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Discrimination of Herbal Products from Zingiberaceae Family Using Electric Nose Combined with Chemometric Techniques

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Many herbs from the Zingiberaceae family are widely used as raw materials in herbal products. Some of them have a similar colour for their rhizomes and are quite hard to distinguish from each other, so it is possible to misidentify one for the other. Therefore, it is necessary to find a rapid and reliable method for discrimination of Zingiberaceae-based products. The present work investigates the possibility of applying electric nose (e-nose) combined with chemometric techniques for discrimination of herbal products from the Zingiberaceae family. Nine powdered samples consisting of *Alpinia galanga*, *Alpinia conchigera*, *Curcuma longa*, *Curcuma zedoaria*, *Curcuma xanthorrhiza*, *Zingiber officinale*, *Zingiber zerumbet*, *Kaempferia galangal*, and *Kaempferia pandurata* were analysed by using e-nose. As a result, e-nose identified several volatile compounds for each sample. Next, the responses from e-nose were used for principal component analysis (PCA) and discriminant function analysis (DFA) to visualise the dataset using score plots. The result demonstrates that the combination of e-nose and PCA and DFA could discriminate the samples, in which DFA gave clearer discrimination between all samples. This combination can be used for rapid discrimination of Zingiberaceae-based products, which could contribute to the quality control in the herbal industry's supply chain.

Key words: Chemometric; electric nose; herbal product; volatile compounds; Zingiberaceae

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The Zingiberaceae family is well known for its medicinal value and is widely distributed throughout the tropical areas. About 23 genera and 200 species in Peninsular Malaysia have been reported in a previous study [1]. Zingiberaceae has gained scientific attention to its curative property for many chronic diseases like osteoarthritis, inflammatory pain and major depressive disorder (MDD) [2]. Based on their beneficial nature, the rhizomes of Zingiberaceae are commonly used as raw materials in various herbal products [3]. The most widely utilised genera in this family are the Alpinia, Curcuma, Kaempferia and Zingiber varieties [4].

Every species of Zingiberaceae has its unique effects upon human consumption. Therefore, it is vital to ensure that the species used is correct before processing. Accurate identification of herbal species is a major concern since it directly impacts on the safety and effectiveness of the herbal products [5]. However, the identification and discrimination of Zingiberaceae species are quite hard due to narrow differences in the physical appearance and colour of the rhizomes, especially after processing such as drying, grinding and isolating [6].

Electric nose (e-nose) is a device that "smells" or detects a sample's odour [1]. At present, e-nose provides some versatility in applications of various fields [7]. This technology's strengths are short analysis time, cost effectiveness, and high sensitivity [8]. Recently, there have been studies of e-nose for effective discrimination of wine [9], tea [10] [11], cheese [12] and milk [13]. To the best of our knowledge, the combination of e-nose and chemometric techniques has not yet been applied to discriminate herbal products from the Zingiberaceae family. Therefore, this study attempts to evaluate this technology's applicability combined with chemometric techniques to discriminate nine herbal products from the Zingiberaceae family.

MATERIALS AND METHODS

1. Experimental Materials

This study was performed on nine species samples from the Zingiberaceae family which consisted of *Alpinia galangal*, *Alpinia conchigera*, *Curcuma longa*, *Curcuma zedoaria*, *Curcuma*

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xanthorrhiza, *Zingiber officinale*, *Zingiber zerumbet*, *Kaempferia galangal* and *Kaempferia pandurata*. Fresh plants were collected from Kompleks Pertanian Serdang in February 2020. After cleaning and sorting, only the rhizomes of these herbal plants were used in this study. All the rhizomes were cleaned and cut into small pieces before being dried overnight in the oven (37°C). Then, the dried rhizomes were ground by using a blender (7011HS, Waring, USA) into powder size (250 µm). The samples were stored in airtight containers and placed at room temperature until future analysis.

