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Curcuminoid content of *Curcuma longa* L. and *Curcuma xanthorrhiza* rhizome based on drying method with NMR and HPLC-UVD

S Hadi[1], A N Artanti[1], Y Rinanto[2], D S C Wahyuni[1*]

[1]Pharmacy Department, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Indonesia, [2]Biology Education Program, Faculty of Teacher Training and Education, Universitas Sebelas Maret, Indonesia

*E-mail: dinarsari_cw@staff.uns.ac.id

Abstract. Curcuminoid, consisting of curcumin, demethoxycurcumin and bis demethoxycurcumin, is the major compound in *Curcuma longa* L. and *Curcuma xanthorrhiza* rhizome. It has been known to have a potent antioxidants, anticancer, antibacteria activity. Those rhizomes needs to be dried beforehand which influenced the active compounds concentration. The present work was conducted to assess the curcuminoid content of *C. longa* L. and *C. xanthorrhiza* based on drying method with Nuclear Magnetic Resonance (NMR) and High Pressure Liquid Chromatography (HPLC)-UVD. Samples were collected and dried using freeze-drying and oven method. The latter is the common method applied in most drying method at herbal medicine preparation procedure. All samples were extracted using 96% ethanol and analyzed using NMR and HPLC-UVD. Curcuminoid as a bioactive compound in the sample exhibited no significant difference and weak significant difference in *C. xanthorrhiza* and *C. longa* L., respectively. HLPC-UVD as a reliable analytical method for the quantification is subsequently used to confirm of the data obtained by NMR. It resulted that curcuminoid content showed no significant difference in both samples. This replied that curcuminoids content in both samples were stable into heating process. These results are useful information for simplicia standardization method in pharmaceutical products regarding to preparation procedure.

1. Introduction

Curcuma longa L. and *Curcuma xanthorrhiza Roxb* are representative of Indonesian traditional medicine as carminative, laxative, anthelmintic and a cure for liver ailment. [1]. They are also widely used in cosmetics and as a color dye. The main active components include curcuminoids, monoterpenoids and sesquiterpenoids [2]. Curcumin, demethoxycurcumin and bisdemethoxycurcumin belong to curcuminoids as the pharmacologically significant compounds and their chemical structures are shown in Fig. 1. These three curcuminoids have been proved to show strong anti-oxidant [3], anti-inflammatory [4], anti-bacterial [5], and anti-carcinogenic activities [6,7].

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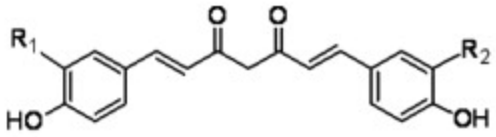

Compound	R_1	R_2
Curcumin	OCH_3	OCH_3
Demethoxycurcumin	OCH_3	H
Bisdemetoxycurcumin	H	H

Fig. 1: Structures of the three curcuminoids.

The post harvest processing of *Curcuma longa L.* and *Curcuma xanthorrhiza Roxb* rhizomes involves many steps, such as washing, cleaning, and drying. Drying is one of the most important methods of preservation and production of rhizomes, with major aim to prolong its storage life. Unfortunately, changes in the physical and biochemical structure are inevitable because the rhizomes are treated with thermal treatments.

Different types of dryers and drying methods have been adopted. Traditional drying method by direct sun drying could result in the degradation of the herb quality, for example loss of the of volatile oil (up to 25 per cent) by evaporation [8]. The traditional drying are also risky and result in mold growth, destruction of some heat sensitive pungent properties, and also influences on levels of bioactive compounds of sample. Previous study on turmeric rhizomes collected from 30 locations showed that conventional processing could maintain the intrinsic quality of sample up to a certain level, but extrinsic quality of product could not be achieved [9]. By considering the above point, it is necessary to investigate of different drying method as an effective alternative to traditional open sun drying. In this case, there is an underlying assumption that freeze-drying properly preserves the medicinal qualities of plants, and is superior to other drying methods. However, little systematic research has been done to verify these assumptions.

This study thus aimed to compare the freeze drying and oven drying method of fresh rhizomes of *Curcuma longa L.* and *Curcuma xanthorrhiza Roxb* with the specific objectives comparative evaluation of curcuminoids level of the sample. For this purpose, Nuclear Magnetic Resonance (NMR) and High Performance Liquid Chromatography-Ultra Violet (HPLC-UVD) Detector analytical method are applied for determination of curcuminoids dried by oven and freeze-drying method. NMR has been known as a simple preparation and produced good reproducibility; on the other hand, it has low sensitivity. In contrast to that, HPLC shows high sensitivity and resolution [10].

