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Cymbopogon citratus AND *Cymbopogon nardus* ESSENTIAL OIL COMPONENTS – FTIR, CHEMOMETRICS ASSESSMENT AND IDENTIFICATION USING GC-MS

(Komponen Minyak Pati *Cymbopogon citratus* dan *Cymbopogon nardus*- Penilaian FTIR, Kemometrik dan Penentuan Menggunakan GC-MS)

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Abstract

Cymbopogon citratus and *Cymbopogon nardus* essential oil are high-value natural products due to their special qualities and commercial significance. In this work, the volatile compounds of the *C. citratus* and *C. nardus* natural essential oils obtained by hydro distillation and commercial sources using gas chromatography-mass spectrometry (GC-MS), Fourier transformation infrared spectroscopy (FTIR) and chemometrics methods were systematically detected and identified. The GC-MS results indicated that the main compound in the natural essential oil of *C. citratus* is citral while citronellal commercial samples of both *Cymbopogan* species are positively significant. Based on the results of GC-MS, citral was the major component in *C. citratus* while citronellal was the major component in *C. nardus*. Analyzing the FTIR data using principal component analysis (PCA), hierarchical cluster analysis (HCA), and discriminant analysis (DA) further revealed that the chemical composition of natural essential oils *C. citratus* and *C. nardus* samples were significantly different from commercial samples. This study revealed the first insight into metabolite compositional differences among *C. citratus* and *C. nardus* using quick and affordable analytical procedures.

Keywords: Fourier transformation infrared spectroscopy, chemometrics, principal component analysis, hierarchical cluster analysis, *Cymbopogon*

Abstrak

Minyak pati *Cymbopogon citratus* dan *Cymbopogon nardus* ialah produk semula jadi bernilai tinggi kerana kualitinya yang istimewa dan kepentingan komersialnya. Dalam kajian ini, sebatian meruap minyak pati semula jadi *C. citratus* dan *C. nardus* yang diperoleh melalui penyulingan hidro dan sumber komersial dikenalpasti dengan menggunakan kromatografi gas spektrometri jisim (GC-MS), spektroskopi inframerah transformasi Fourier (FTIR) dan kaedah kemometrik. Keputusan GC-MS menunjukkan bahawa sebatian utama dalam sampel komersial bagi kedua-dua spesies *Cymbopogan* secara signifikan berkorelasi positif dengan minyak pati semulajadi. Berdasarkan keputusan GC-MS, sitral adalah komponen utama dalam *C. citratus* manakala sitronelal adalah komponen utama dalam *C. nardus*. Melalui analisis data FTIR menggunakan analisis prinsip komponen (PCA) dan analisis kluster hierarki (HCA) seterusnya mendedahkan bahawa komposisi kimia minyak pati semulajadi *C. citratus* dan *C. nardus* sampel adalah berbeza dengan ketara daripada sampel komersial. Kajian ini memberikan keputusan yang jelas tentang perbezaan komposisi metabolit antara *C. citratus* dan *C. nardus* menggunakan prosedur analisis yang cepat dan berpatutan.

Kata kunci: Spektroskopi inframerah transformasi Fourier, kemometrik, analisis prinsip komponen,analisis kluster hierarki, *Cymbopogon*

Introduction

Essential oils from the *Cymbopogon* species are widely used in the perfumery, cosmetic, food and pharmaceutical industries. The most widely used species in food processing is *C. citratus*. Among aromatic species that are also important are *C. fexuosus* (East Indian *C. citratus*), *C. martini* (palmarosa), and *C. nardus* (*C. nardus* grass) [1]. Essential oils contain different volatile compounds and the most common compounds found in *Cymbopogon* essential oil are nerol, geranial, citronellol, geraniol, and piperitone [2]. Phytochemical studies of different species showed the presence of terpenoids, alkaloids, phenolic acids, tannins, and carotenoids, and the genus *Cymbopogon* was reported as a rich source of C-glycosyl flavonoids [2]. Most of the research done has focused on the species of *C. citratus*, with less emphasis on other species in the genus [3].

Numerous studies have been conducted to see the differences in chemical composition and pharmacological effects on *Cymbopogon* species. Essential oil products from the *Cymbopogon* species are also widely available in the market, but until now the validity of commercial products with natural essential oils has not been well distinguished. Difficulty discriminating between *Cymbopogon* species may also occur due to similar physical and morphological characteristics. To avoid confusion, a rapid and reliable method to discriminate *Cymbopogon* species is urgently needed. This is important for quality control, safety and effectiveness of essential oils from the *Cymbopogon* species.

