# DISCRIMINATION OF ZINGIBERACEAE MEDICINAL HERBS USING ANALYTICAL METHODS COMBINED WITH CHEMOMETRIC TECHNIQUES


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## ABSTRACT

Zingiberaceae is one of the well-known families that are widely used as herbal medicines due to its various beneficial values. However, substitution with closely related species has become a serious issue in the herbal industry due to similarities in their physical appearance. Therefore, the development of a method for herbal discrimination is crucial to ensure its accurate identity and authenticity. This study aims to evaluate the ability and effectiveness of analytical methods that consist of Fourier Transform Infrared (FTIR) spectroscopy and electronic nose based on gas chromatography (e-nose-GC) combined with chemometric techniques to discriminate selected Malaysian medicinal herbs from the Zingiberaceae family. Nine powdered rhizomes of the Zingiberaceae family consisting of *Alpinia conchigera, Alpinia galanga, Curcuma longa, Curcuma xanthorrhiza, Curcuma zedoaria, Kaempferia galanga, Kaempferia pandurata, Zingiber officinale* and *Zingiber zerumbet* were used in this study. The best discrimination method was determined based on its accuracy percentage of discrimination performance. Results show that all samples were successfully discriminated against using both analytical methods with the aid of chemometric techniques. The best method was conducted by a combination of e-nose-GC and discriminant factor analysis (DFA) with an accuracy of 99.02 %, and followed by a combination of e-nose-GC and principal component analysis (PCA) with an accuracy of 98.17 %. In conclusion, the combination of FTIR spectra and e-nose-GC data with chemometric techniques can be applied for a rapid, accurate and reliable methods for the discrimination of medicinal herbs from the Zingiberaceae family as well as herbal authentication and quality control of herbal products.

# ABSTRAK

Zingiberaceae adalah salah satu keluarga terkenal yang sering digunakan sebagai ubatan herba disebabkan nilai manfaatnya yang pelbagai. Penggantian dengan spesies yang berkait rapat telah menjadi masalah yang serius dalam industri herba kerana persamaan dalam penampilan fizikal mereka. Oleh itu, pembangunan kaedah untuk diskriminasi herba adalah penting untuk memastikan ketepatan identiti dan keasliannya. Kajian ini bertujuan untuk menilai kemampuan dan keberkesanan kaedah analisis yang terdiri daripada spektroskopi Inframerah Fourier Transformasi (FTIR) dan hidung elektronik berdasarkan kromatografi gas (*e-nose-GC*) dengan kombinasi teknik kemometrik untuk membezakan beberapa herba ubatan Malaysia yang terpilih daripada keluarga Zingiberaceae. Sembilan serbuk rizom daripada keluarga Zingiberaceae yang terdiri daripada *Alpinia conchigera, Alpinia galanga, Curcuma longa, Curcuma xanthorrhiza, Curucma zedoaria, Kaempferia galanga, Kaempferia pandurata, Zingiber officinale* dan *Zingiber zerumbet* digunakan dalam kajian ini. Kaedah diskriminasi terbaik ditentukan berdasarkan peratusan ketepatan dari pelaksanaan diskriminasi. Keputusan menunjukkan bahawa semua sampel berjaya didiskriminasikan menggunakan kedua-dua kaedah analisis dengan bantuan teknik kemometrik tersebut. Kaedah terbaik telah dilakukan oleh gabungan analisis *e-nose-GC* dan faktor diskriminan analisis (DFA) dengan ketepatan 99.02 %, dan diikuti dengan gabungan *e-nose-GC* dengan analisis komponen utama (PCA) dengan ketepatan 98.17 %. Kesimpulannya, gabungan spektrum FTIR dan data *e-nose-GC* bersama teknik kemometrik dapat digunakan untuk kaedah yang cepat, tepat dan boleh dipercayai untuk diskriminasi herba ubatan dari keluarga Zingiberaceae serta pengesahan herba dan kawalan kualiti produk herba.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | | |
|---|---|---|
| % | - | Percent |
| °C | - | Degree celcius |
| $cm^{-1}$ | - | Inverse centimetre/ Reciprocal wavelength |
| m | - | metre |
| s | - | Second |
| µL | - | microlitre |
| µm | - | micrometre |
| ANOVA | - | Analysis of variance |
| ATR-FTIR | - | Attenuated Total Reflectance-Fourier Transform Infrared |
| CA | - | Clustering analysis |
| $CO_2$ | - | Carbon dioxide |
| DA | - | Discriminant analysis |
| DFA | - | Discriminant factor analysis |
| E-nose-GC | - | Electronic nose based on gas chromatography |
| FTIR | - | Fourier Transform Infrared |
| HCA | - | Hierarchical cluster analysis |
| IR | - | Infrared |
| LTPRI | - | Linear temperature-programmed retention index |
| PCA | - | Principal component analysis |
| PLS-DA | - | Partial least square-Discriminant analysis |
| RT | - | Retention time |
| WHO | - | World Health Organization |

