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Research Article
GC-MS and ATR-FTIR Spectroscopy Coupled with Chemometric Analysis for Detection and Quantification of White Turmeric (*Curcuma zedoaria*) Essential Oils Adulteration

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Abstract

Background and Objective: White turmeric essential oil (WTEO) is known to have high commercial value since it has been used to improve immunological function, increase blood circulation, ease toxin clearance and stimulate digestion. However, there is no standard to regulate the specific characteristics of white turmeric essential oil. Therefore, the objective of this research was to develop an analytical technique for WTEO authentication from vegetable oils, namely palm oil (PO), coconut oil (VCO) and soybean oil (SO), using FTIR spectroscopy and chemometrics, as well as GC-MS spectroscopy. **Materials and Methods:** The WTEO was obtained by hydrodistillation method. Pure WTEO and vegetable oils were scanned in the MIR region (4000-650 cm^{-1}) of FTIR spectroscopy and the spectra were further analyzed using chemometrics. **Results:** The extraction yielded 0.103% v/w WTEO, a dark purple color with a specific pungent odor. Discriminant analysis separated pure WTEO and adulterated WTEO with 100% accuracy at wave numbers 4000-650 cm^{-1}. The best PLS regressions to quantify SO, VCO, PO and concentration in WTEO were at wave numbers 4000-1100, 1400-1050 and 2100-650 cm^{-1}, respectively. **Conclusion:** The FTIR and chemometrics combination effectively authenticates white turmeric essential oil from any possible adulterants, such as vegetable oil.

Key words: White turmeric, chemometrics, instrumental analysis, GC-MS analysis, essential oil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The high demand and acceptance of essential oil (EO) encouraged adulteration practices to maximize profit and make competitive prices. Adulterated oil can significantly reduce its quality and have harmful effects on those who consume it. Thus, the quality of essential oils should refer to specific international or national standards, namely International Standard for Standardization (ISO) or Indonesian National Standard (SNI). However, only a few oils have standard quality. Therefore, authentication of EOs could be an essential part of ensuring the quality and safety of EOs. There are various ways to authenticate essential oils (EOs), including physicochemical techniques and analytical methods using chromatography and spectroscopy[1]. Both spectroscopy and chromatographic methods, such as Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transformation Infrared (FTIR) spectroscopy coupled with chemometrics analysis, have been used to authenticate essential oil[2-7].

Chemometrics offers advantages by producing faster and more economical analysis. Chemometrics could be used for qualitative purpose through pattern recognition methods such as using discriminant analysis. In contrast, Partial Least Square (PLS) were used for quantitative analysis which aimed to establish a model that allows the analysis of an unknown sample[8,9].

White turmeric essential oil (WTEO) was reported to have various biological activities such as cytotoxic, antimicrobial and larvicidal, etc.[10,11]. The variation in chemical constituents of WTEO could be due to geographical conditions and plant variation[12]. Previous reviews reported that *C. zedoaria* essential oil could be divided into 2 clusters. First, it was dominated by curzerenone/epi-curzerenone and others dominated by 1,8-cineole[13]. White turmeric oil or zedoary oil could also be extracted from different species such as *Curcuma phaeocaulis* valeton, *Curcuma zedoaria*, *Curcuma kwangsiensis* S.G. Lee & C.F. Liang and *Curcuma wenyujin* described by Chen *et al.*[14]. However, there is no standard to regulate the specific characteristics of white turmeric essential oil. Thus, the study aimed to develop rapid and simple analytical technique to authenticate white turmeric essential oil (WTEO) using FTIR spectroscopy and chemometrics, as well as GC-MS spectroscopy.

MATERIALS AND METHODS

Study area: The study was conducted for 4 months in Laboratorium of Natural Product Chemistry, Faculty of Pharmacy, Universitas Andalas Padang, West Sumatra, Indonesia.

Sample collection and physical characterization: About 10 kg of white turmeric (*Curcuma zedoaria* Rosc.) fresh rhizome was obtained from the Sitiung region, Dharmasraya, West Sumatra, Indonesia. A botanist at the Andalas University Herbarium (ANDA), located in the Department of Biology at the Faculty of Mathematics and Natural Sciences in Andalas University, Padang, was identified the rhizome. The fresh rhizomes were washed thoroughly with water to remove any dirt. The clean rhizomes were then cut with a thickness of 2-3 mm and extracted by hydrodistillation method using a Clevenger-type apparatus for 4-6 hrs. The essential oil was collected into dark bottle. To remove any remaining water, anhydrous Na_2SO_4 was added. Then, the essential oil was stored at 4°C for further used. The physical properties of WTEO were assessed, which included its color, odor, specific gravity, refractive index and optical rotation.

