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Research Article

Effect of Ethanol Concentration on the GC–MS Phytochemical Profile of *Curcuma longa* Rhizome Extract

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Abstract

Curcuma longa L. rhizome is a highly valuable medicinal plant that is very rich in essential secondary metabolites, particularly phenolic compounds and sesquiterpenes, which actively exhibit a wide variety of biological activities. The phytochemical composition of these turmeric extracts is strongly influenced by solvent polarity, making ethanol concentration a highly critical factor in the overall extraction process. Therefore, this specific study aimed to evaluate the precise effect of different ethanol concentrations on the phytochemical profile of turmeric extracts using the Gas Chromatography Mass Spectrometry method. Turmeric rhizomes were extracted through a maceration technique using 70% and 96% ethanol, and the resulting extracts were immediately analyzed. Extracted compounds were identified through comparison with the mass spectral library, and their relative abundances were determined using chromatographic peak areas. The comprehensive analysis revealed that both samples contained diverse bioactive phytochemicals predominantly dominated by sesquiterpenes. Major constituents were ar-turmerone, turmerone, curlone, 2-methoxy-4-vinylphenol, caryophyllene, and bergamotol. Consequently, the 96% extract produced significantly higher concentrations of ar-turmerone (33.30%) and curlone (12.11%) compared to the 70% extract (21.16% and 11.78%). Conversely, 2-methoxy-4-vinylphenol was found to be more abundant in the 70% ethanol extract (3.91%) than the 96% ethanol extract (3.00%). These findings successfully validate solvent importance.

Keywords: *Curcuma longa,* Ethanol concentration, GC–MS, Phytochemical profile, Turmerone.

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1 Introduction

Indonesia is one of the world's richest countries in terms of biodiversity and possesses enormous potential for the development of natural product-based medicines. One of the most widely used medicinal plants is turmeric (*Curcuma longa* L.) from the Zingiberaceae family. Turmeric rhizome has long been utilized in traditional medicine because of its anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and potential anticancer properties, which are attributed to its diverse bioactive constituents, including curcuminoids, sesquiterpenes, monoterpenes, phenolic compounds, and essential oil components [1–3].

The phytochemical composition of turmeric rhizome is influenced by several factors, including plant variety, cultivation conditions, harvesting time, extraction method, and the type and concentration of the extraction solvent. Ethanol is one of the most commonly used solvents for natural product extraction because it is relatively safe, readily available, and capable of extracting a broad range of secondary metabolites. Variations in ethanol concentration, particularly 70% and 96%, may affect extraction efficiency and the composition of phytochemical constituents due to differences in solvent polarity [4,5].

Phytochemical characterization is commonly performed using Gas Chromatography–Mass Spectrometry (GC–MS), a highly sensitive and selective analytical technique for identifying volatile and semi-volatile compounds. Compound identification is based on retention time and comparison of mass spectra with standard libraries, such as the National Institute of Standards and Technology (NIST) database, enabling comprehensive characterization of the chemical composition of plant extracts. Such information is essential for extract quality standardization and the development of herbal and phytopharmaceutical products.

Previous studies have reported that turmeric rhizome contains major bioactive compounds, including ar-turmerone, turmerone, curlone, curcumin, and various other sesquiterpenes that contribute to its biological activities [1–3]. However, studies specifically comparing the effects of 70% and 96% ethanol on the phytochemical profile of turmeric rhizome extracts using GC–MS analysis remain limited. Such information is important for determining the most appropriate extraction conditions to obtain specific bioactive compounds for the development of herbal medicines and phytopharmaceutical products.

Therefore, this study aimed to compare the phytochemical profiles of *Curcuma longa* L. rhizome extracts prepared using 70% and 96% ethanol based on GC–MS analysis. The findings are expected to provide insights into the influence of ethanol concentration on phytochemical composition and serve as a scientific basis for selecting suitable extraction solvents in the development of turmeric-based herbal and phytopharmaceutical products.

