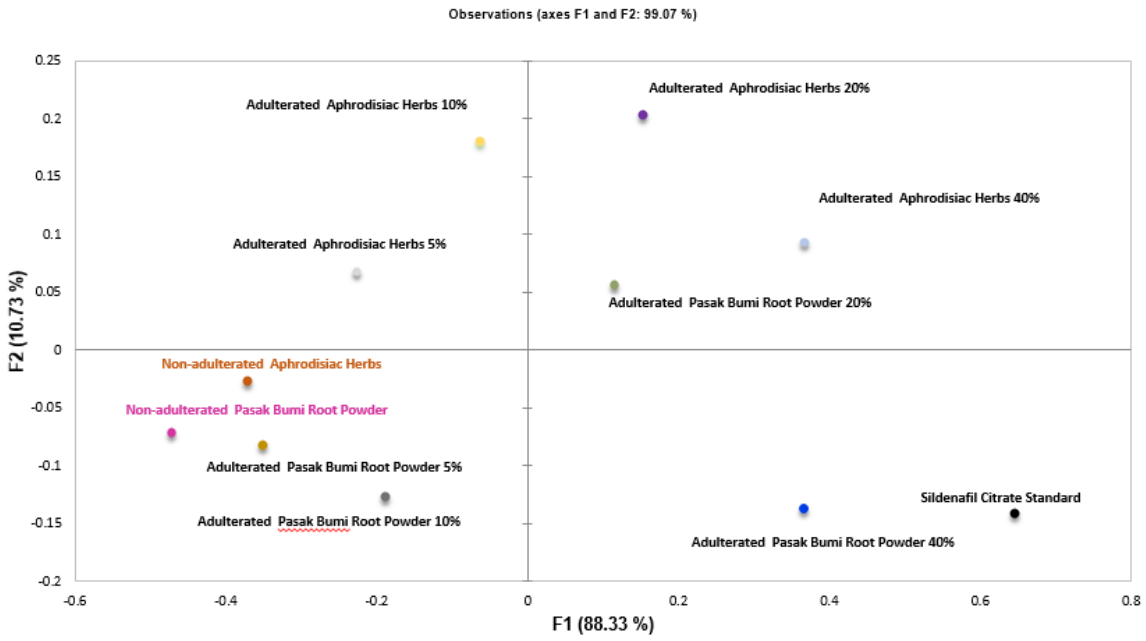

Figure 7. Score plot F1 (proportion 88.33%) and F2 (proportion 10.73%) for non-adulterated and adulterated pasak Bumi (*E. longifolia* Jack.) root powder and aphrodisiac herbs samples.

Figure 8 shows the visualization of hierarchical clusters of sildenafil-adulterated and non-adulterated samples in the form of a dendrogram. The dendrogram of AHC analysis shows that non-adulterated aphrodisiac herb, non-adulterated pasak Bumi root powder, and adulterated aphrodisiac herbs (5%) formed the first cluster. Adulterated aphrodisiac herbs (5, 10, and 20%), and adulterated pasak Bumi root powder (10 and 20%) formed the second cluster. Adulterated aphrodisiac herbs (40%) and adulterated pasak Bumi root powder (40%) formed the third cluster. The hierarchical clustering by AHC indicates that the powder of non-adulterated and adulterated pasak Bumi (*E. longifolia* Jack.) root powder and aphrodisiac herbs samples are clearly dissimilar.