Preparation of binary mixture white turmeric essential oil and vegetable oils: The binary mixture contained combinations of WTEO and vegetable oils (palm oil, virgin coconut oil and soybean oil) in varying concentrations ranging from 0 to 100% v/v, as indicated in Table 1.

Table 1: Binary mixture of white turmeric essential oil and vegetable oils

	Percentage (%v/v)	
Binary mixture	WTEO	Vegetable oils
1	100	0
2	90	10
3	80	20
4	70	30
5	60	40
6	50	50
7	40	60
8	30	70
9	20	80
10	10	90
11	0	100

Chemical composition of white turmeric essential oil by Gas Chromatography-Mass Spectroscopy (GC-MS): The Gas Chromatography-Mass Spectroscopy (Shimadzu GCMS-QP 2010 SE) was used to determine the components of white turmeric oil (WTEO). The experimental conditions were identical to those previously described by Syafri *et al.*[10]. Identification of the compound was done by referring to the "WILEY library" in the GC-MS program.

Analysis of pure and binary mixture of white turmeric essential oils by Attenuated Total Reflection Fourier Transform-Infra Red (ATR-FTIR): The Shimadzu FTIR spectrometer (Shimadzu Corp) was used to acquire the FTIR spectra. The measurements were taken at a temperature of 25°C. Samples were placed on the Smart iTR ATR surface and scanned in the MIR region with a 4000-650 cm^{-1} wave number range. Before measuring the WTEO spectra, the air background was measured. The spectral measurements were repeated three times. The spectra analysis was performed using OMNIC software version 9.

Chemometric analysis: The chemometric analysis was performed using TQ analyst™ version 9. Discriminant analysis was used to classify the pure and adulterated WTEO. At the same time, Partial Least Square (PLS) analysis was utilized for quantitative analysis to predict the authenticity of white turmeric essential oil. The observed parameters are latent variables, correlation coefficients (R^2 values), standard errors (RMSEC and RMSEP) and outlier diagnoses.

RESULTS

Physical characterization of white turmeric essential oil: Figure 1 shows essential oil extracted from fresh rhizome of the white turmeric. The extraction of white turmeric rhizomes obtained 0.103% of essential oil. Table 2 provides information on the physical characterization including yield, color, refractive index, specific gravity and optical rotation.

Chemical constituents of white turmeric essential oil: The GC-MS chemical analysis revealed 70 compounds consisting of monoterpene, sesquiterpene and aliphatic compounds. The WTEO contained seven major compounds including isovelleral, germacrone, camphor, eucalyptol, camphene, β-elemene and curzurene (Fig. 2).

Table 2: Physical characterization of white turmeric essential oil

Parameter	Current study	Indonesian pharmacopeia by KKRI[15]	Previous study by Rosa *et al.*[16]
Color	Specific, pungent	-	Specific, pungent
Odor	Dark purple	-	Brown
Yield	0.103% v/w	Not less than 0.10% v/w	0.66% v/w
Specific gravity (g/ml)	1.03 g/mL	-	0.98 g/mL
Refractive index	1.52	-	1.50
Optical rotation	+14.99°	-	+15.19°

Fig. 1: White turmeric essential oil

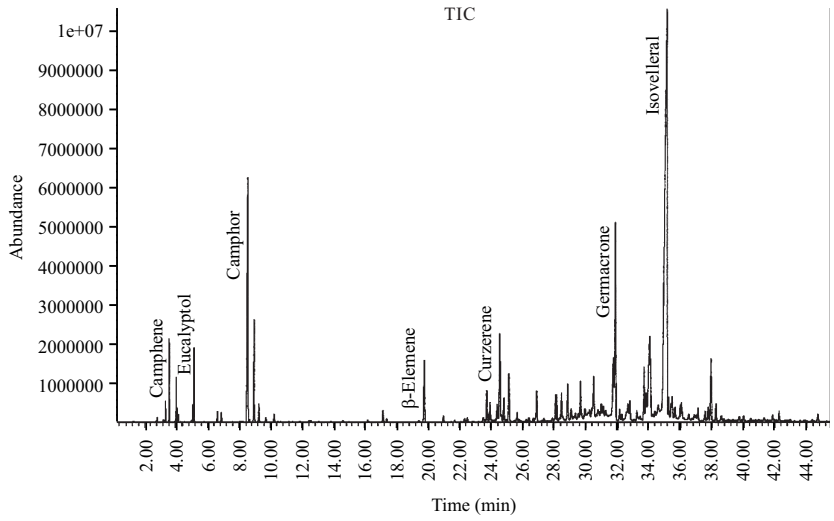

Fig. 2: Total ion chromatogram (TIC) of white turmeric essential oil

Table 3: Chemical composition of WTEO (present study and previous study)