2. Methods

2.1 Chemicals and reagents

Standards of curcuminoids were purchased from CV Chemonic Pratama as commercial standard. All the other chemicals were of analytical grade and were purchased from E. Merck (Germany). The sample of *Curcuma longa L.* and *Curcuma xanthorrhiza Roxb.* was collected from local farmer in Tanjungrejo village, Nguter, Sukoharjo.

2.2 Drying experiment

The sample preparation protocol consisted of washing the rhizomes in running tap water in order to remove all the soil particles adhering to the rhizomes water. the rhizomes cut into piecesand finely powdered using a blender. Fresh powder sample was dried by two different drying methods, i.e. oven and freeze drying. For oven drying, temperature was set at 50°C and run for 3×24 hours. For frezee drying, a VirTis BenchTop Pro Freeze Dryer (SP Scientific), was set at temperature -109°C and run for 3×24 hours.

2.3 Extraction of plant materials for NMR analysis

Three replicates of each species were used for NMR profiling. The standard protocol of sample preparation and 1H-NMR profiling described by Kim et al. [9] was applied. Samples of 30 mg freeze-

dried plant material were weighed into a 2 ml microtube and extracted with 1.5 ml of a mixture of phosphate buffer (pH 6.0) in deuterium oxide containing 0.05% trimethylsilylproprionic acid sodium salt-d4 (TMSP) and methanol-d4 (1:1). Samples were vortexed at room temperature for 1 min, ultrasonicated for 20 min and centrifuged at 13000 rpm for 10 min. An aliquot of 0.8 ml of the supernatant was transferred to 5 mm NMR tubes for 1H-NMR measurement.

2.4 NMR analysis

^{1}H-NMR spectra was recorded at 21^{0}C on 400 MHz Agilent spectrometer. Deurated water was used as the internal lock. Each ^{1}H-NMR spectrum consisted of 128 scans requiring 10 min and 26 s acquisition time with the following parameters: 0.16 Hz/point, pulse width (PW) of 60^{o} (11.3 μs), and relaxation delay (RD) of 2s. Two-dimensional J-resolved NMR spectra were acquired using 8 scans per 128 increments for F_1 (chemical shift axis) and 8 k for F_2 (spin-spin coupling constant axis) using spectral widths of 66 Hz and 5000 Hz respectively. Both dimensions were multiplied by sine-bell functions (SSB = 0) prior to double complex Fourier transformation. J-resolved spectra were tilted by 45°, symmetrized about F_1, and then calibrated to TMSP. ^{1}H-^{1}H correlated COSY spectra were acquired with a 1.0 s relaxation delay and 6361 Hz spectral width in both dimensions. The window function for the COSY spectra was Qsine (SSB = 0). MestRenova version 11.0.0 applied in order to identify metabolites in sample**s.**

2.5 HPLC analysis

The HPLC system was consisted of a binary pump sytem, 20 μL Rheodyne sample injector, and UVD detector (LabAlliance). A Waters BioSuite^{TM} pC_{18} column (4.6 mm×150 mm, 7 μm) is used for separation. The mobile phase was composed of methanol and run in isocratic mode. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The mobile phase was degassed and filtered through a 0.22 μm membrane filter before use. The column temperature was maintained at 30 °C. The UVD wavelength was set at 420 nm.

Prior HPLC analysis, 0.1 g sample was quantitatively weighed and extracted with 10 mL methanol in an ultrasonic apparatus for 20 min at room temperature. After sonication, the supernatant liquid was filtered through a 0.45 μm membrane filter and used for subsequent chromatography analysis. External calibration curve was used as quanification method.

3. Results and discussion

3.1 NMR analysis

Curcuminoid was identified using 1D and 2D NMR spectra. Signals at δ 7.58 (d, J = 15.47 Hz), δ 7.15 (dd, J = 8.58, 2.83 Hz), δ 7.09 (d, J = 2.4 Hz), δ 6.90 (d, J = 8.31 Hz) and δ 6.70 (d, J = 15.47 Hz). A correlation between signals of proton δ 7.58 and δ 6.70 was observed in the COSY spectrum.

The results of effect of drying methods on curcuminoid contents of *Curcuma longa L.* and *Curcuma xanthorrhiza Roxb.* are tabulated in Figure 2. The curcuminoid between drying method did not show significant difference in C. xanthorrhiza (F=0.302, df=1, P=0.612). The curcuminoid exhibited weak significant difference in C. longa L between drying method (F=0.9.153, df=1, P=0.039). It has half level higher in freeze-drying method than in oven-drying method.