# LIST OF APPENDICES

# LIST OF PUBLICATION

**Aslia Natasha Pauzi**, Norhayati Muhammad, Norazlin Abdullah and Nurkhalida Kamal. 2021. Discrimination of herbal products from Zingiberaceae family using electric nose combined with chemometric techniques. Malaysian Journal of Chemistry, 23(2), 205-212. (Scopus)

# CHAPTER 1

# INTRODUCTION

## 1.1 Background study

In this era, herbal-based products have gained a significant demand among consumers of all ages in the global marketplace (Larsen, 2002). The global herbal product market is expected to reach a valuation of USD 411.2 billion (RM 1, 715.32 billion) by the year 2026 (Delaware, 2020). In 2013, Malaysian Agricultural Research and Development Institute (MARDI) substantiates that the gross profits of local herbal market are more than RM 5.4 billion per year (Ahmad & Othman, 2013). In addition, the Ministry of Natural Resources and Environment estimated that the local herbal market could grow by 15 % a year starting from RM 7 billion in 2010 (Ahmad *et al*., 2015). According to the credible sources, the market value of the herbal products in Malaysia is able to reach RM32 billion in 2020, with an annual growth of 8 % to 15 % (Naharul, 2019). The significance of marketable herbal products is demonstrated by an increase in the percentage of product registration by 20 % with the National Pharmaceutical Control Bureau (NPCB) to meet the increasing demand (Fadzil *et al*., 2018). These statistics concurrently indicated that the herbal industry will be one of the most in-demand sectors in Malaysian agriculture (Nordin, Othman & Che Mat, 2008).

Zingiberaceae is one of the well-known family of herbal plant that has been widely used in the production of herbal products attributed to its various beneficial values (Ahmad & Othman, 2013). Zingiberaceae consists of 53 genera and about 1300 different species, mainly distributed in South-east Asia, which have close similarities between the species from the same family (Barbosa *et al*., 2017). Many herbs from this family have found applications in various industries such as food, pharmacy, perfume and cosmetics due to their diverse chemical composition as well as their organoleptic properties (Zhang *et al.*, 2020). They are versatile species that are cultivated as medicinal herbs (Lianah, Krisantini, & Wegener, 2020). Notably, the *Alpini*, *Curcuma*, *Kaempferia*, and *Zingiber* are economically important genera in the Zingiberaceae family, as their rhizomes have been utilised as traditional medicines, spices and herbs as well as natural colourant for food and fabric (Kumar *et al*., 2013). In addition, some parts of Zingiberaceae are commonly utilised as in the preparation of Thai food, vegetables, side dishes which contribute to the distinctive aroma of aromatic tea (Rachkeeree *et al*, 2018).

