

Optimization of ar-Turmerone Isolation Method from Turmeric Oleoresin using Silica Gel Adsorbent

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Abstract: Ar-turmerone is one of the curcumin derivatives contained in turmeric oleoresins. Ar-turmerone has various pharmacological effects, including antivenom, antidepressant, antiepileptic, antidermatophyte, antitumor, and antiplatelet activity. This research aimed to develop an isolation method of ar-turmerone from turmeric oleoresin using silica gel adsorbent, which is relatively low cost. Ar-turmerone was isolated by adsorption process using silica gel adsorbent and followed by elution process using a suitable solvent. The UV-visible spectrum of the isolated compounds showed a maximum wavelength at 237 nm. FTIR spectrum values were found at 2300 cm^{-1} and 2935 cm^{-1} (for aromatic C=C stretching), 1445 cm^{-1} and 1735 cm^{-1} (for C=O stretching), and 990 cm^{-1} and 1100 cm^{-1} (for C-H bending). GC-MS spectrum showed a main peak at a retention time of 12.884 minutes and a percent area of 27.74%, which was identified as ar-turmerone (molecular weight of 216). The optimum condition for the isolation of ar-turmerone from turmeric oleoresin using silica gel as adsorbent was the use of turmeric oleoresin:silica gel with a ratio of 1:3 without being affected by adsorption time. The optimum eluent for the elution process was 96% ethanol.

Keywords: ar-turmerone; turmeric oleoresin; silica gel; adsorption.

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1. Introduction

Turmeric (*Curcuma longa* L.) is a typical plant cultivated in tropical and subtropical areas of Asia, Australia, and South America. Currently, turmeric is widely grown in various Asian countries, such as India, Bangladesh, Thailand, Malaysia, Indonesia, and the Philippines [1]. Turmeric has been widely used in traditional and modern medicine because it has antimicrobial, anti-inflammatory, antioxidant, anticancer, neuroprotective, hepatoprotective, cardioprotective, and hypoglycemic activities [2-10]. The main part of turmeric is the rhizome [11]. Turmeric rhizome contains several major chemical compounds, including curcumin, demethoxycurcumin, bis-demethoxycurcumin, bisacurone, α -turmerone, β -turmerone, and ar-turmerone [1,12-14]. Chemical content in turmeric can be obtained in the form of turmeric powder, turmeric oil, oleoresin, and curcuminoid isolates.

Turmeric oleoresin is a by-product obtained from the extraction process of turmeric using organic solvents. Although it is a by-product that is rarely used, turmeric oleoresin still contains many compounds, including 37-55% curcuminoids and up to 25% essential oil [15]. Curcumin, one of the curcuminoids, is a phenolic compound that has various health benefits and can prevent various diseases. One of the curcumin derivatives also widely contained in turmeric oleoresin is ar-turmerone. Ar-turmerone also has various pharmacological effects, as

well as curcumin. For example, Ar-turmerone has strong antivenom activity against snake bites [16]. Ar-turmerone also has anti-inflammatory, anti-plasmodial, neuroprotective, and antiaging activities [17-20]. In addition, with a certain dose, ar-turmerone also has pharmacological activities such as antidepressant, antiepileptic, anti-dermatophyte, antitumor, and antiplatelet [21].

Various pharmacological effects of ar-turmerone have led to the development of research on ar-turmerone isolation methods. Ar-turmerone is one of the main compounds contained in turmeric essential oil. In previous studies, turmeric essential oil has been extracted using various methods, such as hydrodistillation and extraction involving lyophilization [22-27]. Purification of ar-turmerone has been carried out using preparative high-performance liquid chromatography (HPLC) [28]. In addition, purification of ar-turmerone has also been carried out using GC (Gas Chromatography) with chloroform as solvent [29]. However, it is still considered a relatively difficult and expensive method for preparative purposes. Therefore, this research will develop a method of isolation of ar-turmerone from turmeric oleoresin using silica gel adsorbent, which is relatively low cost. Previous studies have shown that silica gel is a suitable adsorbent for several terpenes [30]. In addition, the use of a variety of non-polar solvents can cause the silica adsorbent to be more selective in absorbing ar-turmerone. So it is hoped that ar-turmerone isolates with high purity can be obtained at a relatively low cost. This study aims to obtain the optimum conditions for isolating ar-turmerone from turmeric oleoresin using silica gel as an adsorbent.