The combination of spectroscopic and chemometric techniques is gaining attention because of its ability to quickly distinguish various plant species [4]. Infrared spectroscopy provides information about the vibrational of functional groups in the studied molecules and the absorption of infrared radiation by the sample [5]. The chromatographic technique is also a common method used in determining the quality of herbal medicine. Recently, the combination of chromatographic and chemometric techniques in the identification of varieties and the discovery of herbal chemical markers has increased widely [6]. Most researchers focus on establishing GC-MS fingerprints to reveal differences in the composition and chemical content of various botanical and geographical origins of plants [7]. Chemometrics tools have not been widely used in the analysis of volatile compounds for geographic origin differentiation and discrimination.

In this study, a simple and fast method for various identification and selection of chemical markers will be developed. FTIR spectroscopy and GC-MS chromatography coupled with chemometrics were used to discriminate the different botanical origins as well as the authenticity of commercial products. Furthermore, for the first time, the potential of functional groups and volatile compounds that contribute to the differences between *C. Citratus* and *C. nardus* species and commercial samples were determined through PCA and HCA models built from FTIR data.

Materials and Methods

Materials

The samples for *C. citratus* and C. *nardus* were collected from three different origins from the state of Pahang, Malaysia and the natural essential oils obtained by hydro distillation were labelled as ECC1, ECC2, ECC3 for essential oils of *C. citratus* and ECN1, ECN2, ECN3 for essential oils from *C. nardus*. Commercial essential oils purchased from three different companies and were labelled as CCC1, CCC2, CCC3 for commercial samples of *C. citratus* and CCN1, CCN2, CCN3 for commercial samples of *C. nardus*.

Sample preparation and essential oil extraction

The stalks and leaves of *C. citratus* and *C. nardus* are washed with tap water and cut into small pieces before being stored overnight in the oven (50 °C). The dried *C. citratus* and *C. nardus* are ground into small sizes. Thirty grams (30 g) of each of *C. citratus* and *C. nardus* are placed in a 2000 mL distillation flask connected directly to the Clevenger apparatus containing distillation water (1000 mL). The samples are heated at 100 °C for 2 hours. The extracted essential oils are dried

over anhydrous sodium sulphate and filtered. The volatile oils are obtained and kept for further analysis.

GC-MS analysis

A 7890A Agilent Technologies Gas Chromatography with a 5760N Mass Spectrometer equipped with an HP-5 ms (5% phenyl methyl siloxane) fused silica capillary column (30 m 0.25 mm internal diameter, film thickness 0.25 m) was used to conduct the GC-MS analysis of samples of essential oils. The temperature of the injector is fixed at 250 °C. For three minutes, the initial oven temperature of 40 °C was maintained. The temperature was then raised to 100 °C gradually at a rate of 8 °C/min. The temperature increased to 200 °C at a rate of 5 °C/min and to 250 °C at a rate of 10 °C/min. For 10 minutes, the temperature was maintained at 250 °C. At a rate of 1.0 ml/min, helium was used as the carrier gas.

FTIR analysis

The procedure begins with the crystal being wiped and cleaned with acetone to ensure precise detection. Small amounts of samples were placed onto the crystal surface in contact with the ATR at ambient temperature (25 °C). Then, after placing the essential oil on the crystal region, the pressure arm over the crystal/sample area was setup. All spectra were background corrected using an air spectrum, which was renewed after each scan. After each measurement, the ATR base was thoroughly cleaned using acetone and the surface was allowed to dry before measuring the next sample. Spectra were recorded in the range 600-4000 cm^{-1} at a resolution of 4 cm^{-1} and each spectrum consisted of 64 co-added scans. The FTIR spectrum of each sample was measured in triplicate to assess precision and ensure the reproducibility of each sample. The spectra were recorded as absorbance values.

Chemometrics

All the data obtained from the application of FTIR was subjected to chemometrics data analysis. Principal component analysis (PCA), hierarchical cluster analysis (HCA) and discriminate analysis (DA) were used to discriminate samples from different origins. XLSTAT software was used to perform chemometric analysis of FTIR and GC-MS data of *C. citratus* and *C. nardus* essential oils and commercial samples. The scattering pattern of authentic and commercial samples was displayed using two-dimensional PCA plots. HCA is a method of cluster analysis that aims to create a hierarchy of groups. The HCA dendrogram indicated significant differences and similarities. For analysis of FTIR data, the spectral ranges of 1000-3300 cm^{-1} were employed.

Results and Discussion

Identification of volatile compounds in *C. citratus* and *C. nardus*

Cymbopogon essential oils (n = 6) and commercial samples (n = 6) were analysed utilising GC-MS techniques in the current research investigation. Samples of *Cymbopogon* essential oils included a total of 111 constituents. Monoterpenes, sesquiterpenes, aldehydes, ketones, alcohol hydrocarbons, alkanes, oxygenated monoterpenes, and oxygenated sesquiterpenes comprise the volatile constituent profile of *C. citratus* essential oil Overall, the main compound identified in essential oil and commercial samples of *C. citratus* were citral and linalool (Table 1). The major volatile compound in *C. citratus* essential oil was citral, which is present almost in all the analysed samples. Prior study indicated that citral plays a crucial role in the development of the plant's taste and other food recipes [8]. Citral exhibits various important therapeutic properties like antimicrobial, antioxidant, anticancer, anti-diabetic and anti-inflammatory [9].