2. Electric Nose Analysis

This study used an electronic nose (Heracles, Alpha M.O.S., France) to discriminate the odour patterns of the different samples. First, 0.5 g of each sample was accurately weighed in a 20 mL vial. The vial was then tightly capped with a silicone seal to prevent the penetration of environmental odours. The headspace in the vial was tgen examined by the e-nose based on the parameters shown in Table 1 [14]. Each sample was run in six replicates with an empty vial (blank run) between each replicate. Alkane solution C_6 to C_{16} (Restek, Bellefonte, USA) was used as the standard. All the response data from the e-nose analysis was further analysed by AroChemBase software (V6, Alpha M.O.S, Toulouse, France) which was integrated with the Heracles e-nose. AroChemBase is a Kovats indices library featuring chemical characterisation with sensory information linked to the NIST database. Lastly, the volatile compounds of the samples were identified by comparing the relevant indices given to each volatile compound by the same software and then comparing the experimental Kovats indices with the Kovats indices found in the AroChemBase library.

3. Chemometric Data Analysis

Chemometric techniques are essential to extract important information because of the large number of peaks obtained from the e-nose analysis [15]. The responses from the e-nose analysis were analysed chemometrically using principal component analysis (PCA) and discriminant function analysis (DFA). All data analyses were carried out using the AlphaSoft software (v12.4, Alpha M.O.S., Toulouse, France). This software has a full chemometrics package for data processing, including PCA and DFA. These techniques were performed to explore the presence of outliers [14]. Both have their specific advantages and characteristics.

The primary goal of PCA is to reduce the variables of valuable information from overlapping chemical information [16]. This unsupervised statistical model helps to visualise data patterns of the data obtained from analytical analysis [14]. In contrast, DFA is a supervised pattern recognition method based on the sample's proximity to the group's centre of gravity [17]. It is used to determine which continuous variables discriminate between two or more naturally occurring groups [18].

RESULTS AND DISCUSSION

1. Detection of Volatile Compounds by Electric Nose Analysis

The application of e-nose was suitable for the detection of volatile compounds in herbal products due to its selectivity and sensitivity. This type of e-nose is based on dual flash gas chromatography technology to analyse both olfactory (smell and aroma) fingerprint of the sample and its chemical compounds [7]. With the provided database library incorporated in the e-nose instrument software, the volatile compounds were identified and classified according to their Kovats indices. Besides the chemical data, the sensory information for each volatile compound in the sample was also identified. Table 2 shows the volatile compounds and sensory information in the nine Zingiberaceae samples detected using e-nose.

Table 1. Analysis parameters for e-nose system.

Parameter	Condition
Column length (m)	10
Injection volume (µL)	1000
Injection speed (µL/s)	125
Incubation temperature (°C)	50
Incubation time (min)	20
Initial oven temperature (°C)	50
Trapping temperature (°C)	40
Valve temperature (°C)	259
Acquisition duration (s)	110
Acquisition period (s)	0.01
Agitation speed (rpm)	500
Carrier gas	Hydrogen

Table 2. Volatile compounds detected in the samples of Zingiberaceae.

Retention time (min)	Kovats index (exp)[i]	Kovats index (library)[ii]	Volatile compound	Sensory information	Occurrence[iii]
54.31	939	937	3-Hepten-2-one	Green	*Z. officinale*
54.35	940	943	1S-α-Pinene	Herbaceous, terpenic	*C. zedoaria*, *C. xanthorrhiza*
56.64	969	979	3-Ethyloctane	Green, musty, turpentine, woody	*Z. officinale*, *Z. zerumbet*
56.76	970	970	β-Pinene	Green, musty, turpentine, woody	*A. galangal*, *A. conchigera*, *C. zedoaria*, *C. xanthorrhiza*
58.31	990	988	1,5-Octadienone	Earthy, musty	*Z. zerumbet*
58.68	995	996	Ethyl hexanoate	Fruity, wine gum	*C. longa*, *C. xanthorrhiza*, *K. galangal*, *K. pandurata*
58.72	997	997	1,3,5-Trimethylbenzene	Aromatic, herbaceous	*Z. zerumbet*
59.01	999	998	Myrcene	Fruity, metallic, soapy	*A. conchigera*
60.43	1020	1023	Acetypyrazine	Nutty, roast	*C. zedoaria*
62.72	1055	1060	γ-Terpinene	Fruity, sweet, turpentine, terpenic	*A. galangal*, *A. conchigera*, *C. zedoaria*
66.03	1106	1106	2-Acetylhiazoline	Roast	*K. galangal*
69.36	1165	1149	Benzoic acid	Urine, winey	*C. longa*
71.63	1205	1200	Dodecane	Fusel	*K. pandurata*
75.08	1272	1270	(e)-Cinnamaldehyde	Cinnamon, paint, spicy	*C. longa*, *K. galangal*
81.85	1411	1398	Propyl nonanoate	Fermented	*K. galangal*
82.65	1428	1426	α-Ionone	Floral, fruity, woody	*C. longa*
85.87	1499	1500	Pentadecane	Fusel, mild green	*A. conchigera*, *K. galangal*
86.19	1506	1510	2-Tridecanol	Sweet fruity	*Z. officinale*
90.18	1600	1600	Hexadecane	Fruity, sweet	*C .longa*