2. Materials and Methods

2.1. Materials.

Turmeric rhizome, ethanol (Merck), n-hexane (Merck), silica gel 60 (0.2-0.5 mm) for column chromatography (Merck), acetonitrile (Fulltime), ethyl acetate (Fulltime), toluene (Merck), TLC silica gel 60 F₂₅₄ (Merck).

2.2. Turmeric oleoresin extraction.

Turmeric rhizome was macerated for 72 h using 96% ethanol in a ratio of 1:10. After 72 h accompanied by occasional stirring, filtration was carried out, followed by filtrate evaporation with rotary evaporator until a thick extract was obtained. The thick extract was added with n-hexane in a ratio of 1:2 and then stirred until all the thick extracts were homogeneous. The mixture is allowed to stand until the insoluble part is precipitated and then filtered. The n-hexane filtrate was evaporated to obtain turmeric oleoresin.

2.3. Turmeric oleoresin characterization.

Turmeric oleoresin as raw material was characterized by solubility test, specific gravity, Fourier transform infrared (FTIR), UV-visible spectrophotometry, thin-layer chromatography (TLC), and gas chromatography-mass spectrometry (GC-MS). FTIR spectrum was taken in Nicolet iS5 Thermo Fisher Spectrometer using the resolution of 4 cm⁻¹ in the 4000-400 cm⁻¹ range. Maximum wavelength was identified using Shimadzu UV-1800 Spectrophotometer. Chemical content in turmeric oleoresin was analyzed using Shimadzu GCMS-QP2010 Ultra.

2.4. *ar-Turmerone isolation using silica gel adsorbent.*

Turmeric oleoresin was mixed with silica gel, with a certain ratio (variation of turmeric oleoresin:silica gel ratio = 1:1, 1:2, and 1:3). The mixture was then homogenized and allowed to continue shaking using an orbital shaker at 100 rpm with variations of adsorption time for 2 h, 4 h, and 6 h. After the adsorption process, the silica gel was eluted with a limited volume of acetonitrile solvent. The acetonitrile filtrate was collected in a vial. The silica gel residue was dried and then re-eluted with 96% ethanol, ethyl acetate, toluene, and n-hexane. Each filtrate was collected in vials for further characterization.

2.5. *Characterization of filtrate.*

The filtrate was characterized by Fourier transform infrared (FTIR), UV-visible spectrophotometry, and gas chromatography-mass spectrometry (GC-MS), under the same conditions as turmeric oleoresin characterization.

3. Results and Discussion

3.1. *Characterization of turmeric oleoresin.*

In the present work, a yellow form of turmeric oleoresin with a characteristic odor was obtained as a by-product from turmeric extraction using ethanol, followed by extract purification using n-hexane. The density of turmeric oleoresin obtained in this work is 0.8152 g/ml. The solubility of turmeric oleoresin was characterized using various organic solvents (Table 1). Solubility of turmeric oleoresin in acetonitrile, ethanol 96%, ethyl acetate, toluene, and n-hexane was 2.625 g/100 ml, 4.5 g/100 ml, 4.846 g/100 ml, 12.6 g/100 ml, and 1.8 g/100 ml, respectively. These results indicated that turmeric oleoresin has the highest solubility in toluene.

Table 1. Solubility of turmeric oleoresin in various organic solvents.

Solvent	Solubility (g/100 ml)
Acetonitrile	2.625
Ethanol 96%	4.5
Ethyl acetate	4.846
Toluene	12.6
n-hexane	1.8

Thin-layer chromatography (TLC) was carried out to identify *ar-turmerone* in turmeric oleoresin. TLC was performed using toluene:ethyl acetate (8:2) as mobile phase. The result showed a spot at R_f of 0.43 (Figure 1).



Figure 1. TLC of turmeric oleoresin.

The FTIR spectrum for turmeric oleoresin showed stretch vibration bands of aromatic C=C at 2359.87 cm^{-1} , stretch vibration bands of C=O at 1729.29 cm^{-1} , and bend vibration bands of C-H at 1116.47 cm^{-1} (Figure 2). These results showed the typical functional groups found in ar-turmerone.