The frequently identified compounds of *C. nardus* samples (Table 2) were citronellal, citronellol, and D-limonene. The main constituents of the essential oil in *C. nardus*, commonly known as citronellal, and citronellol are also found in this study [10]. The absence of citronellal from Origin 3 and citronellol from Origin 1 is due to various factors related to the plant, interaction with the environment (soil type and climate, etc.), the maturity of the plant concerned, even at harvest time during the day and related to the extraction method [11]. *C. nardus* and *C. citratus* essential oils were considered important products in the food and cosmetic industry. Consumer safety greatly depends on the ability to trace the quality of *C. nardus* and *C. citratus* essential oils. The development of new analytical techniques that are robust, dependable, effective, and affordable is crucial for determining the authenticity of essential oils. There

is currently interest in chemometrics and innovative analytical techniques.

The current research uses FTIR and GC–MS techniques for the characterization of *C. nardus* and *C. citratus* essential oils. The findings demonstrate that the authenticity of *C. nardus* and *C. citratus* essential oils can be determined by FTIR and chemometrics (HCA & PCA). It takes little sample preparation to distinguish both essential oils from commercial samples. As far as we are aware, this is the first example in which the FTIR technique and chemometrics have been employed to assess the authenticity of *C. nardus* and *C. citratus* essential oils. Additionally, recent research has demonstrated the significant potential of vibrational spectroscopy in conjunction with chemometrics to deliver precise and trustworthy results for determining the authenticity of essential oils. Based on the data collected using HCA and PCA, all samples could be unambiguously identified with 100% accuracy.

Analysis of FTIR spectra of *C. citratus* and *C. nardus*

The typical FTIR spectrum of *C. citratus* and C. *nardus* essential oils obtained by hydro distillation and commercial samples are presented in Figures 1(a) and (b), respectively. In the infrared (IR) region, molecular vibrations matching a specific vibration frequency show the functional group of each sample including its molecular identification [8]. In this analysis, the differences between the samples were determined based on the wavenumber of the absorption band in the IR.

The absorption bands in Figure 1(a) at 1672 to 1612 cm^{-1} were assigned to C=C stretching vibrations of the alkenes functional group. Citral has conjugated double bonds (C=C-CHO), which are frequent in acyclic monoterpenes, as evidenced by the intense peak seen at 1672 -1612 cm^{-1} caused by vibrations of C=C (*cis* and *trans*). As can be seen in the spectrum, the intensity of the essential oil *C. citratus* peak at 1672-1612 cm^{-1} was higher than those in commercial samples. The result might be useful for faster determination of the quality of commercial products *C. citratus* essential oil. The intensity difference might due to the low content of marker compounds in *C. citratus* which was citral. The peak around 1442 to 1377 cm^{-1} could be assigned to the C-H bending vibrations of the alkanes functional group. Bands at 1194, 1154, 1153, 1120 and 1121 cm^{-1} resulted from the C-O stretching vibrations of phenolics. The peak at 1043 cm^{-1} was attributed to the stretching vibrations of C-O. The peak at 984 cm^{-1} is assigned to the C-H bending absorption of the alkenes functional group. The peak at 864, 842, 820, 747 and 748 cm^{-1} are assigned to aromatic rings =CH vibration absorption [12]

Table 1. Chemical compositions of essential oils from *Cymbopogon citratus* of different origins and commercial samples