2 Method

The plant material, Turmeric (*Curcuma longa* L.) rhizomes, was botanically authenticated by a taxonomist at the Botanical Garden, Biotechnology Study Program, Agung Putra University, Gunungpati, Central Java, Indonesia. The dried rhizomes reached a final moisture content of 5.0 % - 10.0% prior to extraction. The chemicals used in this study included 70% and 96% ethanol as extraction solvents and analytical-grade methanol for sample preparation prior to GC–MS analysis. The rhizomes were washed thoroughly under running water to remove adhering impurities, sliced into thin pieces, and dried in a cabinet dryer at 45–50°C until a constant weight was achieved. The dried rhizomes were then ground into a fine powder using a grinder and stored in airtight containers at room temperature until extraction.

Extraction was performed using the maceration method with 70% and 96% ethanol. Dried turmeric powder (100 g) was macerated with the respective solvent at a solvent-to-material ratio of 1:10 (w/v) for 72 h at room temperature with occasional stirring to facilitate the diffusion of bioactive compounds. After filtration, the residue was re-macerated twice with fresh solvent under the same conditions, resulting in a total of three maceration cycles. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at temperatures below 50°C to obtain crude extracts,

Doi: 10.30872/jtpc.v10i2.440

which were subsequently stored at 4°C until further analysis. The extraction yield was calculated as the percentage of crude extract weight relative to the initial dry weight of the turmeric powder.

The phytochemical profiles of the turmeric extracts were characterized using Gas Chromatography–Mass Spectrometry (GC–MS). Prior to analysis, each extract was dissolved in analytical-grade methanol and filtered through a 0.22 µm membrane filter. A 1 µL aliquot of each sample was injected into the GC–MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250°C, and samples were introduced in split mode (split ratio 10:1). The oven temperature was initially held at 60°C for 2 min, increased to 280°C at a rate of 10°C/min, and then held at 280°C for 10 min. Mass spectrometric detection was performed using electron ionization (EI) at 70 eV, with the ion source maintained at 230°C and the quadrupole temperature at 150°C. Mass spectra were acquired over an m/z range of 40–600. Compound identification was carried out by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library. The relative abundance of each compound was determined based on the percentage of chromatographic peak area. The relative peak area (%) represents the proportion of an individual compound relative to the total chromatographic peak area of all detected volatile compounds within the same sample and should not be interpreted as an absolute concentration or quantitative measurement of the compound.

The GC–MS data were analyzed descriptively by comparing the number of identified compounds, retention times, and relative peak areas (%) between the 70% and 96% ethanol extracts. Relative peak area (%) was used as a semi-quantitative indicator of the relative distribution of volatile constituents within each extract rather than their absolute concentrations. Compounds with the highest relative peak areas were considered the dominant constituents and were further classified according to their chemical groups to evaluate the effect of ethanol concentration on the phytochemical profile of *Curcuma longa* rhizome extracts.

3 Results and Discussion

3.1 GC-MS Chromatographic Profile of *Curcuma longa* Rhizome Extracts

GC–MS analysis was performed to characterize the phytochemical profiles of *Curcuma longa* L. rhizome extracts obtained using 70% and 96% ethanol. The chromatograms of both extracts exhibited similar patterns, with multiple peaks distributed over a retention time range of approximately 5–31 min. The most intense peaks in both chromatograms were detected at retention times between 14 and 15 min (Figures 1 and 2), indicating the presence of major volatile constituents. Although the overall chromatographic profiles were comparable, noticeable differences in peak intensities suggested that ethanol concentration affected the relative abundance of several phytochemical constituents extracted from turmeric rhizomes.