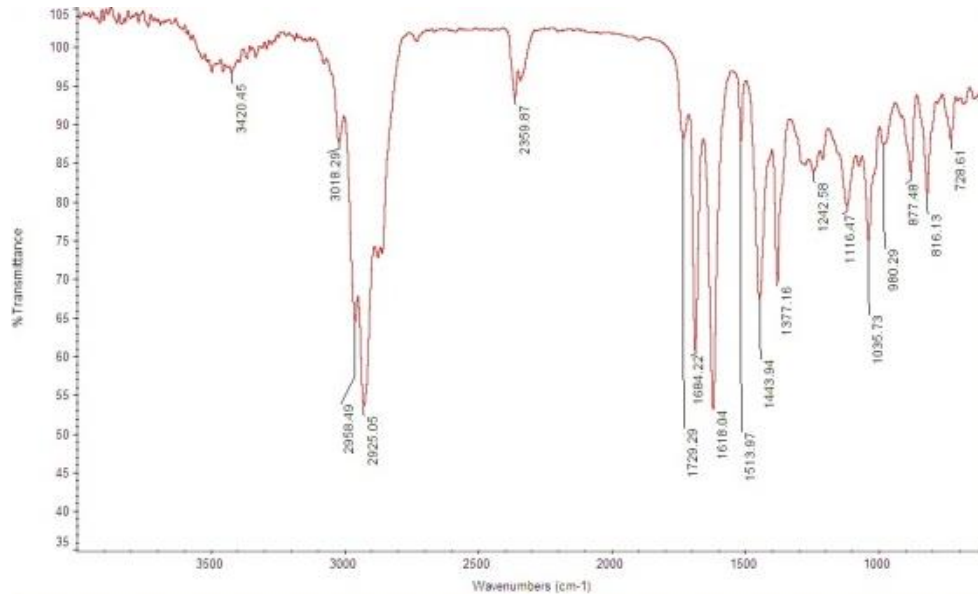


Figure 2. FTIR spectrum of turmeric oleoresin.

Turmeric oleoresin characterization was also carried out by determining the maximum wavelength. The UV-visible spectrum showed a maximum wavelength of 233 nm (Figure 3). This result was in line with previous studies, where the maximum wavelength of ar-turmerone was 233.5 nm [23].

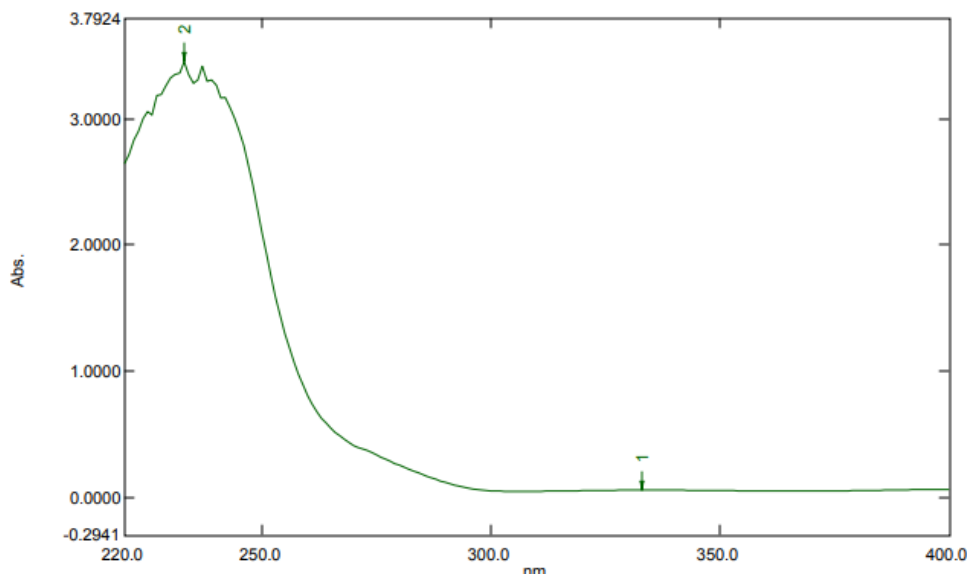
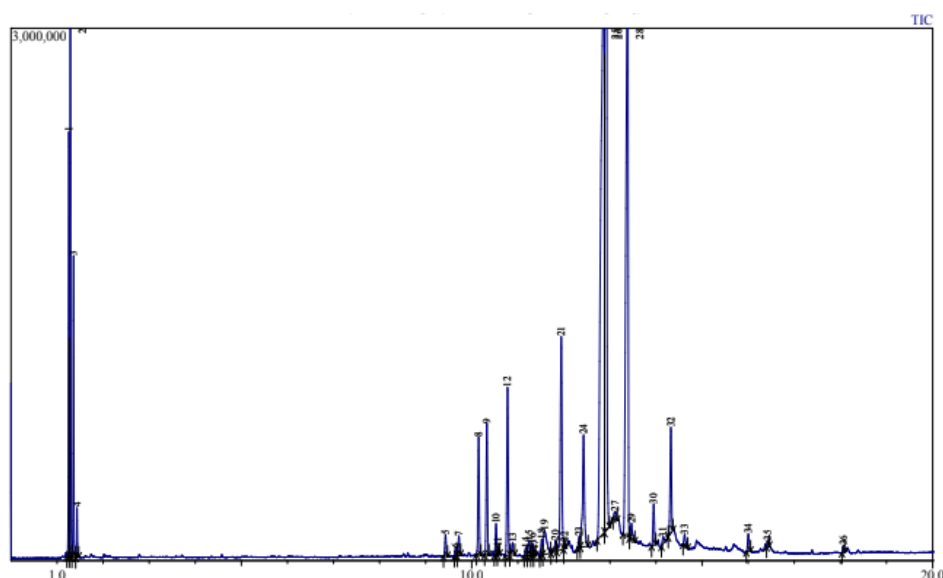


