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Standardization Methods for the Determination of Curcumin in Turmeric and Its Anticancer Activities : A Review

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ABSTRACT

Cancer is one of the deadliest diseases in the world. Indonesia is a country with cancer rates that ranks 8th in Southeast Asia. Cancer that has the highest number of sufferers in Indonesian men is lung cancer, as much as 19.4 per 100,000 population, with an average death rate of 10.9 per 100,000. Meanwhile, for women, breast cancer is the highest cancer incidence rate, which is 42.1 per 100,000 population, with an average death rate of 17 per 100,000 population. Although chemotherapy remains a highly efficient way to treat cancer, it is the drawbacks related to its side effects that are often feared. Natural products such as plants are more beneficial for improving treatment results because they are safe and can minimize side effects on healthy cells. Herbal plants that are often associated with anticancer drugs are turmeric. Currently, several standardized methods have been developed to analyze curcumin in turmeric, and from these methods, there are advantages and disadvantages of each method. Therefore, selecting the analytical method is very important to get better results.

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1. INTRODUCTION

Cancer is one of the deadliest diseases in the world. Cancer mainly occurs due to gene mutations, and the condition is aggravated by other carcinogenic agents. Both gene mutations and carcinogenic agents then affect and change cell function and metabolism so that the replication and spread of cancer cells become uncontrollable. Cancer cells grow and multiply rapidly, thus damaging the normal cells around them[1]. Indonesia is a country with cancer rates that ranks 8th in Southeast Asia. Cancer that has the highest number of sufferers in Indonesian men is lung cancer, as much as 19.4 per 100,000 population with an average death rate of 10.9 per 100,000 population. Whereas for women, breast cancer is the highest cancer incidence rate, which is 42.1 per 100,000 population, with an average mortality rate of 17 per 100,000 population [2]. Although chemotherapy remains a very efficient way to treat cancer, the drawbacks associated with side effects are often feared. Natural products such as plants are more beneficial for improving treatment results because they are safe and can minimize side effects on healthy cells. Herbal plants that are often associated with anticancer drugs are turmeric [3]. Based on research conducted by Mirani et al (2011), turmeric rhizome extract against HeLa cancer cells has an LC50 value of 0.657 µg/ml. If the LC50 value is ≤ 30 µg/ml, these results indicate that turmeric has the potential as an anticancer [4]

Turmeric is a spice that is in great demand both from the medical/scientific world and from the culinary world. Turmeric is a herbaceous perennial plant rhizomatous (*Curcuma longa*) from the ginger family. The medicinal properties of turmeric, a source of curcumin, have been known for thousands of years, but its ability to determine the exact mechanism of action and to determine bioactive components

has only recently been investigated [5]. Turmeric consists of 3 curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin). In addition, there are also several other ingredients, such as sugar, protein, essential oils (natlanton, tumeron, and zingiberon), and resin. Curcumin has the pure chemical structure of 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, consisting of two ferulic acid residues connected by a methylene bridge. Commercially, curcumin contains 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin [6]. Currently, several analytical methods have been developed to detect curcumin in turmeric, and from these methods, there are advantages and disadvantages of each method. Therefore, the selection of the analytical method is very important to get better results. There have been many reviews on the chemical, biological, and pharmacological properties of curcumin, including its anticancer activity, but reviews regarding the analytical method are still very limited. Therefore, we will summarize several methods of analyzing curcumin in turmeric so that readers can choose a suitable analytical method according to the desired criteria.

2. METHOD

The method used in writing this article is a literature study obtained from various journals regarding theories that are relevant to the content of the topic of discussion in this review article.

3. RESULTS AND DISCUSSION

Currently, several analytical methods have been developed to detect curcumin in turmeric, and from these methods, there are advantages and disadvantages of each method. Here are some methods that we summarize from various literature regarding the analysis of curcumin in turmeric.

