Determination Of The Curcumin Pigment In Extract Curcuma Domestica Val From South Sulawesi, Indonesia, By High Performance Liquid Chromatography

Sri Hastati, Veni Hadju, Gemini Alam, Nusratuddin

Abstract: Indonesia is a country endowed with a variety of medicinal plants with strong potential for therapeutic application, curcuma domestica val is called kunyit is one of the most popular medicinal herbs.Its important active ingredient responsible for the biological activityofcurcuma, through the major activity is antyinflammatory. Curcuma has also been reported to possess antioxidant, antiallergic,wound healing,antibacterial,antifungal and anti tumor activity.The yellow colour of curcuma is mainly due to the presence of polyphenolic curcuminoids. This experiment was aimed to determine analysis compound of curcuminoid form analysis using HPLC,LC-10AT,Shimadzu,column C-18 VP-ODS, acetonitril:acetic acid:aquabides as mobile phase,flow rate 1 ml/min and detection at 425nm.Thus the analytical method using HPLC for curcumin were feasible for quantitative analysis. The curcuminoid content from Extract Curcuma domestica Val had the highest curcumin (16.1%) followed metoxycurcumin (3.2%) and bisdemetoxycurcumin 2.8%

Index Terms: curcuma domestica val., turmeric, curcumin, hplc,

1. INTRODUCTION

Turmeric,one of natural ingredients that is widely used especially by people in South Sulawesi to spice, food preservative, skin diseases, wounds,intestinal worms,diarrhea inflammation,and constipation is kunyit (Curcuma Domestica Val.).(Badan POM,2004) [1]. Curcuma Domestica val leaves contain protein, fat,mineral,carbohidrat and moisture (kapoor,1990) [2], Curcuminoid is responsible for the yellow colour and comprises curcumin, demetoksicurcumin and bisdemethoxy curcumin (Vopel, et al , 2010) [3]. Turmeric is native to tropical South Asia and needs temperatures between 20°C and 30°C, and a considerable amount of annual rainfall to thrive (Peret et al.,2005) [4]. Medicinal plant species that produce raw material for pharmaceuticals and phyto-chemicals for manufacturing drugs.In the commercial market,medicinal herbs are used as raw drugs,extracts or tinctures. Turmeric is prepared by grinding of dried rhizomes mainly from Curcuma .of the Zingiberaceae family.These powdered dried rhizomes have at least 76 synonims listed in the 1999 WHO monograph (, WHO 1999) [5] Turmeric is used as traditional medicine in many countries because of the antibiotic and antiseptic effects of curcumin ,an important constituent of turmeric. A yellow-pigmented fraction isolated from the rhizemos of Curcuma contains curcuminoids belonging to the diccinamoyl methane group.Curcuminoids are present to the extent of 3 – 5%(Abinasa, 2000)[6]. The alcoholic extract of turmeric mainly contains three curcuminoids ,namely curcumin (also referred as curcumin I or diferuloylmethane), desmethoxycurcumin (curcumin II),and bisdesmethoxycurcumin (curcumin III)

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Fig.1..The first attempt at curcumin purification was carried out by Vogel and Pelletier in 1815 and its structure was confirmed in 1973 (Roughley and Whiting, [7]and the solution structure was only confirmed in 2007 .(Payton et al., 2007),[8] At most research, Curcumin content of turmeric and curry powders.had relativity small mounts of curcumin present and variability in content was great (Tayyem RF et al.,2006) [9].. Curcumin quantification in dosage forms using high performance liquid chromatography (HPLC) has been carried out.(Pandey A and Katiyar SK.2010) [10]. It has also been reported that turmeric from various regions in Indonesia had a high variation of curcuminoids and volatile oil contents especially in java(Dalimartha S.2001) ,[11] but there is no report concerning the amount of each curcuminoid in the crude curcuminoid extracts from south of sulawesi. Thus, this study was undertaken to determine the amount of curcumin in the crude curcuminoid extracts of Curcuma domestica ,collected from south of sulawesi in Indonesia by HPLC method.