Figure 3. The UV-visible spectrum of turmeric oleoresin.

Further characterization was carried out by gas chromatography-mass spectrometry (GC-MS) analysis. The results of the GC-MS analysis showed that turmeric oleoresin contains 36 components, with the main compound being ar-turmerone, with a retention time of 12.921 minutes and the percent area of 19.19%, and β -turmerone, with a retention time of 12.858 minutes and the percent area of 25.84% (Figure 4).



16	11.316	181301	0.28	71216	2.55	2,5,9-TRIMETHYL-CYCLOUNDECA-4,8-DIENONE
17	11.360	59240	0.09	33153	1.79	1-(1,2,3-TRIMETHYL-CYCLOPENT-2-ENYL)-ETHANONE
18	11.519	195171	0.30	90937	2.15	AR-TUMERONE
19	11.578	683448	1.07	136239	5.02	2,3-DIBROMO-8-PHENYL-P-MENTHANE
20	11.817	169195	0.26	63252	2.67	2,4-DINITRO-6-(2-BUTYL)-PHENYL-.beta.,.beta.-DIMETHY
21	11.945	2970146	4.64	1199913	2.48	Benzene, 1,4-dimethyl-2-(2-methylpropyl)- (CAS) 1,4-Dimethyl-
22	12.015	71762	0.11	37471	1.92	Bicyclo[2.2.1]hept-2-ene, 2,3-dimethyl- (CAS) Santene
23	12.330	157122	0.25	59590	2.64	FARNESOL 1
24	12.429	1929166	3.01	631225	3.06	EPI-ALPHA-PATSCHULENE
25	12.858	16535543	25.84	3247117	5.09	.BETA. TUMERONE
26	12.921	12279022	19.19	4152218	2.96	AR-TUMERONE
27	13.112	136858	0.21	43273	3.16	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis
28	13.380	10621033	16.60	3385837	3.14	ALPHA-TUMERONE

Figure 4. GC-MS spectrum of turmeric oleoresin.

3.2. Characterization of isolated compounds.

Isolated compounds were obtained from the adsorption of turmeric oleoresin on silica gel with various ratios and adsorption times. The silica gel adsorbed the compounds on the turmeric oleoresin was then eluted with various organic solvents, starting from the most polar solvent. Each eluate was then identified using a UV-visible spectrophotometer to determine its maximum wavelength (Table 2). Based on the ratio of silica gel, adsorption time, and type of eluent, each eluate showed a different maximum wavelength. However, the eluate of adsorption results with a 1:3 ratio turmeric oleoresin:silica gel eluted with ethanol showed a consistent maximum wavelength and was not affected by adsorption time. This is due to the polarity of ar-turmerone, which is close to the polarity of ethanol. Thus, in the next process, the eluate of adsorption results with a 1:3 ratio turmeric oleoresin:silica gel eluted with ethanol (hereinafter referred to as ethanol eluate) was used.

Table 2. Maximum wavelength of eluate from the adsorption process of turmeric oleoresin on silica gel.

Turmeric oleoresin-silica gel ratio	1:1			1:2			1:3		
	2	4	6	2	4	6	2	4	6
Acetonitrile	322 259	322	235	236	235	235	234	231	235
Ethanol 96%	236	237	237	237	236	237	237	237	237

Turmeric oleoresin-silica gel ratio	1:1			1:2			1:3		
	2	4	6	2	4	6	2	4	6
Ethyl acetate	255	322	256	254	253	259 223	254	254	323 253
Toluene	291	281	327	280	247	282	283	284	237
n-hexane	254	236	266 238	237	234	234	239	234	233

The FTIR spectrum for ethanol eluate showed stretch vibration bands of aromatic C=C at 2300 cm⁻¹ and 2935 cm⁻¹, stretch vibration bands of C=O at 1445 cm⁻¹ and 1735 cm⁻¹ and bend vibration bands of C-H at 990 cm⁻¹ and 1100 cm⁻¹ (Figure 5). These results aligned with the FTIR spectrum of turmeric oleoresin, which showed the typical functional groups found in ar-turmerone.