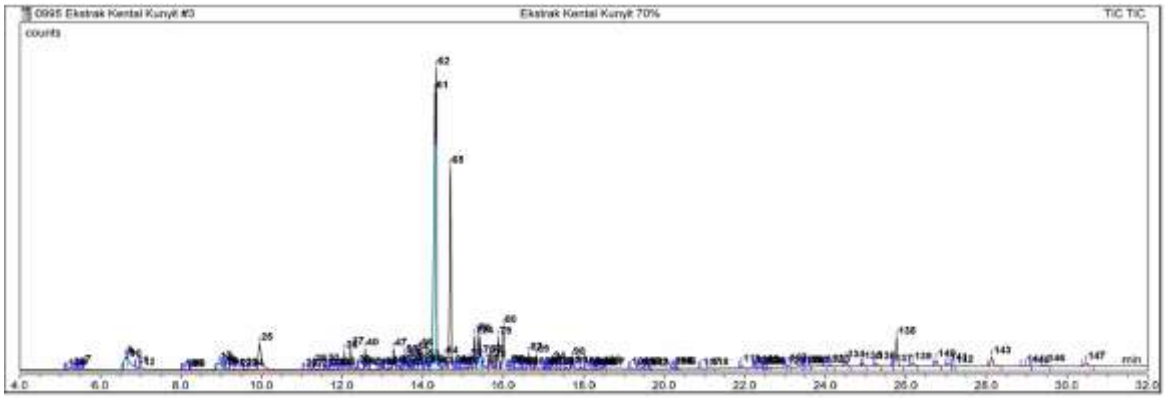

Figure 1 GC–MS chromatogram of *Curcuma longa* L. rhizome extract prepared using 70% ethanol.

Doi: 10.30872/jtpc.v10i2.440

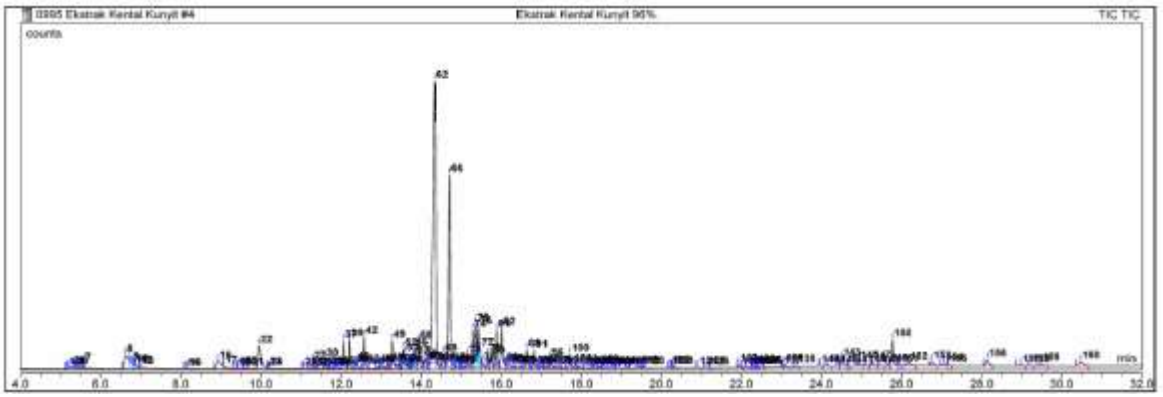

Figure 2 GC–MS chromatogram of *Curcuma longa* L. rhizome extract prepared using 96% ethanol.

3.2. Identification of Phytochemical Constituents

Compound identification was carried out by comparing the obtained mass spectra with the National Institute of Standards and Technology (NIST) mass spectral library. GC–MS analysis identified 20 major compounds in both extracts (Table 1). The detected phytochemicals were predominantly classified as sesquiterpenes, oxygenated sesquiterpenes, sesquiterpene ketones, phenolic compounds, monoterpenes, and other volatile constituents.

Ar-turmerone was identified as the predominant compound, accounting for 21.16% of the total peak area in the 70% ethanol extract and increasing to 33.30% in the 96% ethanol extract. Other major constituents included turmerone, curlone, 2-methoxy-4-vinylphenol, bergamotol, caryophyllene, caryophyllene oxide, and several sesquiterpene derivatives. The predominance of ar-turmerone, turmerone, and curlone represents a characteristic chemical profile of *Curcuma longa* essential oil. These compounds have been widely reported as the major bioactive constituents responsible for the biological activities of turmeric, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [1–3].