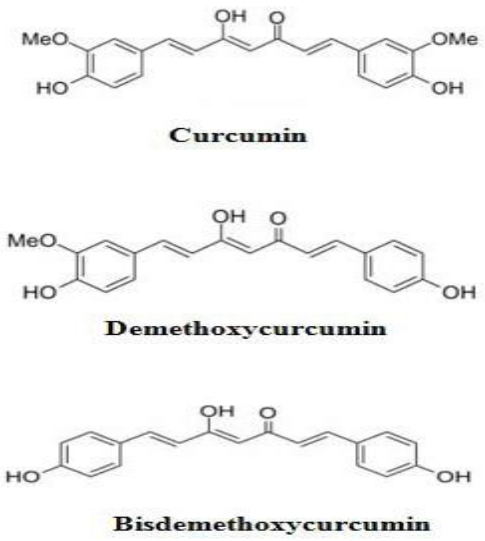

Fig.1. *Molecular structur of curcuminoid*

2. MATERIALS AND METHODS

Materials used in this study were Apis melliferaCurcuma domestica Val., distillated water. acetic acid, Acetonitril, ethanol, Standart Curcumin

2.1 Plant Material

Plant Material The rhizomes of C.domestica were collected from maros distric, during April 2014.The samples were identified were deposited at the department of Fitofarmaka,Faculty of Pharmacy, Hasanuddin University, South of sulawesi,Indonesia Fresh rhizomes were cleaned ,cut in to small pieces.The samples were then further dried in a hot air oven at 50^0C for 24 hours,ground into powder and passed through a sieve (20 mesh). Dried powder of curcuma domestica collected from maros distric was extracted with 70% ethanol. The ethanol extract was dried using a rotary evaporator

2.2 Mobile phase preparation

Mobile phase use acetonitril: acetic acid:Aquabides 50:1:49%. The solusion was then filtered by pump filter; the filterate was placed in Erlenmeyer flask and degasses.The solution was then placed into mobile phase bottle and labeled.

2.3 Instrument Optimation

HPLC by shimadzu,flow rate 1.0 ml/min.PDA detector, Column Shim Pack Vp-Ods. Column used C18 (250x4.6mm)detection wavelength 425nm injection volume 20 ul, column temperature 40°C. HPLC column was cleaned up by elution,filtration and degasion.Elusion run for 1 hour, then the column was washed for 1 hour.After washing step, the column was conditioned by eluting mobile phase for 30 minutes and at the same time it was run for baseline.

2.3 Preparation of standar

2.5mg of standart curcumin was accurately weighed into a 25 ml volumetric Flask and dissolved in the mobile phase with warming and the volume was made up with solvent mixture. Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected

2.4 Preparation of sample

2.5 mg of the sample was accurately weighed into a 25 ml volumetric flask. Dissolvent and diluted to volume and further diluted to inject in HPLC machine

2.5 Linearity and range study

Five concentrations of curcumin standart solution was made :5, 7.5, 10, 12.5, and 15 ppm. Each solution was filtered by syringe filter 0,45 µm. The solution was injected into injector once time for each time and the area under curve was recorded and measured for the r values and r^2 The correlation coefficient in linear regression equation (Y=ax+b)

3. RESULT AND DISCUSSION

Curcumin have biological properties in which curcuminfor many medicinal properties(Basnet P and Skalko NS,2011) ,[12] Turmeric and curcumin have the potencial for the development of modern for the treatment of various diseases(Chattopadhyay I, Biswas K et al., 2004) ,[13] The choice of solvents for extraction is restricted to the few solvents of defined purity allowed by national and international Food laws in the prossesing of food materials. Ethanol was the suitable solvent for extraction of curcumin. Its support by FAO: that ethanol are solvent used sparingly because curcumin is completely soluble in ethanol(Stankovic I,2004) ,[14.] A number of studies are undertaken to separate curcuminoid pigments by thin layer chromatography (TLC) (Pothitirat W and Gritsanapan W,2005) ,[15]. High performance thin layer chromatography(HPTLC) and Column chromatography (CC). In our present study, HPLC(High Performance Liquid Cromatography) HPLC method was sensitive, precise and accurate for detection and quantification of curcuminoid in the extract(Jayaprakasha GK et al.,2002) ,[16] the HPLC method for separation and estimation of curcumin and its structural isomers(Tonnesen H et al,1995) ,[17] A mixture of curcuminoid in the ethanol extract of curcuma domestica val. Were analyzed by HPLC, make C18 column at flow rate of 1.0 ml/min and detected at a wavelength of 425 nm. Well-resolved chromatograms of curcuminoid were obtained with a gradient elution acetonitril:acetic acid:aquabidest 50:49%:1(v/v). The total time required for a single analysis was approximately 10 min.