	Percentage relative (%)	
Chemical constituents	Present study	Previous study[17-19]
Camphene	1.67	1.9
1,8 cineole/eucalyptol	1.88	1.6
Camphor	12.60	19.7
Isoborneol	4.13	5.1
(-)-β-Elemene	3.11	2.1
Germacrene D	1.60	0.8
Curzerene	4.37	5.0
Germacrene B	1.17	1.2
β-Caryophyllene	2.42	0.4
(-)- Spathulenol	1.21	5.0
β-Selinene	1.10	1.5
Epicurzerenone	1.75	7.4
α-Curcumene	1.50	3.9
Germacron	9.74	9.0
Isovelleral	29.30	2.5

The terpenoid compounds constituted 77.55% of the total percentage of WTEO, with 20.28% monoterpenes and 57.27% sesquiterpenes. The oxygenated monoterpene found was 1,8-cineole (1.88%), while monoterpene hydrogen consisted of camphor (12.60%), isoborneol (4.13%) and camphene (1.67%). The highest percentage of the sesquiterpene class component was Isovelleral (29.30%) as seen in Table 2. While, a previous study found Camphor (19.70%) to be the largest percentage followed by germacron and epicurzerenone, as shown in Table 3.

FTIR spectra of white turmeric essential oil and vegetable oil: Figure 3 demonstrates that the FTIR spectrum of white turmeric essential oil (WTEO) has different pattern from vegetables oil. However, the FTIR spectra of vegetables oil revealed that palm oil (PO), virgin coconut oil (VCO) and soybean oil (SO) gave nearly the same pattern except for the absorbance values of each peak, which indicated that the chemical compounds contained were almost the same but differed in concentration.

Most of the peaks and shoulders found in the FTIR spectra of essential and vegetable oils were caused by specific functional groups responsible for absorbing infrared radiation. Each compound's functional group absorbs IR radiation and would be visible as a peak at one particular wave number (Table 3). Based on Table 4, the most substantial peak observed at 1743 cm^{-1} was associated with the stretching vibration of the carbonyl (C = O) found in vegetable oil,

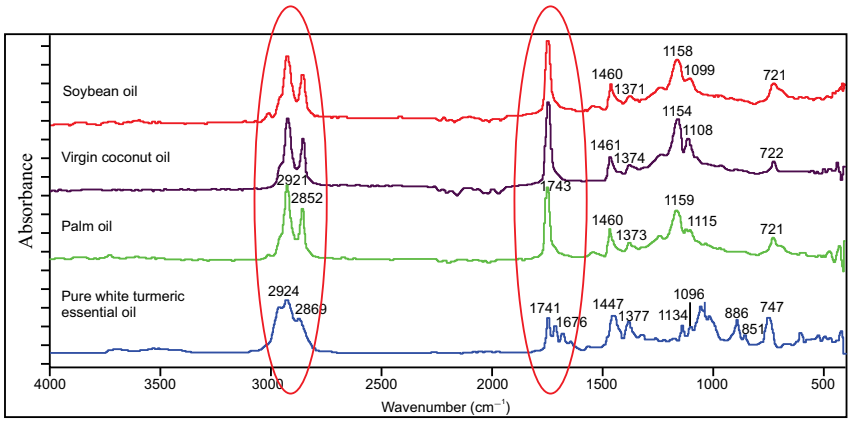

Fig. 3: FTIR spectra of pure white turmeric essential oil (WTEO), palm oil (PO), virgin coconut oil (VCO) and soybean oil (SO)

Table 4: Functional group that absorbs infrared light at wave numbers 4000-650 cm^{-1}

Wavenumber (cm^{-1})	Functional group
2924 and 2850	Asymmetrical and symmetrical stretching vibration of methylene (-CH_2) group
1743	C = O stretching vibration
1676	C = C stretching vibration
1446 and 1378	Bending vibrations of methyl (-CH_3) dan methylene (CH_2)
1159	C-O ether bending vibrations
1115, 1108, 1099 and 1096	C-O ether stretching vibration
886 and 851	Bending out of plane vibrations of-HC = CH- (trans) and -HC = CH- (cis)- HC = CH- (trans) and -HC = CH- (cis)