Herbal-based products in Malaysia are commonly available in the form of raw materials, dried plants, powder, herbal extracts, creams and essential oils (Aziz *et al*., 2005). These products have been widely utilised as medicines, and many other products over the years (Zakaria *et al*., 2016). The main advantages highlighted by herbal products are their efficacy, safety, authenticity, lower toxicity and side effects compared to synthetic drugs, placing herbal products in high demand (Kalia, 2005). Due to the overwhelming market demand for herbal products, the Malaysian herbal industry will encounter a shortage of local raw materials for the large scale commercialisation of herbal-based products, resulting in increased adulteration practices by unscrupulous manufactures (Keshari, 2021). According to Sagar (2014), adulteration practice is defined as mixing or substituting original materials with adulterants such as other parts of the same plant or closely related species, which do not conform to the correct standard. Consumers are often unaware of this adulteration and purchase herbs that have a resemblance to the authentic herbs. As a result, herbal products with high degree of adulterants could negatively impact human health and local herbal industry (Ekar & Kreft, 2019). The reports about some of adverse effects associated with the consumption of herbal products also appearing from time to time largely due to poor quality of the finished products, contamination with toxic materials and adulteration with drugs or other herbal species (Zhang *et al*., 2012).

In this regard, there is an urgent need to identify the correct species by prior use as raw materials in herbal products (Mishra *et al*., 2016; Leonti, 2013). However, herbal discrimination tends to be a difficult work as many categories of medicinal herbs have close physical appearances after pre-processing (Zhan *et al*., 2018). Previous studies have highlighted that most of the herbal species can be identified and discriminated from their adulterants via a few methods including morphology and microscopy examination and traditional chemical fingerprinting (Parveen *et al*., 2016; De Boer, Ichim & Newmaster, 2015). Those methods are subjective in the aspect of nature or require experienced users, thus barely meeting the criteria needed to support their widespread use (Fu *et al*., 2015; Boothe & Arnold, 2002). The approaches mentioned also have several disadvantages such as the accuracy is highly dependent on individual experience and skills, difficult to differentiate between closely related substitutes or morphological similarities, complex and imprecise, impractical practices with various standards (Fadzil *et al*., 2018).

Recently, the application of Fourier Transform Infrared (FTIR) spectroscopy and electronic nose based on gas chromatography (e-nose-GC) on herbal discrimination is now a new platform on herbal discrimination. FTIR spectroscopy is simple, rapid, and environmentally friendly (Fadzillah, Che Man & Rohman, 2014). Therefore, it has been widely used in the food and herbal industry (Mazivila & Olivieri, 2018; Hua-Bin *et al*., 2012). In addition, the application of e-nose-GC to measure the aroma profiles of the sample for food quality inspection has also been discovered (Baby *et al*., 2005; Yu *et al*., 2008). E-nose-GC allows for combining the features of gas chromatography (GC) with advantages of a sensor-based e-nose (Wiśniewska *et al*., 2016). Typically, the volatile molecules react with the sensing materials of the gas sensor and cause irreversible changes in electrical related properties, such as conductivity. These changes are then detected and characterized by pattern recognition algorithms to perform discrimination of samples. Compared with traditional gas analytical equipment such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS), this type of e-nose is a relatively inexpensive and less time-consuming approach (Tan & Xu, 2020). Moreover, with the advancement in instrument sensitivity and computational capabilities, non-targeted analysis methods coupled with chemometric technique have become the mainstream approach to fingerprint the overall chemical composition of a sample (Schwanz *et al.*, 2019).