[i]Kovats index from e-nose analysis, [ii]Kovats index from AroChemBase library data, [iii]Occurance: Volatile compound detected in the sample.

As shown in Table 2, a total of 19 volatile compounds were identified, which consisted of 3-hepten-2-one, 1S-α-pinene, 3-ethyloctane, 1,5-octadienone, β-pinene, ethyl hexanoate, 1,3,5-trimethylbenzene, myrcene, acetylpyrazine, γ-terpinene, 2-acetylhiazoline, benzoic acid, dodecane, (e)-cinnamaldehyde, propyl nonanoate, α-ionone, pentadecane, 2-tridecanol, and hexadecane. The most common compounds present in the Zingiberaceae samples are β-pinene, ethyl hexanoate, and γ-terpinene. Salehi *et al*. [19] reported that β-pinene is a well-known representative of the monoterpenes group found in many herbal plants. This compound exhibits various biological activities, leading to multiple uses such as antimicrobial agents, fungicides, fragrances, and flavourings [20]. β-Pinene is a vital compound found in *A. galangal*, *A. conchigera*, *C. zedoaria*, and *C. xanthorrhiza*. Ethyl hexanoate was found in all *Kaempferia* and *Curcuma* species except for *C. zedoaria*. It contributed a fruity aroma to the samples. However, this fatty acid ethyl ester enhances the sweet and fruity aroma only when in combination with other compounds [21]. In addition, the presence of γ-terpinene in Zingiberaceae samples also gave a fruity and sweet aroma. It is a monoterpene that has a role as an antioxidant [22].

All the sensory information of each volatile compound detected are reported in Table 2. Based on this, it can be concluded that each compound contributed to the odour of the sample. The green odour in most Zingiberaceae samples is due to the presence of several compounds, which are 3-hepten-2-one, 3-ethyloctane, β-pinene, and pentadecane. Furthermore, the typical odour of Zingiberaceae is turpentine. However, based on the e-nose analysis, three out of nine of the samples did not have this odour, which were *C. longa*, *K. galanga*, and *K.*

pandurata. The turpentine odour is caused by various volatile compounds such as 3-ethyloctane, β-pinene, and γ-terpinene. Several reports have shown that the turpentine-like odour of the Zingiberaceae species is strongly due to β-pinene [23] [24]. However, in reviewing the literature, no data was found on the association between 3-ethyloctane or γ-terpinene with turpentine odour.

2. Discrimination of Herbal Products from the Zingiberaceae Family by PCA

The slight differences in the chemical composition between herbal products from Zingiberaceae, which are difficult to interpret with naked eyes, could be easily identified by using chemometric techniques. Therefore, PCA was first conducted to evaluate the responses from the e-nose and then for the discrimination of the nine herbal products of Zingiberaceae. It was also used to identify the relationship between each dataset [26].

The score plot on the first two first principal components (PC) which are PC1 and PC2 that illustrate nine different clusters, is shown in Figure 1. Each sample was clearly distinguished from the other samples except for three sample groups: *K. galangal*, *K. pandurata* and *Z. officinale*. By looking at the 2D PCA plot (Figure 1), these three samples seem to overlap with each other. However, they are actually separate but are very close, and careful observation is required to distinguish them well. The differences between the clusters can also be visualised by 3D PCA plot (Figure 2). It predicts some important compounds for each sample that causes a clear gap between the clusters.

Next, the critical part of PCA is the discrimination index. The discrimination index indicates the discrimination effectiveness between the samples on the PCA score plot [27]. Figure 1 shows that the discrimination index of this study is 90, which indicates a good discrimination of the nine herbal products of Zingiberaceae by PCA. According to the statistical model, a successful discrimination model should have an index between 80 and 100 [28].