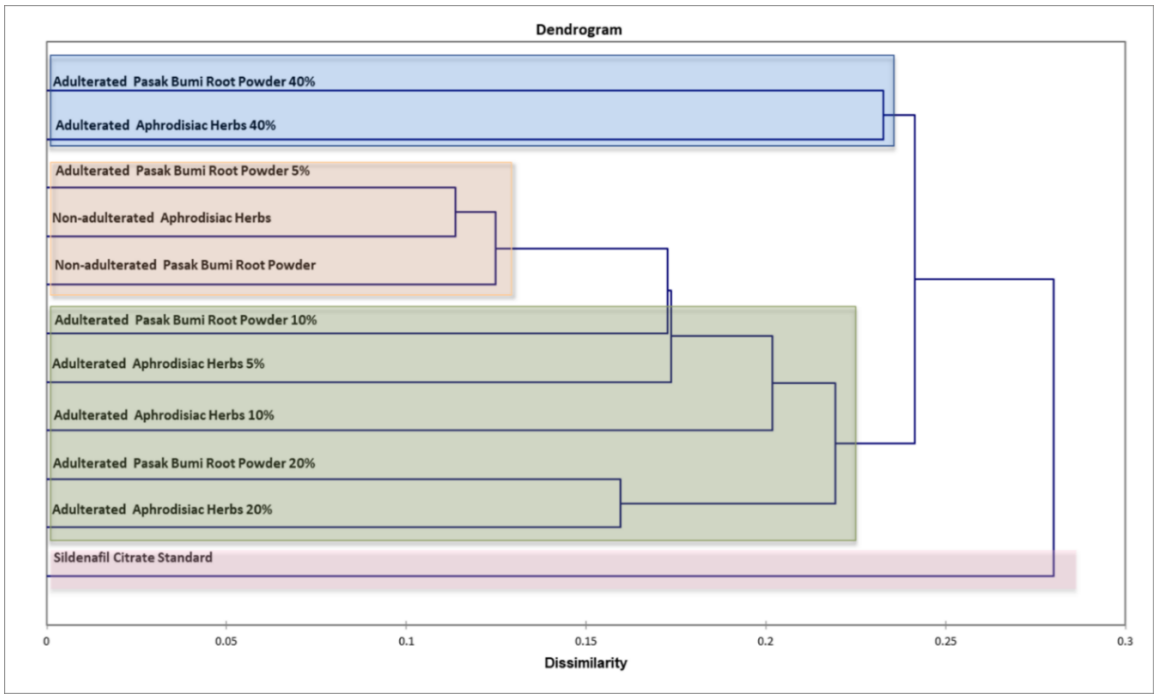

Figure 8. The AHC dendrogram for sildenafil citrate standard, non-adulterated and adulterated aphrodisiac herbs (5, 10, 20, 40%), and non-adulterated and adulterated pasak Bumi root powder (5, 10, 20, and 40%).

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Non-adulterated and phenylbutazone-adulterated samples

PCA was also performed for non-adulterated and adulterated samples of muscle pain herbs and black pepper (*P. nigrum* L.) powder. The wave numbers used for PCA were in the range of 3050 to 461 cm^{-1}, which were informative of the functionalities of the samples (Figure 3). The score plot analysis is presented in Figure 9. For these sets of samples, F1 component represented 93.02% of the total data variance. Whereas F2 component represented 3.73% of the total data variance. Hence, they accounted for 96.75% of the total variance.

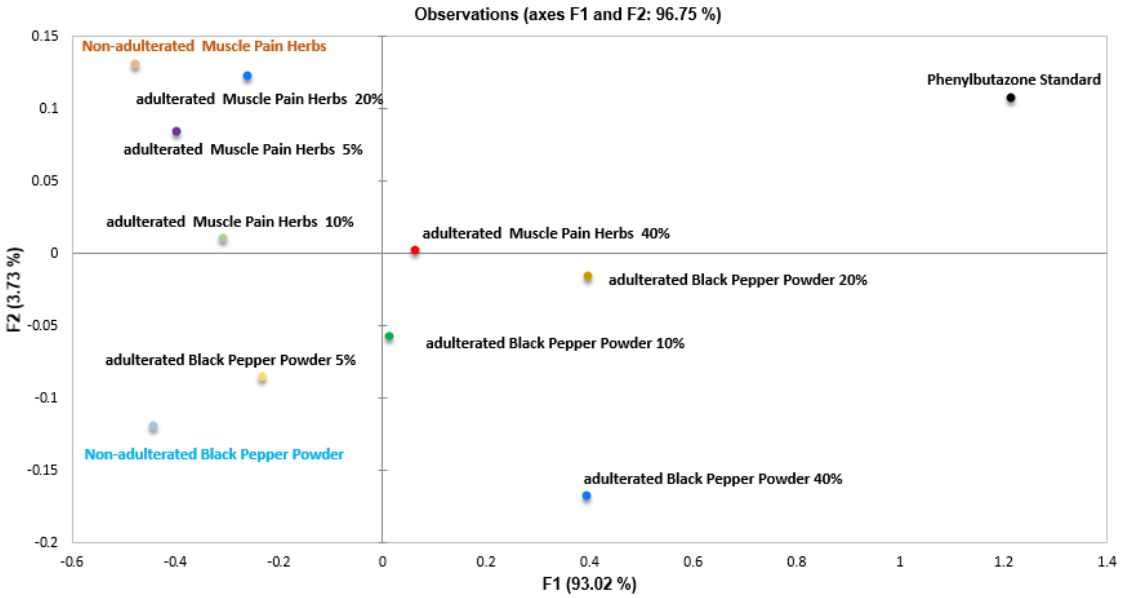

Figure 9. Score plot F1 (proportion 93.0%) and F2 (proportion 3.7%) for non-adulterated and adulterated black pepper (*Piper nigrum* L.) powder and muscle pain herbs samples