Singh *et al*. (2013) explained that the chemometric technique is an application of mathematics and statistics for processing a large number of samples and providing important information by analysing the chemical data. Chemometric technique has been reported as one of the most successful statistical methods for qualitative and quantitative studies (Fadzlillah *et al*., 2014). Since the technique is relatively simple, rapid, and non-destructive, it can be used to solve technological and legislative food control problems efficiently (Sim, 2003). Therefore, this study aims to apply FTIR spectroscopy and e-nose-GC combined with chemometric techniques for the analysis and discrimination of medicinal herbs from four genera of the Zingiberaceae family consisting of the nine powdered rhizomes of *Alpinia galanga*, *A. conchigera*, *Curcuma longa*, *C. xanthorrhiza*, *C. zedoaria*, *Kaempferia galanga, K. pandurata*, *Zingiber officinale*, and *Z. zerumbet.*

## 1.2 Problem statement

Adulteration and substitution either of closely related species from same family or unwanted materials by immoral manufacturers has become a common practice in the herbal industry (Poornima, 2010; Ali, Gyulai & Al-Hermaid, 2015). A total of 59,440 of herbal products were seized in Malaysia that were due to several offences from several years, starting from 2008 to 2014. Within seven years of data, majority of seized adulterated herbal products were originated from neighbouring countries outside Malaysia such as Indonesia, China and Middle East (Ariffin *et al*., 2021). The worst-case scenario is closely related species may be extremely similar to each other, in terms of their physical appearances. In fact, experienced botanists are challenged to safely distinguish species that can be identified only by almost invisible characteristics (Müller et al., 2016). The real situation happened when *Curcuma longa,* a medicinal herb known for its various health benefits, is often adulterated by *C. aromatica*, which cheaper one for the purpose of reducing the production cost (Sasikumar, 2019). In addition, since the substituted plants, especially in powder form, are often morphologically indistinguishable from the native ones, making the identification and discrimination assessments against these species are difficult, and therefore could be erroneously supplied to food and pharmaceutical companies (Zhan *et al*., 2018; Ghosh *et al*., 2011; Rohaeti *et al.*, 2015). Consequently, the therapeutic efficiency of herbal products is markedly reduced and, in some cases, poses threats to public health (Basak *et al*., 2019). This can erode consumer confidence in herbal or natural products (Parvathy *et al*., 2015).

In line with this, the World Health Organisation (WHO) has updated its regulations by stating that the identification of pre-market herbal medicines is a fundamental and compulsory assay to ensure quality and discriminate them from other related species or potential misuse (WHO, 2007). Due to that, herbal product manufacturers often seek faster and cost-effective identification or discrimination methods (Gopal, 2014). Unfortunately, the similarities between herbal appearance necessitates a large amount of time for discrimination as well as identification and authentication (Fu *et al*., 2015). However, most current analytical methods are reportedly to be unable to cater the demands for rapid product testing due to the high similarities of physical appearance among herbs, especially from the same genus or family (Foley & Grant, 2007; Fu *et al.*, 2015). The high similarities among herbs in terms of morphological can cause confusion and prone to misidentifications (Seeland *et al*., 2019).

Morphological examination is the most common discrimination and authentication method. Nonetheless, this technique is often confusing (Ghosh *et al*., 2011; Basak *et al*., 2019) and has proven giving low accuracy (De Boer *et al*., 2015). Some of current analytical methods for discrimination include the use of thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), gas chromatography (GC), deoxyribonucleic acid (DNA) methods, and near-infrared (NIR) spectroscopy (Jiang *et al*., 2010). These methods require competent personnel (Fu *et al*., 2015) due to high handling skills (Tanaka *et al*., 2008). In addition, some of these methods apply targeted analysis that focuses on the detection of one or more specific compounds in a specimen (Cheah & Fang, 2020), which are generally complicated and not always effective for analysing unknown samples (Kaufmann *et al*., 2015). DNA-based methods have proven to be useful in identifying important components in herbal products (Wenzl *et al*., 2002; Hilt *et al*., 2003; Yin *et al*., 2005; Mejia *et al*., 2007; Pizarro *et al*., 2007). Although there are considerable values in various experiments, these methods are not suitable for daily and high volume sample testing (Dhanya & Sasikumar, 2010).

As the alternative to previous discrimination works, the combination between analytical methods consist of FTIR and e-nose-GC, and chemometric techniques allow for a rapid, accurate and reliable methods for discrimination of nine medicinal herbs from Zingiberaceae family.