Compounds	**Molecular Formula**	***Cymbopogon citratus***											
		Origin 1		**Origin 2**		**Origin 3**		**Company 1**		**Company 2**		**Company 3**	
		RT	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**
β-Myrcene	$C_{10}H_{16}$	6.619	1.09	6.604	0.93	-	-	-	-	-	-	-	-
D-Limonene	$C_{10}H_{16}$	-	-	-	-	-	-	7.388	0.71	7.366	0.92	7.377	0.23
β-(Z)-Ocimene	$C_{10}H_{16}$	7.579	0.30	8.517	0.77	-	-	-	-	-	-	-	-
Linalool	$C_{10}H_{18}O$	8.522	0.45	-	-	8.523	0.47	8.534	0.55	-	-	8.523	0.18
Citronellal	$C_{10}H_{18}O$	9.365	0.20	9.371	1.07	-	-	-	-	-	-	-	-
Nerol	$C_{10}H_{18}O$	-	-	-	-	-	-	10.533	0.64	-	-	10.522	0.36
6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$	9.516	0.75	-	-	-	-	-	-	-	-	-	-
Cyclohexane, ethenyl-	C_8H_{14}	9.814	1.08	-	-	-	-	-	-	-	-	-	-
3-Octyne	C_8H_{14}	-	-	10.791	31.90	10.802	10.20	-	-	-	-	-	-
Cis-citral	$C_{10}H_{16}O$	-	-	-	-	-	-	10.780	4.57	-	-	10.780	1.42
Citral	$C_{10}H_{16}O$	-	-	11.246	42.37	11.274	16.32	11.207	2.74	11.235	6.92	11.212	1.28
2,3-Epoxycarane, (E)-	$C_{10}H_{16}O$	-	-	-	-	11.364	10.80	-	-	-	-	-	-
1-Propyne, 1-(methylthio)-	C_4H_6S	-	-	-	-	-	-	11.319	2.97	-	-	-	-
γ-Picoline, N-oxide	C_6H_7NO	11.381	41.32	-	-	-	-	-	-	-	-	-	-
β-Cymene	$C_{10}H_{14}$	-	-	-	-	-	-	-	-	11.414	4.52	-	-
1,4-Benzenediol, 2-(1,1-dimethylethyl)-	$C_{14}H_{22}O$	-	-	11.796	3.72	11.864	10.58	-	-	11.847	4.93	-	-
Geranic acid methyl ester	$C_{11}H_{18}O_2$	-	-	11.875	0.77	11.920	0.70	-	-	-	-	-	-
Geranyl acetate	$C_{12}H_{20}O_2$	12.622	0.66	-	-	12.627	2.66	-	-	-	-	-	-
Caryophyllene	$C_{15}H_{24}$	13.380	0.43	-	-	-	-	13.386	2.19	-	-	13.397	1.66
Butylated Hydroxytoluene	$C_{15}H_{24}O$	-	-	-	-	-	-	14.357	0.93	-	-	14.362	0.43
Caryophyllene Oxide	$C_{15}H_{24}O$	15.564	0.23	-	-	15.564	0.44	-	-	-	-	-	-
γ-Gurjunene	$C_{15}H_{24}$	16.075	3.95	-	-	16.609	0.56	-	-	-	-	-	-
α-Cadinol	$C_{15}H_{26}O$	16.412	0.87	-	-	16.407	0.52	-	-	-	-	-	-
γ-Selinene	$C_{15}H_{24}$	16.614	0.74	-	-	16.070	2.70	-	-	-	-	-	-
Juniper Camphor	$C_{15}H_{26}O$	16.990	0.39	-	-	-	-	-	-	-	-	-	-
Benzyl benzoate	$C_{14}H_{12}O_2$	-	-	-	-	-	-	17.996	1.52	17.984	0.45	-	-
Isopropyl Myristate	$C_{17}H_{34}O_2$	-	-	-	-	-	-	18.220	15.01	18.192	2.64	-	-
Versalide	$C_{18}H_{26}O$	-	-	-	-	-	-	18.928	1.77	18.911	0.48	-	-

Table 2: Chemical compositions of essential oils from *Cymbopogon nardus* of different origins and commercial samples

Compounds	**Molecular Formula**	***Cymbopogon nardus***											
		Origin 1		**Origin 2**		**Origin 3**		**Company 1**		**Company 2**		**Company 3**	
		RT	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**	**RT**	**Area %**
D-Limonene	$C_{10}H_{16}$	7.394	0.52	7.383	2.57	7.355	44.04	7.355	0.19	7.388	1.20	7.388	0.31
Eucalyptol	$C_{10}H_{18}O$	-	-	-	-	-	-	7.444	0.79	7.484	3.34	7.484	1.77
Camphor	$C_{10}H_{16}O$	-	-	-	-	-	-	9.488	0.22	9.494	0.65	-	-
Linalool	$C_{10}H_{18}O$	8.534	0.34	8.523	0.34	8.528	1.10	-	-	-	-	-	-
Citronellal	$C_{10}H_{18}O$	9.477	21.95	9.393	3.59	-	-	9.376	1.02	9.387	2.73	9.404	2.33
Citronellol	$C_{10}H_{20}O$	-	-	10.533	3.33	10.583	18.31	10.516	0.41	10.516	1.03	10.533	0.85
Citral	$C_{10}H_{16}O$	10.780	0.17	11.218	0.31	10.774	0.33	-	-	-	-	11.207	0.42
2,6-Octadiene, 2,6-dimethyl	$C_{10}H_{18}$	12.206	0.48	12.201	1.61	12.201	0.34	-	-	-	-	-	-
Geranyl acetate	$C_{12}H_{20}O_2$	12.622	0.44	12.644	4.97	12.622	0.58	-	-	-	-	-	-
β-Elemene	$C_{15}H_{24}$	12.886	3.86	12.874	2.23	-	-	-	-	-	-	-	-
δ-Cadinene	$C_{15}H_{24}$	14.615	3.49	-	-	-	-	-	-	14.598	0.35	14.609	0.24
Germacrene D	$C_{15}H_{24}$	-	-	-	-	14.183	0.42	-	-	-	-	14.205	0.27
Elemol	$C_{15}H_{26}O$	-	-	-	-	15.036	1.31	-	-	-	-	15.036	0.26
γ-Eudesmole	$C_{15}H_{26}O$	14.671	2.87	16.126	0.55	-	-	-	-	-	-	-	-
Myristic acid	$C_{14}H_{28}O_2$	-	-	-	-	-	-	18.310	25.29	18.299	39.32	-	-
Isopropyl Myristate	$C_{17}H_{54}O_2$	-	-	-	-	18.181	1.71	-	-	18.074	1.15	18.389	46.39
Cis-Pinane	$C_{10}H_{18}$	18.321	0.21	-	-	18.321	0.34	-	-	-	-	-	-