The score plots of F1 and F2 of the standard phenylbutazone were clearly separated from non-adulterated samples of black pepper (*P. nigrum* L.) powder and muscle pain herbs (Figure 9). The score plots of non-adulterated samples of black pepper powder and muscle pain herbs and adulterated muscle pain herbs at concentrations 5, 10, 20, and 40% were of the same group, however, the plots came up apart, showing clear separation. Likewise, the score plots for non-adulterated black pepper powder and adulterated black pepper powder at concentrations of 5, 10, 20, and 40% had the tendency to be of the same group although good separation was clearly observed.

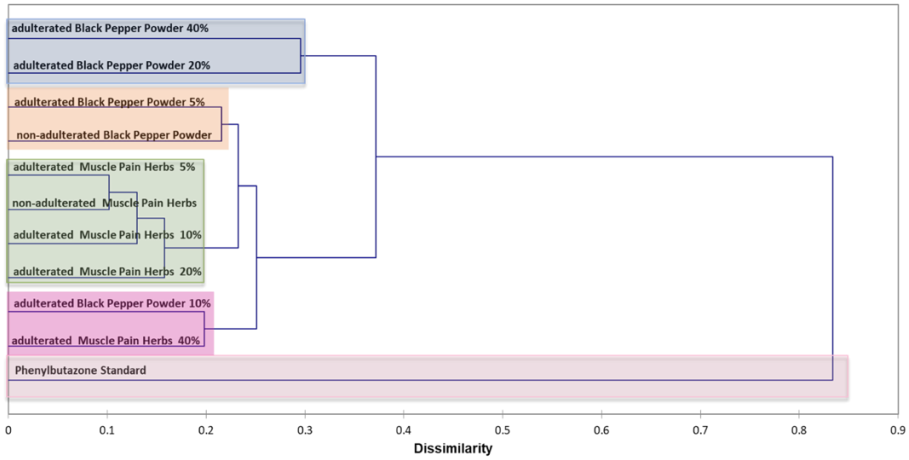

Figure 10. The AHC dendrogram for phenylbutazone standard, non-adulterated and adulterated muscle pain herbs (5, 10, 20, 40%), and non-adulterated and adulterated black pepper powder (5, 10, 20, and 40%).

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The AHC dendrogram of these samples shows clustering at different stages. Non-adulterated muscle pain herbs and adulterated muscle pain herbs at concentrations 5, 10, and 20% formed the first cluster. Adulterated black pepper powder 10% and adulterated muscle pain herbs 40% formed the second cluster. Adulterated black pepper powder 5% and non-adulterated black pepper powder formed the third cluster. Adulterated black pepper powder at 20 and 40% formed the fourth cluster. It is noted that non-adulterated, adulterated black pepper (*P. nigrum* L.) powder 40% and muscle pain herbs 20% samples show dissimilarities (in Figure 10).

Non-adulterated and sibutramine-adulterated samples

PCA was also applied to assess spectral data of non-adulterated and adulterated samples of West Indian elm/jati Belanda (*G. ulmifolia* Lamk.) powder and slimming herbs samples. The vibrational peaks in the range of 3417 – 599 cm^{-1} were used for PCA analysis. The calculated variance of F1 accounted for 88.56% of the total data variance, and F2 represented 8.58% of the total variance (Figure 11). Thus, the components accounted for 97.14% of the total data variance, suggesting only 2.86% of the variance was not accounted for.