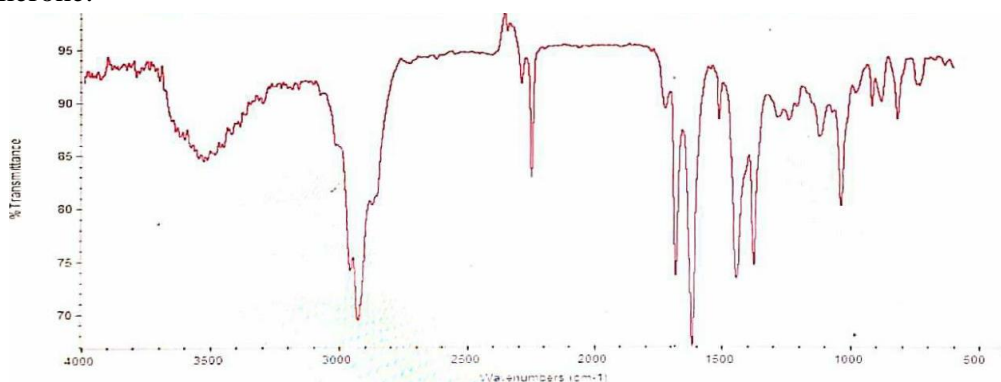
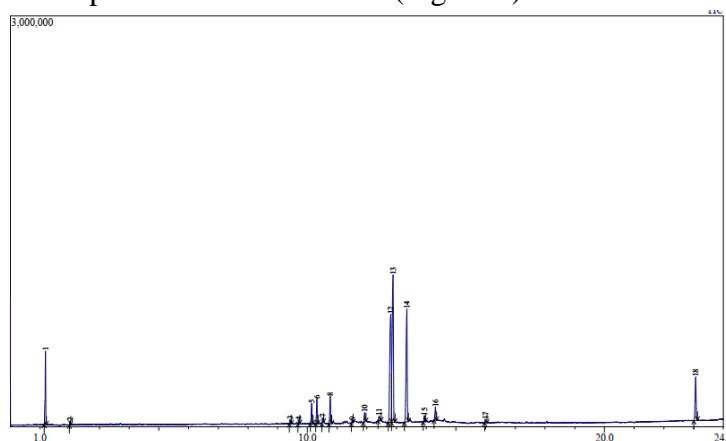


Figure 5. FTIR spectrum of ethanol eluate.

GC-MS analysis was also carried out to identify isolated compounds in ethanol eluate. The results of the GC-MS analysis showed that ethanol eluate containing 18 components, with the main compound was ar-turmerone, with a molecular weight of 216, the retention time of 12.884 minutes, and the percent area of 27.74% (Figure 6).



Peak	R.Time	Area	Area%	Height	A/H	Name
13	12.884	3165975	27.74	1077285	2.94	AR-TUMERONE
14	13.348	2171786	19.03	823696	2.64	ALPHA-TUMERONE
15	13.940	89196	0.78	43770	2.04	(6R,1'R)-6-(1',5'-DIMETHYLHEX-4'-ENYL)-3-METHYLCYCLOPENTANE
16	14.310	186096	1.63	95183	1.96	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-
17	15.997	49829	0.44	20557	2.42	Methyl pentachlorostearate
18	23.071	753076	6.60	316938	2.38	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bisphenol A

Figure 6. GC-MS spectrum of ethanol eluate.

This result showed that the adsorption process of turmeric oleoresin on silica gel is selective to increase the purity of the compound, which is indicated by the decrease in the type of compound after adsorption. In addition, the adsorption process on silica gel obtained ar-turmerone compound with a higher percent area.

4. Conclusions

In this study, the optimum condition for isolating ar-turmerone from turmeric oleoresin using silica gel as adsorbent was the use of turmeric oleoresin:silica gel with a ratio of 1:3 without being affected by adsorption time. The optimum eluent for the elution process was 96% ethanol. Based on this process, the ar-turmerone compound was obtained with a molecular weight of 216 and a percent area of 27.74%.

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Conflicts of Interest

The authors declare no conflict of interest.

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