Based on the PCA plot, most of the sample groups were plotted in the second and third quadrants except for *C. xanthorriza*. *C. xanthorriza* had the largest distances from the other samples and was plotted in the fourth quadrant. This is due to the volatile compound content in that sample. The higher the difference of the sample's volatile compound content, the larger the distance between the clusters [29]. In this study, the difference of the intensities and amount of compounds in *C. xanthorriza* was the reason for this observation.

Based on the PCA finding, the total percent of data variance of the first two PCs was 98.17%, which was high enough to present all the samples' variables. PC1 and PC2 accounted for 96.036% and 2.134% of volatile variability, respectively. Based on a previous study by Zhu *et al*. [27], the first two PCs with 95% of the total variance could provide sufficient information to explain the difference of the sample. Generally, when the PCs have more than 85% of the total variance, it proves that the data obtained is reliable and can be used for further analysis [30], such as for discrimination or classification purposes. Other than its accuracy, PCA can visualise the cluster of the samples on a score plot and reduce the time spent on discrimination. This statement is supported by a previous study by Sarker and Nahar [31]. With advanced computational techniques, PCA has become a rapid tool for analysing the enormous data obtained and shortens the analysis time [32]. Therefore, this combination with PCA improves the existing analytical capability, making it a fast method [33].

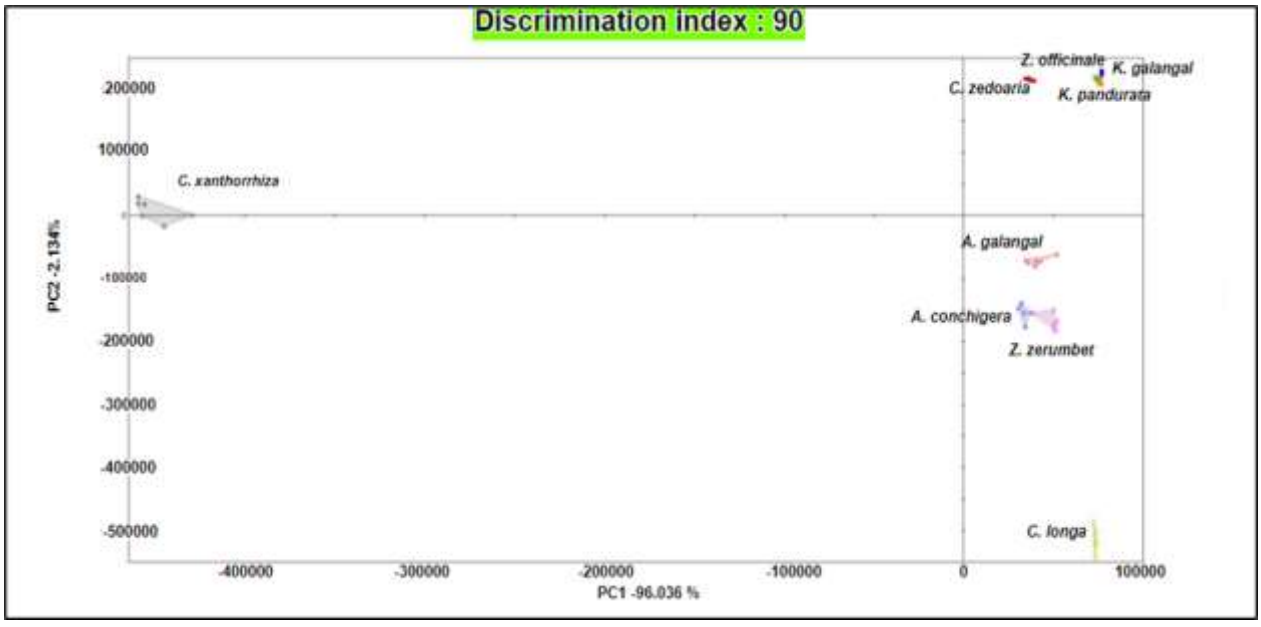

Figure 1. PC1 vs PC2 plot of nine herbal products of Zingiberaceae.