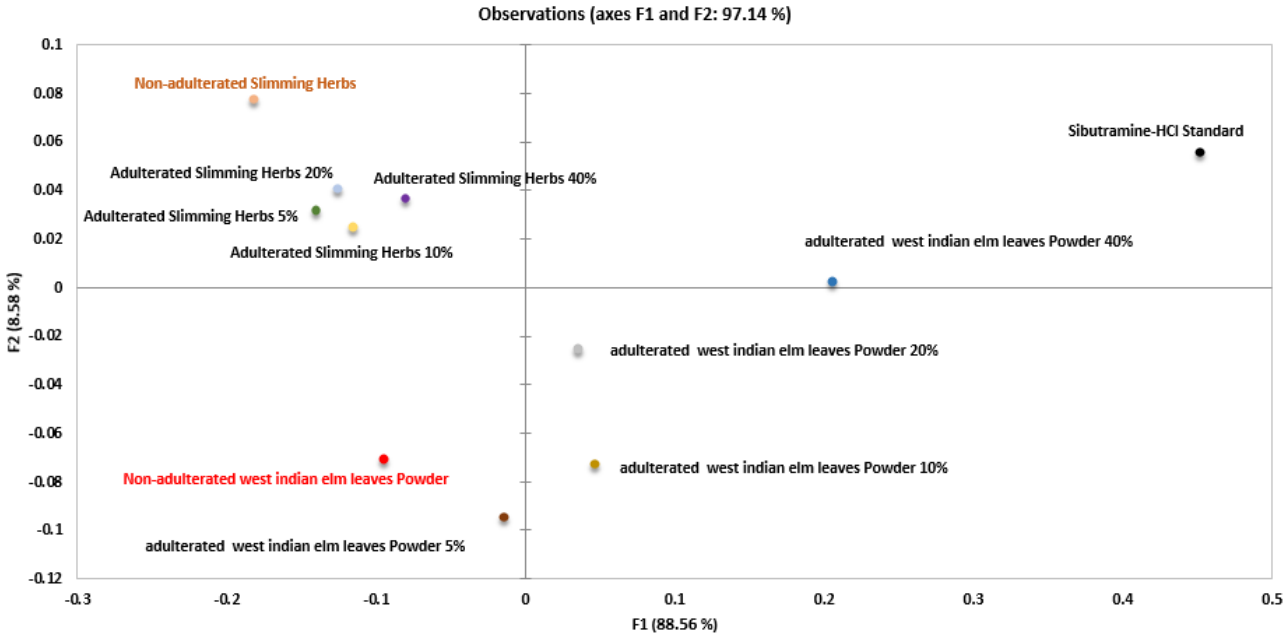

Figure 11. Score Plot F1 (proportion 88.56%) and F2 (proportion 8.58%) for non-adulterated and adulterated West Indian elm/jati Belanda (*G. ulmifolia* Lamk.) and slimming herbs samples.

As can be seen in Figure 12, adulterated West Indian elm leaf powder of concentrations 5, 10 and 20 % and non-adulterated West Indian elm leaf powder formed the third cluster. The observation clearly indicates that non-adulterated and adulterated slimming herbs and adulterated West Indian elm leaf powder have dissimilarity.

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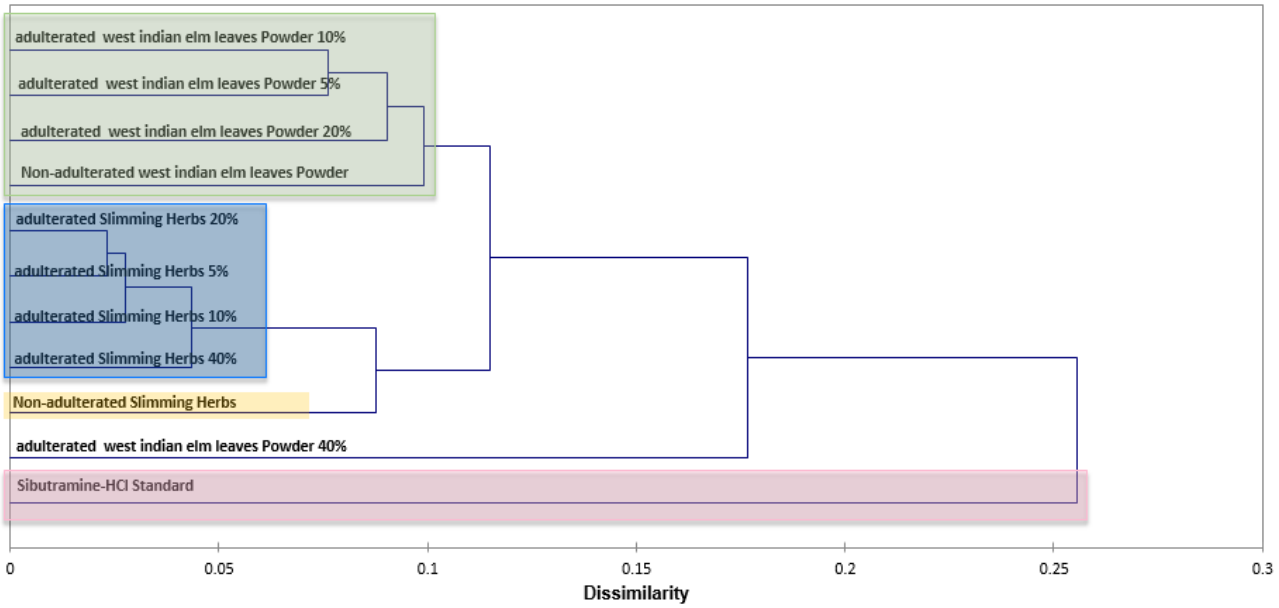