Table 5: Best PLS model for each binary mixture

			Calibration		Validation	
Model	Wavenumber (cm^{-1})	Spectra	R^2	RMSEC	R^2	RMSEP
PLS (WTEO-palm oil)		Normal	0.9602	0.0884	0.9781	0.0659
	2100-650	**1st derivative**	**0.9970**	**0.0246**	**0.9913**	**0.0449**
		2nd derivative	0.9980	0.0199	0.9812	0.0626
PLS (WTEO-soybean oil)		Normal	0.9922	0.0393	0.9952	0.0372
	4000-1100	**1st derivative**	**0.9994**	**0.0110**	**0.9970**	**0.0297**
		2nd derivative	0.9906	0.0432	0.9933	0.0658
PLS (WTEO-virgin coconut oil)		Normal	0.9825	0.0589	0.9815	0.0619
	1400-1050	1st derivative	0.9977	0.0212	0.9896	0.0480
		2nd derivative	**0.9799**	**0.0630**	**0.9899**	**0.0474**

*Bold indicated the best model

identified as triglyceride[20]. However, the peak was less prominent in pure white turmeric essential oil than in vegetable oil. The vibrations at 2924 and 2850 cm^{-1} were observed for vegetable oil and WTEO, but the patterns were not identical. The peak of 1676 cm^{-1} was only observed in white turmeric essential oil, indicating C = C stretching vibrations. The broad peak at 1159 cm^{-1} observed in palm oil, coconut oil and soybean oil was associated with the C-O ether bending vibration.

Chemometric analysis: Discriminant analysis was employed to distinguish pure WTEO and adulterated WTEO with vegetable oil at wave numbers of 4000-650 cm^{-1}. Figure 4(a-c) reveals the Cooman plot of WTEO and vegetable oils. Discriminant analysis successfully classified pure WTEO and adulterated WTEO with 100% accuracy without misclassification.

In addition, the quantification of adulterants in a binary mixture of WTEO was quantified using PLS regression. A reliable and satisfactory PLS model was characterized by low RMSEC and RMSEP values, representing the deviation between predicted and measured values. These values provide an overall assessment of the model's accuracy and how well it predicts the same sample used to develop it. The best PLS model for each binary mixture as shown in Table 5.

DISCUSSION

The characteristics of essential oils determine their quality, ultimately determining their value or price. Parameters