A. Thin Layer Chromatography Method

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixtures. In principle, thin-layer chromatography is performed on a sheet of glass, plastic, or aluminum foil coated with a thin layer of adsorbent material (silica gel, aluminum oxide, or cellulose (ink paper)). This adsorbent layer is known as the stationary phase. After the sample is dappled onto the plate, the solvent or mixture of solvents (known as the mobile phase) is drawn onto the plate by capillary action. Because different analytes move up the TLC plate at different rates, separation is achieved [7]. High-Performance Thin Layer Chromatography (HPTLC) is an advanced and automated form of thin-layer chromatography (TLC) with better separation efficiency and detection limits. HPTLC is also known as High-Pressure Thin Layer Chromatography / Planar Chromatography or Flatbed Chromatography. HPTLC is a powerful analytical method suitable for both qualitative and quantitative analysis tasks [8] The stationary phase, mobile phase, and solvents that have been used in several studies on the analysis of curcumin content in Turmeric Rhizome can be described as follows.

Table 1. The stationary phase, mobile phase, and solvent have been used in several studies on the analysis of curcumin content in Turmeric Rhizomes.

Sample	Stationary phase	Mobile phase	Solvent	Wavelength	Reference
TLC method					
Turmeric Rhizome powder	silica gel 60 F254	Chloroform : methanol (95:5 v/v)	Methanol	426 nm	[9]
Turmeric Rhizome	silica gel 60 F254	Chloroform : methanol (95:5 v/v)	Aceton	420 nm	[10]
Turmeric Rhizome	silica gel 60 F254	Dichloromethane : methanol (97:3 v/v)	Ethanol	420 nm	[11]
Turmeric Rhizome	silica gel 60 F254	Chloroform : methanol : formic acid (94:3:3 v/v)	Methanol	427 nm	[12]
HPTLC method					

Turmeric Rhizome	silica gel 60 F254	Chloroform : methanol : acetic acid (9.5: 0.5: 0.1 v/v/v)	n-Hexan, dichloromethane, ethyl acetate, acetone, ethanol	254 and 366 nm	[13]
Turmeric Rhizome	silica gel 60 F254	Chloroform : methanol (97:3 V/V)	Methanol	420 nm	[14]
Turmeric Rhizome	silica gel 60 F254	Chloroform : methanol (97:3 V/V)	Methanol	254, 366, and 427 nm	[15]

The TLC method has several advantages, namely its low cost and easy process. However, the TLC method also has disadvantages, namely long separation times and poor resolution in the case of turmeric analysis [16].

HPTLC can analyze samples in small quantities up to the nano-gram range with good sensitivity. In addition, HPTLC also has a minimum of human error because the machine is automated. However, there are also disadvantages of HPTLC, which include large instruments that require a large space, require a dust-free environment and controlled temperature conditions, and require technically skilled people with knowledge to operate the instruments [8].

B. UV-Visible Spectrophotometry Method

The working principle of the UV-Vis Spectrophotometer is that when monochromatic light passes through a medium (solution), some of the light is absorbed (I), some is reflected (lr), and some are emitted (It). [17]. Some of the advantages of this method include high sensitivity, requiring only a small number of samples, linearity over a wide range of concentrations, and can be used with gradient elution. Whereas the weaknesses of this method include the lack of sensitivity and selectivity, only for compounds that absorb light at UV-Visible wavelengths, the sample must be in the form of a solution, the mixture of substances creates difficulties for analysis and requires prior separation, and generates significant heat and requires external cooling [18]. The following is a series of processes carried out by Yanlinastuti et al (2016) in analyzing curcumin using the UV-Visible spectrophotometry method.

1) Preparation of 1000 ppm curcumin standard stock solution

Curcumin standard stock solution was prepared by dissolving 10 mg of curcumin standard with 10 mL of 96% ethanol to obtain a curcumin standard stock solution with a concentration of 1000 ppm. Then dilution was carried out into five series, namely 110, 120, 130, 140, and 150 ppm.