Figure 12. The AHC dendrogram for sibutramine standard, non-adulterated and adulterated slimming herbs (5, 10, 20, 40%), and non-adulterated and adulterated West Indian elm leaf powder (5, 10, 20, and 40%).

4. CONCLUSION

In the present study, ATR-FTIR spectroscopy was used in combination with two chemometric techniques (PCA and AHC) for the detection of sildenafil, phenylbutazone, and sibutramine adulteration in herbal herbs and herbal powder, intended for aphrodisiac, pain relief, and slimming agents, respectively. The methods can differentiate the variations in the concentration of chemical adulterants included in herbal herbs and pulverized herbal materials. The study recommends the use of FTIR spectral data for rapid screening for synthetic adulterants in herbal herbs and plant medicine preparations.

5. MATERIALS AND METHODS

5.1. Standards, herbal products, and herbal powder

Standards sildenafil citrate, phenylbutazone, and sibutramine HCl were purchased from Sigma Aldrich (St Louis, USA). Herbal products included in the present study i.e. aphrodisiac, muscle pain-relieving, and slimming herbs. The roots of pasak Bumi (*E. longifolia* Jack), fruits of black pepper (*P. nigrum* L.), leaves of West Indian elm (*G. ulmifolia* Lamk.), each plant material was dried and pulverized using a grinder until a fine powder was obtained.

5.2. Sample preparation

Three sets of samples were prepared consisting of standard synthetic drugs, non-adulterated samples (herbal products and herbal powder), and adulterated samples (Figure 1). The adulterated sample set was prepared by intentionally adulterating aphrodisiac herb and pasak Bumi leaf powder with sildenafil citrate, muscle pain-relieving herb, and black paper fruit powder with phenylbutazone, and slimming herb and West Indian elm leaf powder with sibutramine HCl. Adulterated samples were prepared by mixing and crushing each component in a mortar to obtain homogenous preparations at different concentrations (5, 10, 20, and 40% (w/w)). The mixtures were placed in aluminum-coated vials and stored in a dry place until further use.

5.3. Data acquisition

A Bruker (type of instrument) FTIR equipped with a single bounce diamond crystal ATR accessory was used to record IR spectra. The IR spectra were recorded from 4000 to 525 cm^{-1} at a spectral resolution of 4 cm^{-1}, accumulating 32 scans per spectra. Spectrum scanning for each sample was repeated thrice to obtain an average spectrum. This arrangement must be maintained throughout the measurement of all samples and

standards. The sample or standard powder (approximately 5 mg) was placed on an ATR crystal plate. Following each sample measurement (in Figure 13), the crystal was cleaned with methanol and dried at ambient temperature. A blank sample was then taken to check for contamination. In between samples, a background air spectrum was read under the same instrument conditions as for the samples.