Table 1 Major phytochemical compounds identified in *Curcuma longa* L. rhizome extracts prepared using 70% and 96% ethanol by GC–MS analysis.

No	RT (mins)	Compound	Molecular Formula	70% Ethanol (%)	70% Ethanol (%)	Class
1	14.31–14.34	ar-Turmerone	C15H20O	21.16	33.30	Seskuiterpen keton
2	14.34–14.71	Turmerone/Curlone	C15H22O	12.66 / 11.78	12.11	Seskuiterpen keton
3	9.95	2-Methoxy-4-vinylphenol	C9H10O2	3.91	3.00	Fenolik
4	15.37	Atlantone	C15H22O	2.02	–	Seskuiterpen
5	13.28	2-Methyl-6-(p-tolyl)hept-2-en-4-ol	C15H22O	1.37	2.02	Alkohol seskuiterpen
6	6.62	Phenol, 2-methoxy	C7H8O2	1.92	2.32	Fenolik
7	15.42	Benzene,1-ethyl-4-(2-methylpropyl)	C12H18	1.69	–	Hidrokarbon aromatik

Doi: 10.30872/jtpc.v10i2.440

8	6.69	Phenol,2-methoxy	C7H8O2	1.66	–	Fenolik
9	15.29	Turunan Atlantone	C15H22O2	1.58	–	Seskuiterpen
10	12.57	Cyclohexene derivative	C15H24	1.19	1.59	Seskuiterpen
11	12.21	Cyclohexadiene derivative	C15H24	1.16	1.38	Seskuiterpen
12	12.06	Benzene derivative	C15H22	1.09	1.45	Seskuiterpen aromatik
13	13.56	Bergamotol	C15H24O	0.84	1.06	Seskuiterpen alkohol
14	13.61	Santalol	C15H24O	0.64	0.85	Seskuiterpen alkohol
15	13.82–13.83	Dihydro-ar-turmerone	C15H22O	0.92	1.00	Seskuiterpen keton
16	14.56–14.57	Turunan Turmerone	C15H24O	0.93	0.70	Seskuiterpen alkohol
17	14.09	Turunan seskuiterpen	C15H24O	0.72	0.51	Seskuiterpen alkohol
18	11.29	Caryophyllene	C15H24	0.41	0.47	Seskuiterpen
19	15.16–15.17	Bisabolone	C15H24O	0.44	0.50	Seskuiterpen keton
20	12.70	Caryophyllene oxide	C15H24O	0.04	0.07	Seskuiterpen oksida

3.3. Effect of Ethanol Concentration on the Phytochemical Profile

Comparison of the phytochemical profiles demonstrated differences in the relative peak areas of several major compounds between the 70% and 96% ethanol extracts (Figure 3). Ar-turmerone remained the dominant constituent in both extracts, with a relative peak area of 21.16% in the 70% ethanol extract and 33.30% in the 96% ethanol extract. Curlone was also detected in both extracts with relative peak areas of 11.78% and 12.11%, respectively. In contrast, the phenolic compound 2-methoxy-4-vinylphenol showed a higher relative abundance in the 70% ethanol extract (3.91%) than in the 96% ethanol extract (3.00%). Interestingly, atlantone was detected only in the 70% ethanol extract (2.02%) but was not identified in the 96% ethanol extract. This difference may be attributed to the higher polarity of 70% ethanol, which contains a substantial proportion of water that enhances solvent penetration into plant tissues, promotes matrix swelling, and facilitates the release of intracellular constituents. Although atlantone is classified as a sesquiterpene, its extraction efficiency may depend not only on its intrinsic physicochemical properties but also on its interactions with other plant matrix components. The presence of water in 70% ethanol may therefore improve the recovery of certain sesquiterpenes by increasing mass transfer and disrupting cellular structures, whereas the lower polarity of 96% ethanol may preferentially extract more hydrophobic constituents, resulting in the absence of detectable atlantone under the

Doi: 10.30872/jtpc.v10i2.440

analytical conditions employed. These findings suggest that solvent composition influences the qualitative phytochemical profile of *Curcuma longa* extracts by differentially extracting specific volatile constituents.