The main constituents of *C. nardus* essential oil were citronellal, citronellol and geraniol [13]. The characteristic bands of citronellal and citronellol which were the major compounds in *C. nardus* dominated by the C=O stretching of the aldehyde group ascribed by the band between 1740-1700 cm^{-1}. Within the range of 2970–2850 cm^{1}, typical asymmetric and symmetric stretching vibration bands of methyl and methylene groups were found. Figure 1(b) shows low intensities of the peak at 1740-1700 cm^{-1} and 2970-2850 cm^{-1} of commercial samples compared to essential oils of *C. nardus*. Furthermore, around 1670 cm^{-1}, the olefinic unsaturation C=C stretching of conjugated double bonds emerged [14]. Sharp and medium peaks were also seen for the bending of methylene CH_2 (1450-1440 cm^{-1}) and the symmetric bending of methyl CH_3 (1377 cm^{-1}). There were also some small vibrations of functional groups (910–740 cm^{-1}) and C-O stretching (1030-1000 cm^{-1}) [9].

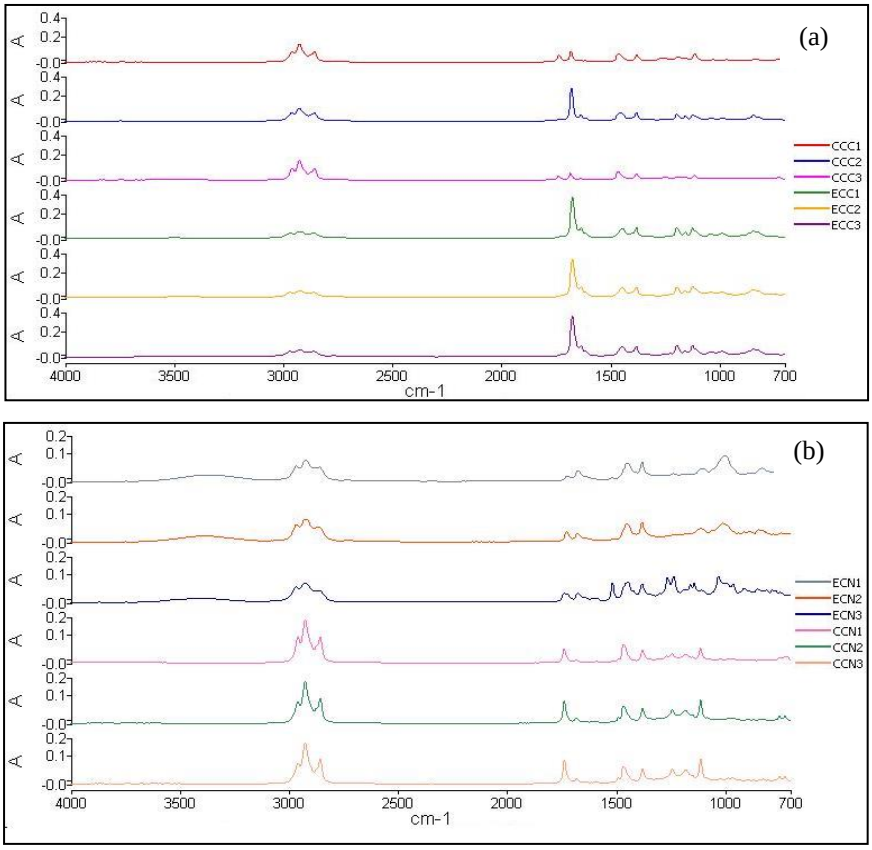


Figure 1. (a) FTIR spectra for essential oils and commercial samples of *C. citratus* (b) FTIR spectra for essential oils and commercial samples of *C. nardus*