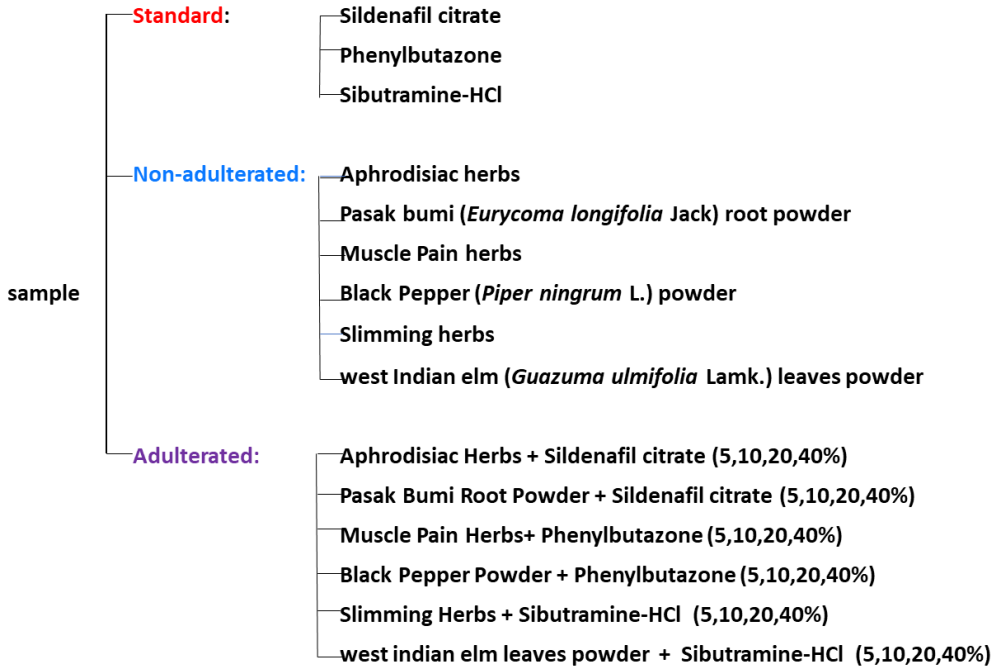

Figure 13. Samples used in this study consisted of synthetic drug compounds, non-adulterated, and adulterated samples.

5.4. Chemometric analysis

In the present study, multivariate methods including agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) were applied to analyze FTIR spectral data for the PCA and clusterization between adulterated and non-adulterated samples [7,22-23]. PCA was performed to evaluate whether adulterated and non-adulterated samples were able to be discriminated based on the content of their respective adulterant. PCA was conducted using Microsoft Excel- XLSTAT software (2019.2.2 Addinsoft, New York, NY, USA). The PCA model was developed using particular spectral ranges which contain significant bands related to the chemical functionalities in the samples.

AHC analyzed ATR-FTIR spectral differences between adulterated and nonadulterated samples by monitoring related clusters and sub-clusters of which the samples were scattered. AHC was carried out using Microsoft Excel- XLSTAT software (2019.2.2 Addinsoft, New York, NY, USA). A hierarchy of clusters and sub-clusters was visualized in dendrogram graphs and Euclidean distance and the Single linkage method were used as the distance measure.

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Conflict of interest statement: The authors declare no conflict of interest.

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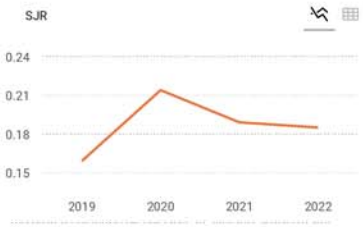

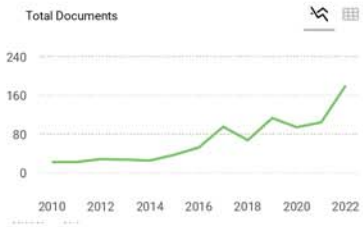

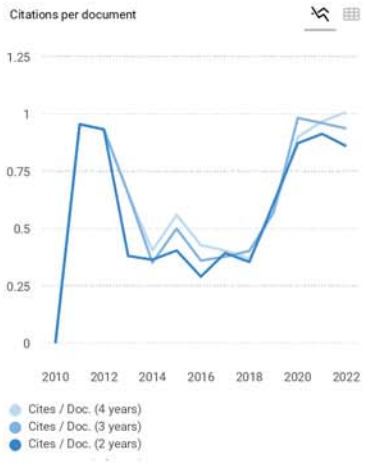

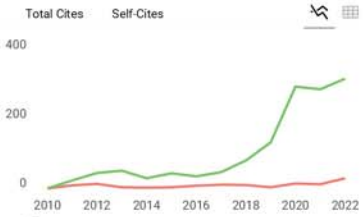

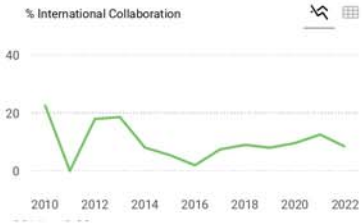

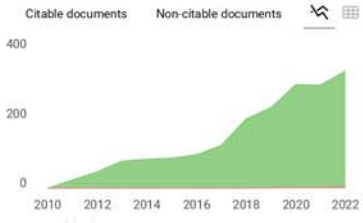

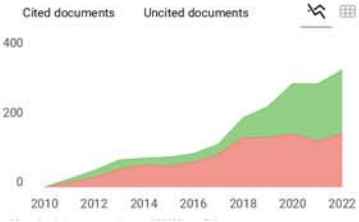

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