In addition, compounds such as atlantone and benzene,1-ethyl-4-(2-methylpropyl) were detected only in the 70% ethanol extract, whereas most sesquiterpenes were identified in both extracts with varying relative abundances. Although drying was performed at 45–50°C to reduce moisture and minimize microbial contamination while preserving thermolabile constituents, some loss of highly volatile monoterpenes cannot be completely excluded. Therefore, the detected volatile profile may partially reflect drying-related changes in essential oil composition.

The observed differences indicate that ethanol concentration influences the composition of phytochemicals extracted from turmeric rhizomes. Ethanol 70%, which contains a higher proportion of water, possesses greater polarity and is therefore more efficient in extracting relatively polar compounds, particularly phenolic constituents. In contrast, 96% ethanol is more suitable for extracting semi-polar to non-polar compounds, including essential oil constituents such as turmerones [4,5].

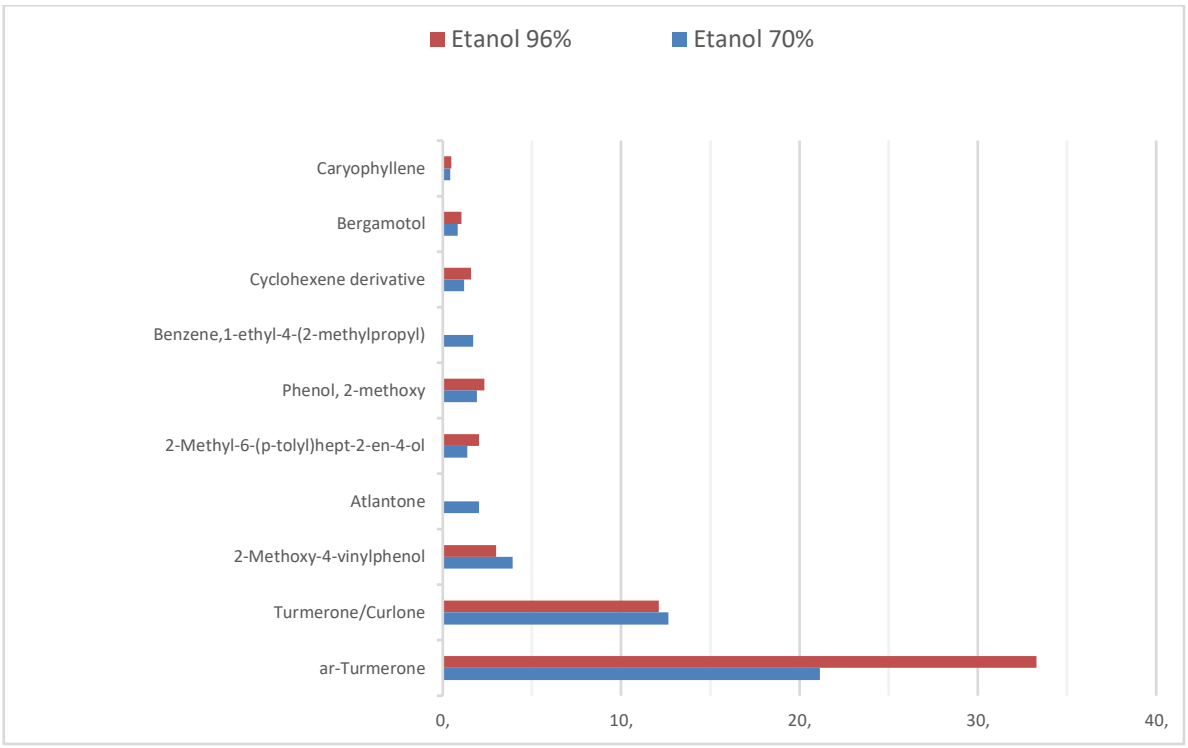

Figure 3 Comparison of the relative peak areas of the ten major compounds identified by GC–MS in *Curcuma longa* L. rhizome extracts prepared using 70% and 96% ethanol.