Discrimination of samples based on FTIR data

The primary sources of variability in a dataset and the relationships between samples and variables are found using PCA analysis. The unsupervised method known as PCA provides pattern recognition data that is used to categorise and differentiate samples based on experimental data. FTIR spectroscopy is a simple, quick, and accurate method for classifying various plant species. Knowing the spectrum information rapidly could be highly important for the authentication of plant products in the market. In the present research study, FTIR data of *Cymbopogo*n essential oils (n=6) and commercial samples (n=6) were used to be distinguished by PCA analysis. The PCA showed two principal components (PCs) with 1400 variables totalling 80.37% of the total variance. PC1 encountered the most

explained variance with 61.70%. The PCA model of FTIR data showed good predictive ability as the total variance value was more than 50% [15]. The PCA analyses were performed to observe classification patterns of essential oils and commercial samples on the basis of their FTIR.

Based on the PCA plot in Figure 2(a), three clusters represented twelve samples. The cluster of ECN1, ECN2 and ECN3 appeared in the second quadrant. Meanwhile, CCN2 and CCN3 were found in the first quadrant where PC1 was negative. In addition, ECC1 and ECC2 had the largest distance from other sample clusters. Essential oils of *C. citratus* must have more distinctive functional groups compared to other samples. The findings are consistent with [16] who agreed that well-separated clusters are determined by distinctive compounds. This proved that the IR spectral characteristics of *C. citratus* essential oil samples were considerably different from those of other samples. Commercial samples of *C. citratus* clusters were located in the third quadrant. Nevertheless, it was found that commercial samples of *C. citratus* and *C. nardus* were unable to be differentiated. Therefore, it seemed quite challenging to observe the FTIR PCA score plot as FTIR can only identify the functional groups of the samples' essential oils, for which almost all samples have close similarities in terms of functional groups.

The PCA variable plot showed the discrimination of *C. citratus* and *C. nardus* essential oils attributed to a different absorbance of wave number present in the sample which was revealed by the fingerprinting FTIR spectroscopy. Based on the variables plot in Figure 2(b), the wavenumber of functional groups for samples of essential oils and commercial samples of *C. nardus* and *C. citratus* were difficult to deduce because they are overlapping each other. The loading plot unravels spectral differences responsible and have a strong impact on their clustering in the respective PC. The result of this observation indicated that the largest distance between *C. citratus* and *C. nardus* were caused by several wavenumbers representing certain functional groups listed in Table 3. Those responsible wavenumbers were determined by comparing the position of observation points in the loading plot with the PCA score plot and had variable influences on the projection (VIP) values in the range of 0.7 to 1.0. In general, variables with VIP values higher than 0.7 are considered significant for the separation of samples [17].

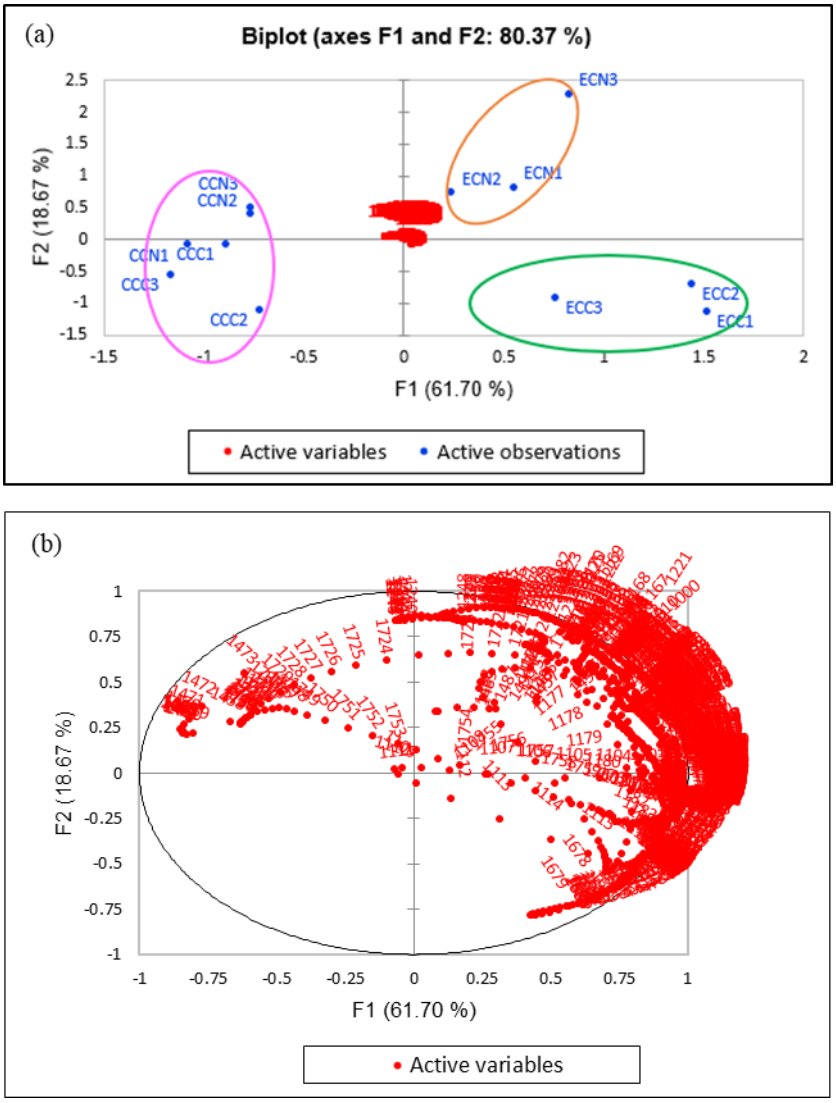
Table 3. Wavenumber responsible for the separation of *C. citratus* and *C. nardus* from PCA analysis on the loading plot