2) Maximum wavelength setting

A standard curcumin solution with a concentration of 110 ppm was pipetted as much as 1 mL in a beaker, and added 20 mg of boric acid and 20 mg of oxalic acid. Then covered with aluminum foil, heated in a water bath for 10 minutes, and cooled. The solution was transferred to a 10 mL volumetric flask and added with acetic acid anhydride up to volume. After that, read the absorbance in the range of 400-600 nm until the wavelength with the highest absorbance is obtained.

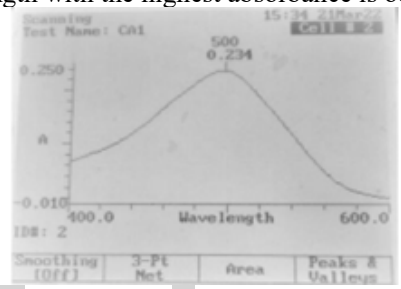

Figure 1 Maximum Wavelength of Standard Raw Solution of Curcumin

The maximum wavelength of curcumin is 500 nm.

3) Standard curve creation

From concentrations of 110, 120, 130, 140, and 150 ppm, pipette 1 mL each in a beaker, and add 20 mg of boric acid and 20 mg of oxalic acid. Then covered with aluminum foil, heated in a water bath for 10 minutes, and cooled. The solution was transferred to a 10 mL volumetric flask and added with acetic acid anhydride up to volume. After that, read the absorbance at the maximum wavelength. Based on the VI edition of the Indonesian Pharmacopoeia, said the squared correlation coefficient ($R^2 \geq 0.98$) indicates linearity.

4) Determination of curcumin levels in samples

The sample was weighed as much as 1 g, then added with 96% ethanol solvent as much as 10 mL, then refluxed for 1 hour. Pipette 1 mL of the filtrate obtained and put it in a beaker glass, add 20 mg of boric acid and 20 mg of oxalic acid. Then covered with aluminum foil, heated in a water bath for 10 minutes, and cooled. The solution was transferred to a 10 mL volumetric flask and added with acetic acid anhydride up to volume. After that, read the absorbance at the maximum wavelength and then calculate the levels. Based on the Indonesian Pharmacopeia IV edition, it is stated that the tolerance limit for determining the wavelength is not more than ± 2 nm from the specified wavelength.

C. FTIR (Fourier Transform Infrared) Spectrophotometric Method

FTIR method can be used in the analysis of medicinal plants qualitatively and quantitatively. The working principle of FTIR is to provide energy in the form of infrared light, which will pass through the gap to the sample, where the gap functions to control the amount of energy delivered to the sample. Some of the energy will be absorbed by the sample, and some will be transmitted through the surface of the sample so that the beam can continue to the detector. The measured signal is then sent to a computer connected to the instrument in the form of a special application which will be recorded in the form of an infrared spectrum. The results of the FTIR analysis have very complex spectral patterns that are not easy to read. Therefore, it is necessary to combine FTIR with chemometric methods such as principal component analysis (PCA), canonical variate analysis (CVA) (Rohaeti et al., 2015), and partial least square regression (PLSR). FTIR analysis of curcumin (CUR), desmethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and total curcuminoids (total C + DMC + BDMC) in turmeric was carried out using the attenuated total reflectance (ATR) method, where turmeric samples (extract or powder) were placed on crystals The ATR spectrum was scanned using an FTIR spectrophotometer and measured in the middle IR band (4000 - 650 〖cm〗^(-1)) with a resolution of 4 〖cm〗^(-1). The FTIR spectrophotometer is set against the background air in the room. The results of the FTIR analysis are in the form of a spectrum with an absorbance value for each peak [19].