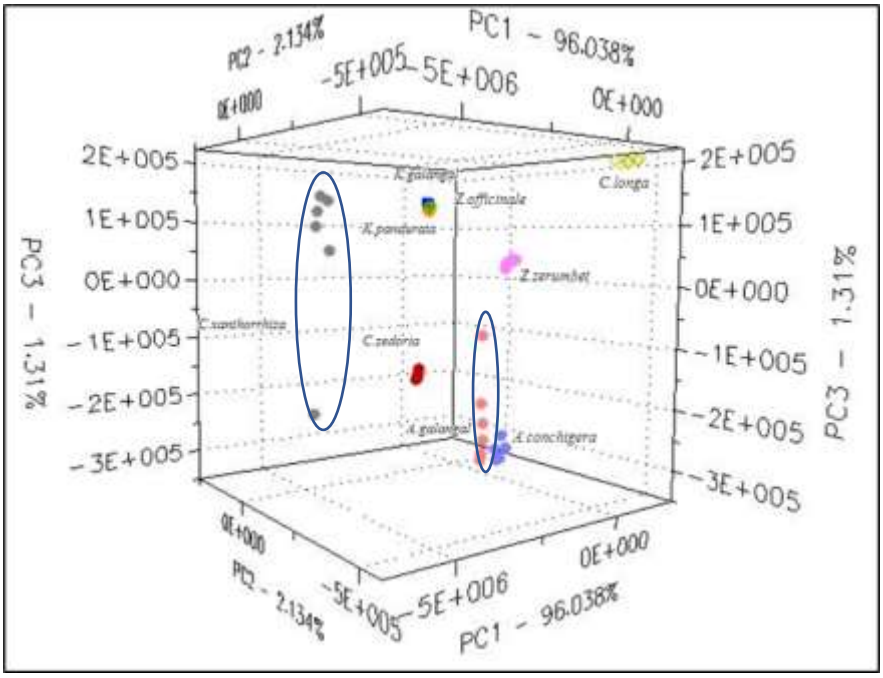

Figure 2. Three dimensional (3D) PCA plots of nine herbal products of Zingiberaceace.

3. Discrimination of herbal products from Zingiberaceae family by DFA

After the application of PCA, a DFA model was built. DFA is another method for grouping data and differentiating between groups [34]. It reduces the distance of each cluster and increases the same sample distance, resulting in better discrimination of all samples [35].

The 2D DFA plot developed from the same data and responses that were used for PCA plot is shown in Figure 3. This method identifies DF1 and DF2, which explains 99.019% and 0.415% of the dataset's variability. As shown in Figure 3, samples from the same genus were located nearby in the DFA plot rather than in the PCA plot, except for the cluster points corresponding to *C. xanthorriza*, which is separated from other groups by a large distance. Overall, it shows a good discrimination as all samples points from the same species were grouped precisely together except for two samples: *C. longa* and *Z. officinale*. Compared to the PCA plot, the 2D DFA (Figure 3) and 3D DFA (Figure 4) plots showed clearer discrimination between each species. The distance between each cluster that represents different species is much greater and has better visualisation. Generally, it indicates the great sensitivity of the e-nose. This is because DFA is a method that maximises the differences between clusters [26]. Moreover, DFA improved the discrimination among these samples and gave clear visualisation compared to the PCA, as has been found by other researchers [14][26].