## 1.3 Significance of the study

Reliable discrimination methods are essential to distinguish Zingiberaceae medicinal herbs from their closely related species to ensure the safety, efficacy and quality of herbal products (Goldman, 2001). In addition, this practice can help prevent misidentification of herbal materials and, hence, reduce fraudulent behaviours such as adulteration practices (Techen *et al*., 2004). Overall, successful discrimination of herbal species will have positive impact on the health of individuals who frequently consume herbal-based products and provides various benefits to society and manufacturers and the national economy directly.

Given the strong interest in fingerprinting, FTIR spectroscopy and e-nose-GC are rapid and cost-effective identification tools in various fields of study (Jurenka, 2009). Kaushik, Pandey and Alok (2014) reported that fingerprinting has the potential to be a powerful method for quality control of herbal products. This study aims to extend the uses of FTIR spectroscopy and e-nose-GC in combination with suitable chemometric techniques for rapid discrimination of the Zingiberaceae family. The findings of this study could provide more insight into the practical approaches to discriminate nine samples from the Zingiberaceae family that are abundant in Malaysia.



## 1.4 Aim and objectives

This study aims to evaluate the ability and effectiveness of analytical methods consist of Fourier Transform Infrared (FTIR) spectroscopy and electronic nose based on gas chromatography (e-nose-GC), combined with chemometric techniques for discrimination of nine medicinal herbs from the Zingiberaceae family. Meanwhile, the objectives are as follows:

i. To identify the specific bands of FTIR spectra of powdered herb samples via FTIR spectroscopy,
ii. To identify the volatile compounds of powdered herb samples via e-nose-GC,
iii. To discriminate different species of nine Zingiberaceae samples via chemometric techniques,

iv. To determine the best method for discrimination of powdered herb samples based on its accuracy percentage.

## 1.5 Scope of study

To achieve the objectives of this study, the scopes of study were identified. The scopes are listed as below:

i. The rhizomes of *Alpinia conchigera*, *A. galanga, Curcuma longa, C. zedoaria, C. xanthorrhiza, Kaempferia galanga, K. pandurata, Zingiber officinale* and *Z. zerumbet* had been collected from Pusat Ujian Varieti Baru Tumbuhan, Kompleks Jabatan Pertanian, Serdang, Selangor.

ii. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) was used to measure the vibration frequencies in the functional groups of chemical bonds, meanwhile, e-nose based on gas chromatography (e-nose-GC) that equipped with capillary column coupled with flame ionization detectors was used to analyse volatile compounds of the herb samples.

iii. The chemometric techniques used were principal component analysis (PCA), clustering analysis (CA) and discriminant analysis (DA).

iv. The best method for the discrimination of nine powdered herbs from the Zingiberaceae family was determined based on the accuracy percentage of discrimination performances.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

This chapter will cover an overview of medicinal herbs, herbal species from the Zingiberaceae family and analytical methods consisting of Fourier Transform Infrared (FTIR) spectroscopy and electronic nose based on gas chromatography (e-nose-GC). In addition, principles and previous studies on the application of those analytical methods for discrimination purposes are also discussed. Lastly, the overview and application of chemometric techniques as statistical data analysis are also briefly explained.

## 2.2 Medicinal herbs

Medicinal herbs are plants that are used for medicinal purpose. Those herbs have been used worldwide for centuries, comprising single or multiple herbs in herbal product preparation. According to the guidelines of World Health Organization (WHO) (2007), herbal medicines can be defined as herbs, herbal materials, herbal preparations and finished herbal products that contain part of plants or active ingredients. Each term is described by Indrayanto (2018) as shown in Table 2.1. Tilburt and Kaptchuk (2008) defined herbal medicines as plant-derived substances with minimal of industrial processing that have medicinal values in local or regional healing practices. Hence, all samples used in this study can be utilised in herbal production.