Some of the advantages of this method include being able to analyze different liquids, powders, gases, semi-solids, and polymers with minimum sample preparation, it is cheaper and provides complete information, data can be stored and re-analyzed, FTIR techniques can perform qualitative analysis and quantitative when combined with chemometric data analysis techniques, as well as all frequencies measured simultaneously. Whereas the weaknesses of this method include not identifying in detail the typical waveform of an object up to the fingerprint level, not being able to identify the type and content of each fatty acid component of a sample with certainty, IR radiation can cause eye irritation to blindness, and the data transfer process takes longer because you can't use the Bluetooth feature.

D. Liquid Chromatography-Mass Spectrometry (LC-MS) Method

Liquid chromatography-mass spectrometry is an analytical technique that combines high-resolution chromatographic separation with sensitive and specific detection of mass spectra [20] . The LC-MS method can detect very low levels of curcuminoids in a given sample matrix. In this method, LC follows the same principles as in HPLC, but connecting the MS detector to the LC makes this method more efficient compared to HPLC and HPTLC methods [16]. First of all, the samples are separated by LC and sprayed into an atmospheric pressure ion source, where they are converted to ions in the gaseous phase. A mass analyzer is then used to order the ions according to their mass-to-charge ratio, and the detector counts the ions emerging from the mass analyzer, which can also amplify the signals generated from each ion. As a result, a mass spectrum is formed that can be used to determine the isotopic properties of sample elements and the masses of particles and molecules and to explain the

chemical structure of molecules [20]. Kotra V et al., stated that to determine very low levels of curcuminoids in any sample matrix, LC-MS/MS is the best choice [16].
Jiang et al. (2006) have conducted a study to investigate the fragmentation behavior of three major curcuminoids by LC/MS/MS using instruments from different manufacturers so as to identify general principles of fragmentation rather than machine-specific idiosyncrasies. Accurate and high-resolution mass spectroscopy, together with sustained off-resonance irradiation fragmentation (Fourier transform ion cyclotron resonance (FTICR) SORI-MS/MS), was also used to confirm the fragmentation behavior of curcuminoids. In this study, the Turmeric Rhizome sample was used. The mobile phases used included (A)buffer (5 mM Ammonium formate, 0.1% Formic acid in H2O and (B) acetonitrile; gradient (in buffer A): 0–2 minutes, 5% B; 2–57 minutes, 5 –100% B; 57–60 minutes, 100% B; 60–65 minutes,100–5% B; 65–75 minutes, 5% B. Flow rate: 0.25 mL/min; temperature, 40°C; injection volume The column used was discovery HS C18. The separation time was 75 minutes. From the results of this study, it was concluded that the flow rate of ion trap instruments from different manufacturers basically produces the same fragmentation pattern[21].

E. High-Performance Liquid Chromatography (HPLC) Method

HPLC (High-Performance Liquid Chromatography) is a technique for separating, identifying, and quantifying components [22] with the addition of using a detector column that is sensitive and very sensitive to the compound being analyzed [23], through this technique would be in line for compounds that are non-volatile, thermally unstable, and of large molecular weight compounds. The working principle of HPLC is the separation of analytes based on polarity, using a column as the stationary phase and the mobile phase being a certain solution. The two phases also function as separators based on adsorption capacity, partition, molecular size, ion size, and vapor pressure [22], [23]. The advantages of this method are its high sensitivity, reusable column, ideal for thermolabile and low volatility substances, and varied separation mechanisms [24]. While the weaknesses of this method are the relatively long analysis time, a lot of consumption of the mobile phase, the stationary phase is relatively expensive, requires a large amount of solvent, and each component is run individually [25].

4. CONCLUSION

Many research reports on turmeric have revealed that curcumin compounds in turmeric have biological activities such as anticancer. Because of these benefits, curcumin is often needed by various medical industries, so in monitoring the quality and quality of curcumin compounds, it is necessary to establish standardization. There are various methods that can be used to standardize curcumin in turmeric, which of course, these methods have their respective weaknesses and drawbacks. Methods that can be used include thin layer chromatography, UV-Vis spectrophotometry, FTIR spectrophotometry, LC-MS method, and HPLC method.

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