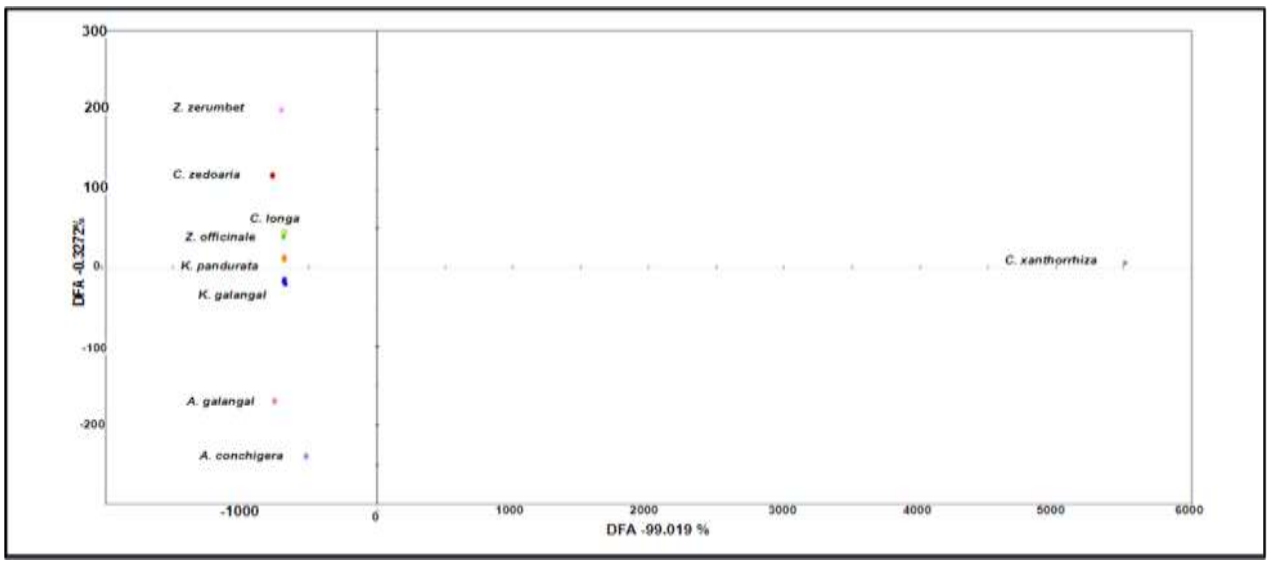

Figure 3. DF1 vs DF2 plot of nine herbal products of Zingiberaceae.

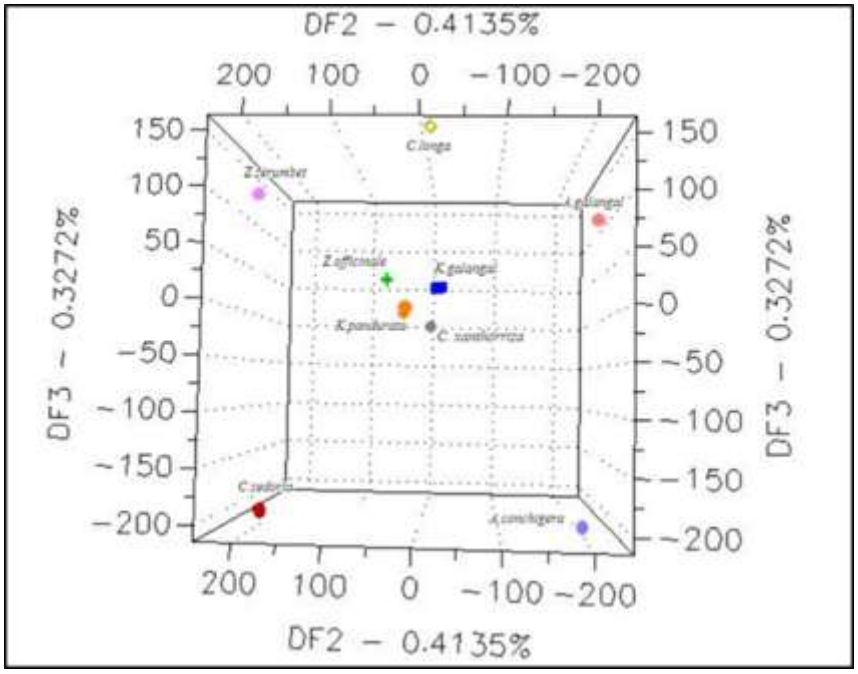

Figure 4. Three dimensional (3D) DFA plots of nine herbal products of Zingiberaceae.

The DFA outcomes demonstrate that this chemometric technique is more appropriate for use as a daily screening tool for discrimination purposes because every sample cluster can be distinguished by analysing and observing its plot.

CONCLUSION

The combination of e-nose with chemometric techniques such as PCA and DFA managed to discriminate nine herbal products from the Zingiberaceae family effectively. The chemometric techniques used in this study, especially DFA, proved their ability to visually discriminate the samples by clustering them on the score plots. Also, this combination of technologies has proven to be fast and accurate in the discrimination process. Lastly, this study could help establish quality control criteria. The combination of e-nose and chemometrics as an identification tool for herbal products could contribute to quality control in the herbal industry's supply chain.

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