Wavenumber (cm^{-1})	**Band Assignment**	**Variable Influences on the Projection (VIP)**
1794	C=O from citronellal /citronellol	0.866
1710	C=O from citronellal /citronellol	0.941
1651	C=C from citral	0.800
1607	C=C from citral	0.812

These responsible wavenumbers are useful and could be used as a marker to discriminate *C. citratus* and *C. nardus*. In conclusion, although the distances of some clusters were very close, different sample species could still be discriminated by PCA. Overall, FTIR-PCA was able to discriminate the sample by visualizing the data on the score plot in contrast to FTIR spectra solely. This combination method could be applied as a non-destructive method for the discrimination of different species of herbs. The results revealed clear discrimination between essential oil of *C. citratus*, *C. nardus* samples and commercial samples. The result of PCA was further confirmed with HCA.

Samples of *C. citratus* and *C. nardus* essential oils and commercial samples were shown to be discriminated by the HCA dendrogram in Figure 2(c). A similar group is represented by the cluster of samples on the HCA dendrogram, as shown in the PCA plot. Commercial samples and essential oil samples were grouped into

three primary categories. On the right branch were two groups of *C. citratus* and *C. nardus* essential oil samples, and on the left branch were commercial samples. As can be observed, the commercial samples were assumed to be low-content of major chemical compounds or mixtures with other chemical substances. The clustering pattern of the examined samples was easily observed by the HCA dendrogram, and the outcomes were completely consistent with those of the PCA.



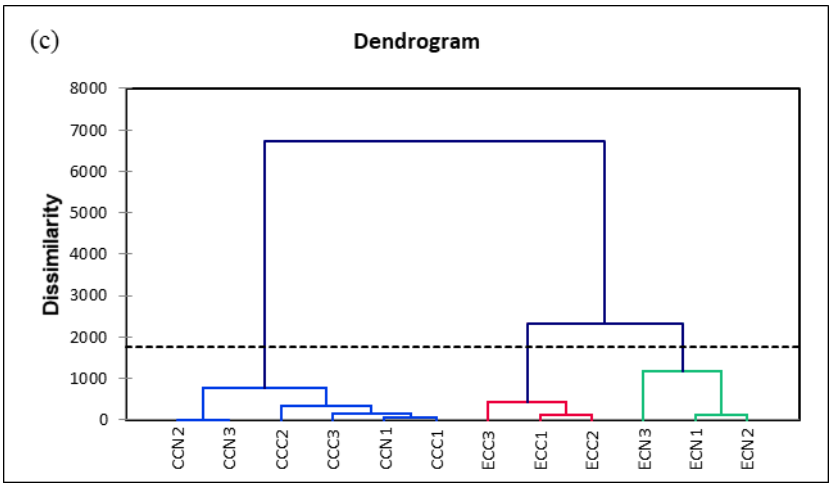


Figure 2. (a) Biplot PCA (b) variables plot and (c) HCA dendrogram of FTIR data of essential oils (n=6) and commercial samples (n=6) of *Cymbopogon* essential oils

This PCA plot was able to discriminate between *C. citratus* and *C. nardus* natural essential oils but not the commercial ones. The discriminant analysis (DA) model was built using the same pre-processed data to classify the samples based on natural essential oils and commercial samples by maximizing the covariance between the data matrix (X) (variables) and samples (Y) (origin of samples). DA is used to discriminate samples using the classification technique. Figure 3 shows the classification pattern of the samples using the DA model with variance explanation 10% and 0% of component 1 and component 2, respectively. The DA score plot showed clearer discrimination of commercial samples from *C. nardus* and *C. citratus* compared to PCA.