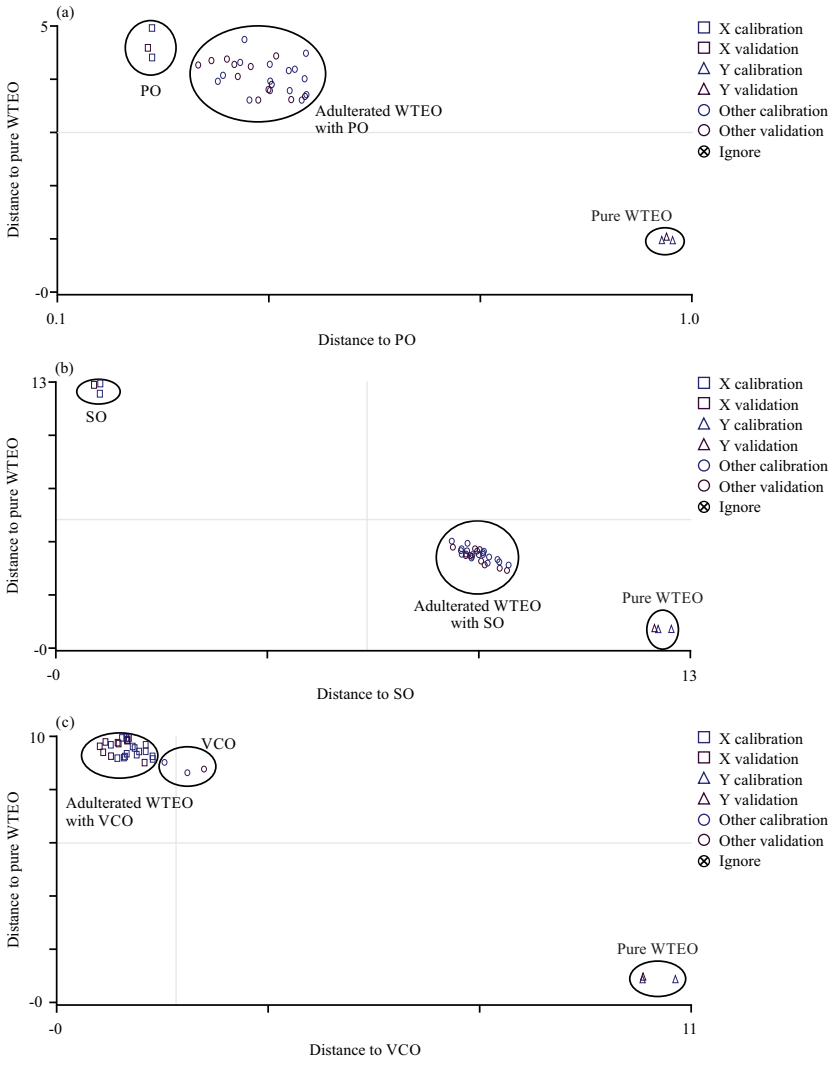

Fig. 4(a-c): Cooman's plot for pure WTEO and adulterated WTEO with (a) Palm oil, (b) Soybean oil and (c) Virgin coconut oil

such as consistency color, odor, specific gravity, optical rotation, refractive index, solubility, ester value, acid value and ester value after acetylation are used to determine physicochemical characteristics. The parameters could refer to the international and national standards[8]. In this study, the white turmeric essential oil yielded 0.103% v/w. However, Indonesian Herbal Pharmacopoeia (FHI) stated that the essential oil content of white turmeric rhizomes was no less than 0.10% v/w[15]. However, a previous study noted that white turmeric essential oil yield was 0.66% v/w[16]. The yield of essential oils obtained in this study was by the requirements in the FHI but lower than in other studies.

The chemical composition of WTEO oil originated from West Sumatra, Indonesia, which is different from other locations. As described in the result section, the highest percentage of the constituents was Isovelleral. A previous study found camphor to be the major component[17-19]. The variation of essential oil constituents were caused by several factors, such as plant age, plant variety, soil fertility, climate, drying method, distillation method and instrument used for extraction[12].

In this study, an analytical method was developed to distinguish white turmeric essential oil from vegetable oils, including palm oil (PO), coconut oil or virgin coconut oil (VCO) and soybean oil (SO) by using a combination of FTIR spectroscopy and chemometric techniques. The FTIR spectroscopy is a reliable approach to quantitatively analyze essential and vegetable oils, as it offers a "fingerprint technique" that allows for comparing IR spectra. Each essential oil has a unique FTIR spectrum, meaning that the number of peaks and their intensities (absorbance) at the maximum peak are distinct. By comparing IR spectra, it's

possible to detect adulteration in white turmeric essential oil[7]. White turmeric essential oil generally consisted of sesquiterpenes and monoterpenes[20], while palm oil (PO), virgin coconut oil (VCO) and soybean oil (SO) generally consisted of triglycerides, glycerol and many other types of fatty acids[21-24].

Discriminant analysis was employed to distinguish pure WTEO and adulterated WTEO with vegetable oil. The classification was done by comparing the similarities between the samples. The Mahalanobis distance was calculated using the absorbance of each peak at wave numbers in the range of 4000-650 cm^{-1}. Then, the Cooman plot was generated from this data. In addition, an analysis was conducted on the quantification of adulterants in a binary mixture of WTEO using PLS regression. Several parameters were assessed, such as the correlation coefficient (R^2), Root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP)[21]. A reliable and satisfactory PLS model is characterized by low RMSEC and RMSEP values, representing the deviation between predicted and measured values. These values provide an overall assessment of the model's accuracy and how well it predicts the same sample used to develop it. Furthermore, a desirable R^2 calibration coefficient value should be close to 1[7].

CONCLUSION

Extraction of white turmeric fresh rhizomes by hydrodistillation method yielded essential oil with Isovelleral as the primary component. Discriminant analysis separated white turmeric essential oil from palm oil, VCO and soybean oil without misclassification. The best PLS regression model for soybean oil, VCO palm oil, was successfully developed at middle infrared (MIR) region. Thus, the combination of FTIR and chemometrics was successfully used to authenticate white turmeric essential oil in vegetable oil.

SIGNIFICANCE STATEMENT

There is currently no utilization of combination of FTIR and GC-MS with chemometrics for authenticating white turmeric essential oil. However, this technique can provide a simple, fast and environmentally friendly analysis, making it a potential alternative for authenticating white turmeric essential oil. Additionally, documenting the physical characterization of white turmeric essential oil can aid ministries or policymakers in collecting information for reference books such as pharmacopeia and national or international standards.

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