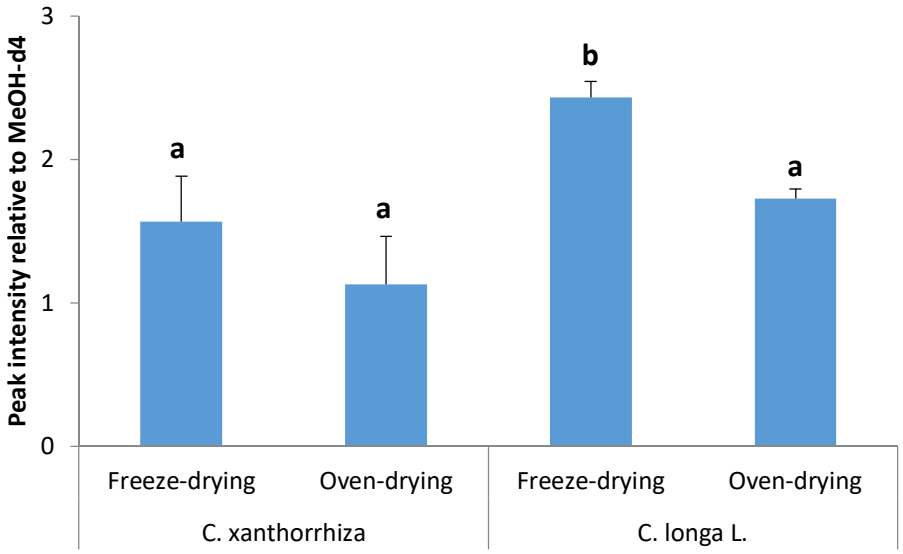

Figure 2: Variation of curcuminoid level of turmeric and temulawak samples dried with two different drying methods. Different letters indicate significant differences between treatment at $P \leq 0.05$

3.2 HPLC analysis

HPLC-UVD is a simple method and low cost for quantitative determination of curcuminoids with high sensitivity. In this study, curcuminoids was extracted from 2 different plant samples. The *C. longa L.* and *C. xanthorrhiza Roxb.* was extracted by sonication method. Peak of total curcuminoids under the optimum conditions described above identified in the chromatogram at t_R of 2.27±0.01 minutes. Isocratic run with methanol produces curcuminoids peak with a symmetry factor of 1,2 and could be well separated with other intererence peaks from matrice and detected within 10 min. Good selectivity and sensitivity were achived by the UVD detection. This method also showed wide linear range, good linearity as shown in **Table 1**.

Table 1: External calibration curve of HPLC-UVD

Compound	Conc. range [ng/µL]	Equation of regression	R^2
Curcuminoid	0.05-0.60	y = 99149x + 34433	0.999

3.3 Effect of drying method

HPLC-UVD analysis for the effects of drying methods on curcuminoids levels of *C. longa L.* and *C. xanthorrhiza Roxb.* are given in **Table 2**. The curcuminoid did not differ between drying method in *C. xanthorrhiza Roxb* and *C. longa* L. (F=0.098, df=1, P=0.770 and F=0.029, df=1, P=0.874).

These results are in accordance to previous study that curcuminoids did not change due to heat treatment [10, 11]. Tangkanakul et al. (2009) studied that curcuminoid content are remain stable due to normal heat treatment in *C. longa* L. (up to 100 °C) [12]. Freeze-drying is an excellent drying method to preserve heat-sensitive compounds. Thus, it is an ideal method to dry herbal plants with less compound degradation. Fortunately, curcuminoid is a stable compound due to heat treatment, therefore oven-drying method of *C. longa* L. and *C. xanthorrhiza* Roxb. as a raw material for herbal medicine can be applied regarding to preparation procedure.

Table 2. Curcuminoids content

Sample	Concentration [% w/w]*	
	Oven-drying	Freezee drying
C. longa L.	0,76 ± 0,06	0,76 ± 0,04
C. xanthorrhiza Roxb.	0,74 ± 0,02	0,73 ± 0,03

*based on dry weight

Conclusion

Curcuminoids as bioactive compounds of *C. longa* L. and *C. xanthorrhiza* Roxb. are remain stable due to heat treatment at preparation procedure. The high content of curcuminoids are important to be maintained during production process. Thus, these results are useful information for simplicia standardization method in pharmaceutical products. Further experiments may focus on other metabolites changing in *C. longa* L. and *C. xanthorrhiza* Roxb. due to heat treatment.

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