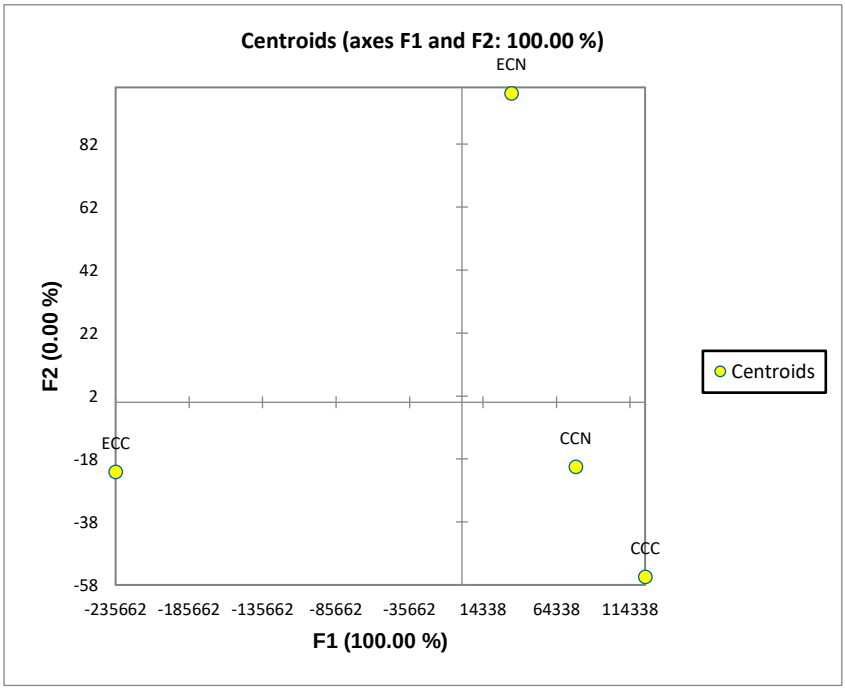


Figure 3. Discriminant analysis plot

A unidimensional test of the quality of the means of the classes identifies the significant variables in the study. Variables with p-value < 0.05 indicate significant variable that plays role in the discriminating model. The Fisher distances test at α 0.05 between the groupings is significantly different. The p-value < 0.01 indicates the natural essential oils of *C. citratus* and *C. nardus* are significantly different but there is no difference between commercial samples of *C. citratus* and *C. nardus* (Table 4). The results obtained revealed the commercial samples were either low-quality or mixtures with other chemical substances.

Table 4. The *p*-values for Fisher distances

Sample	CCC	CCN	ECC	ECN
CCC	1	0.000	< 0.0001	< 0.0001
CCN	0.000	1	< 0.0001	0.000
ECC	< 0.0001	< 0.0001	1	< 0.0001
ECN	< 0.0001	0.000	< 0.0001	1

Conclusion

C. nardus and *C. citratus* essential oils were considered important products in the food and cosmetic industry. Consumer safety greatly depends on the ability to trace the quality of *C. nardus* and *C. citratus* essential oils. The development of new analytical techniques that are robust, dependable, effective, and affordable is crucial for determining the authenticity of essential oils. There is currently interest in chemometrics and innovative analytical techniques. The current research uses FTIR techniques for the characterization of *C. nardus* and *C. citratus* essential oils. The findings demonstrate that the authenticity of *C. nardus* and *C. citratus* essential oils can be successfully determined by FTIR and chemometrics (HCA & PCA). It takes little sample preparation to distinguish both essential oils from commercial samples. As far as we are aware, this is the first example in which the FTIR technique and chemometrics have been employed to assess the authenticity of *C. nardus* and *C. citratus* essential oils.

In summary, it was successful to distinguish the essential oils of *C. citratus* and *C. nardus* from commercial samples using FTIR, and chemometrics. Through HCA and PCA analysis, the classification patterns of different samples may be readily revealed. To the best of our knowledge, this study has been the first to use chemometrics along with FTIR to assess the discrimination of *C. citratus* and *C. nardus* essential oils with commercial samples. This study has the benefit of being a thorough application of simple and easy well-known analytical methods. Nevertheless, the GCMS data obtained complements and confirms the chemical compounds in *C. citratus* and *C. nardus,* particularly the identification of citral and citronellal as major component in each species, respectively. Chemometric analysis through PCA and HCA is a fast, economic, reliable, simple and non-destructive analysis for quality control and authenticity of *C. citratus* and *C. nardus* essential oils. The findings of this study may be used to give a thorough assessment of the quality of *C. citratus* and *C. nardus* commercial products, as well as an optimal evaluation technique for medicinal herb quality management.

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