Table 2.1: Additional information on herbal medicines (Indrayanto, 2018)

| Terms | Details |
|---|---|
| Herbs | May contain crude materials derived from lichen, fungi, algae and parts of higher plant materials, which may be intact, fragmented or powdered |
| Herbal materials | Include fresh juices, gums, fixed oils, essential oils, resins and dry powder of herbs |
| Herbal preparations | Include comminute or cut herbal materials, or extracts |
| Finished herbal products | May contain excipients in addition to the active ingredients |

The pharmacological treatment of disease began long ago with the use of herbs (Schulz, Hänsel &Tyler, 2001). Ancient healing methods around the world commonly used herbs as part of their tradition. Moreover, herbs have also been used to treat various diseases (WHO, 2011). In fact, about 25 % of modern medicines have been developed from medicinal herbs (De Luca, 2012). This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries. It is estimated that up to four billion people which representing 80 % of the world's population, living in the developing world rely on herbal products as a primary source of healthcare (Ekor, 2014).

The market of herbal medicine including herbal products and raw materials is estimated to have annual growth in the range of 5 to 15 % (Baby *et al*., 2005). In addition, research on herbal medicines for therapeutic purposes has increased worldwide and gained attention from all researchers all over the world including Malaysia. Malaysia is known as a country rich in medicinal plant species. In addition to that, Malaysia is also known as one of the countries with mega biodiverse in the world (Von Rintelen, Arida, & Häuser, 2017). To date, about 1300 species of medicinal herbs have been identified in Peninsular Malaysia (Kulip *et al*., 2010). Thereby, it drives Malaysia as one of the countries with great potential to become the world's herbal-based product.

## 2.3 Overview of medicinal herbs from Zingiberacea family

The Zingiberales order has around 68 genera consisting of more than 600 species. Zingiberaceae is one of eight families in Zingiberales order, together with Marantance, Heliconiaceae, Musaceae, Strelitziaceae, Cannaceae, Costaceae and Cannaceae (Wegener, 2020). Zingiberaceae is the largest family with 53 genera and 1200 species.

It is a monophyletic family which is widely cultivated as medicinal herbs (Wegener, 2020). In addition, the Zingiberaceae family is well known as the ginger family with abundant species widely used in Southeast Asia due to their unique flavours, ornamental and also the medicinal attributes (Aggarwal & Shishodia, 2006). They have almost identical morphological characteristics (Wegener, 2020). The worst-case scenario is that most of the common names used to identify them are often inconsistent (Kress *et al*., 2002). In this case, it will be confusing in identifying the herb species.

Table 2.2 summarizes taxonomical description of different parts of typical Zingiberaceae plants, meanwhile Figure 2.1 illustrates the typical herbal plant of Zingiberaceae consisting of whole plant, inflorescence and flower part. They are rhizomatous plant and have zygomorphic flower inflorescences with bractea, stamen as well as filaments that form a staminoid structure (Leong-Škorničková *et al*., 2007). Observations on plant species and genera taxonomy made by common people are still dubious and weak although there are numerous previous studies explaining the characteristics of different parts of Zingiberaceae (Kress *et al*., 2002). Consequently, this will lead to misidentification and adulteration problems among closed-related species.

Table 2.2: Taxonomical description of different parts of typical Zingiberaceae plants (Jatoi *et al*., 2007).

| Parts | Taxonomical description |
|---|---|
| Whole plant | Perennial herbs, aromatic, with fleshy, (non)tuberous rhizomes |
| | Short stems and usually replaced by pseudo stems |
| Rhizome | Main stem of the plant that runs underground horizontally |
| Leaves | Leaf sheath is usually open on the opposite side of the lamina |
| | Leaf blade is usually narrow strap-shaped, rolled longitudinally at the bud and hairy |
| Inflorescence | Either cylindrical or fusiform in shape but sometimes globose and lax to dense with few to many flowers |
| Flower | Bisexual, epigynous and zygomorphic |

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