The present findings are consistent with those reported by Wu et al. (2024), who demonstrated that ethanol extracts of turmeric rhizomes contain various phenolic compounds and sesquiterpenes that contribute to antioxidant and antimicrobial activities. Similarly, Saha (2024) reported that ar-turmerone, α-turmerone, and β-turmerone are the principal constituents of turmeric essential oil and possess a broad range of pharmacological activities, including anti-inflammatory, antioxidant, neuroprotective, antimicrobial, and anticancer properties. These compounds have been shown to modulate several molecular pathways, including NF-κB, MAPK, COX-2, iNOS, Nrf2, and apoptosis-related signaling pathways involved in inflammation, oxidative stress, and cell proliferation, highlighting their potential as active ingredients for herbal medicines and phytopharmaceutical products [6–8].

In addition to sesquiterpenes, the presence of the phenolic compound 2-methoxy-4-vinylphenol (4-vinylguaiacol) may also contribute to the biological activity of turmeric extracts. This compound has

been reported to exhibit antioxidant and anti-inflammatory activities and to inhibit the proliferation of several cancer cell types through modulation of cellular signaling pathways [9,10]. These activities are associated with its ability to suppress reactive oxygen species (ROS) generation and inhibit inflammatory mediators [11]. Therefore, the relatively higher abundance of 2-methoxy-4-vinylphenol in the 70% ethanol extract suggests that this extract may possess greater potential as a natural antioxidant source and as a raw material for phenolic-based herbal products [12].

Conversely, the higher ar-turmerone content observed in the 96% ethanol extract suggests that this solvent is more effective for extracting bioactive sesquiterpenes from turmeric rhizomes. Ar-turmerone has been extensively reported as a major constituent of turmeric essential oil with anti-inflammatory, antimicrobial, neuroprotective, antioxidant, and anticancer activities [13-15]. It exerts these pharmacological effects through modulation of multiple molecular pathways involved in inflammation, cell proliferation, and apoptosis [16,17]. Based on the GC–MS results obtained in the present study, the use of 96% ethanol produced an extract with a higher relative abundance of sesquiterpenes, particularly ar-turmerone, than 70% ethanol. These findings indicate that the 96% ethanol extract has considerable potential for the development of herbal and phytopharmaceutical products targeting anti-inflammatory and anticancer applications, considering the well-documented pharmacological properties of ar-turmerone [10,18,19].

Overall, the present study demonstrates that ethanol concentration not only influences the phytochemical composition of *Curcuma longa* rhizome extracts but may also affect their biological potential. The 70% ethanol extract appears to be more suitable for the development of antioxidant-oriented herbal products because of its relatively higher phenolic content, whereas the 96% ethanol extract is more appropriate for producing sesquiterpene-rich extracts, particularly ar-turmerone, which has promising anti-inflammatory and anticancer properties. These findings provide a scientific basis for selecting extraction solvents according to the intended application in the development of turmeric-based herbal and phytopharmaceutical products.

4 Conclusion

GC–MS analysis showed that turmeric (*Curcuma longa* L.) rhizome extracts prepared with different ethanol concentrations contained diverse phytochemical constituents, predominantly sesquiterpenes, sesquiterpene ketones, phenolics, and other volatile compounds. Ar-turmerone was identified as the major constituent in both extracts, while differences in the relative abundance of several compounds were observed between the extraction solvents. These findings indicate that ethanol concentration influences the phytochemical profile of turmeric extracts, with higher ethanol concentration favoring the extraction of less polar sesquiterpenes and lower ethanol concentration yielding relatively higher levels of phenolic compounds. This information may assist in selecting an appropriate extraction solvent based on the desired phytochemical composition. Further studies are needed to investigate how these differences in phytochemical profiles affect the biological activities of turmeric extracts.

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