Table 1. *Linearity and Range*

No	CURCUMIN	
	Concentration ppm	Peak area
1	5	37285
2	7.5	56868
3	10	76366
4	12.5	112122
5	15	133555

Curcumin present in the extract were analysis by HPLC. Table .1 shown peak areas of standart curcumin. The specificity of the method was ascertained by analyzing the standart and the samples

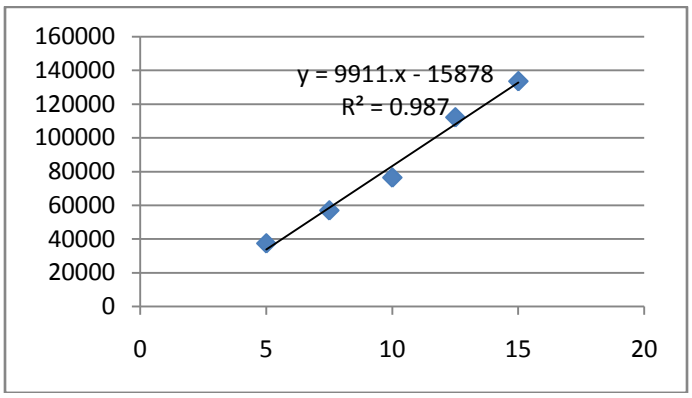

Fig.2. *Linearity curve of curcumin*

Figure 2. *shown The curcumin linear regression curve was Y=9911.7x-15878, with correlation coefficient r=0,9871.*

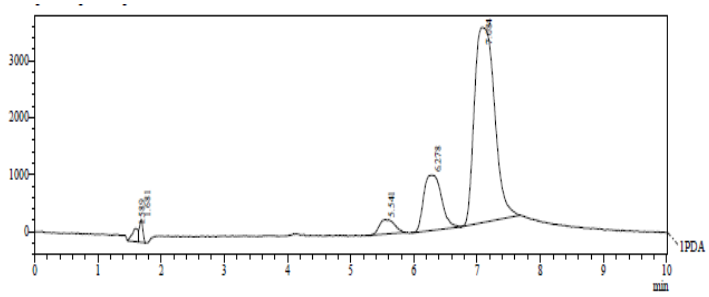

Fig.3 *Standard peak of curcumin at 7.04 retention time monitored at 425 nm wavelength*

PLC analysis profile was obtained for standart curcumin at 7.04 min in 10 min run time at shown in fig.3.

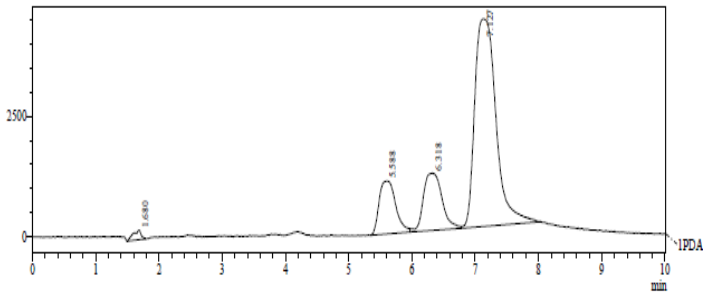

Fig.4 *Peaks of curcumin from ekstrak curcuma domestica val.*

Shown in Fig.4, the identity of each peak were confirmed by determination of retention times and by spiking with standart. Purified samples were injected in a fixed loop in 10 min run time at shown in fig.3. of 20 µl. HPLC analysis profile was obtained for samples at 7.12 min in 10 min run time. The chromatogram from the HPLC analysis showed three major peaks corresponding to curcumin (the biggest) followed demetoxycurcumin and bisdemetoxycurcumin at shown in fig.4 The calculation used linear regression concluded that curcumin in samples was 16.1%, metoxycurcumin 3,2% and bisdemetoksicurcumin 2,8%. The curcumin yield was higher in the present study as compared to another study (Sogi et al,2010),[18], in which were obtained curcumin yields ranging from 4.5 to 12.9%. It might be due to the different composition of curcuma by different sources, extraction condition and analytical technique employed in curcumin quantification, that is Sogi et al used spectrophotometry. In another study, The purity of the curcuminoids was analyzed by an improved HPLC method. HPLC separation was performed on a C_{18} column using three solvents, methanol, 2% AcOH, and acetonitrile, with detection at 425 nm. The total percentages of curcuminoids are 2.34 ± 0.171 to 9.18 ± 0.232%. (Jayaprakasha et al,2005),[19) The separation yielded 1.1 mg of curcumin, 0.6 mg of demethoxycurcumin, and 0.9 mg of bisdemethoxycurcumin (>98% purity). Moreover, the antioxidant effect of curcuminoids was measured by a 1,1-diphenyl-2-picrylhydrazil assay. The order of antioxidant activity was purified curcumin > purified demethoxycurcumin > purified bisdemethoxycurcumin > turmeric powder. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin can be used for various evaluations of their pharmacological activities.(Inoue et al,2008)),[20], The relative standard deviations of intra and inter-day assays by curcuminoids spiked to turmeric powder were less than 6.1%.An HPLC method using fluorescence detection for the quantitation of curcuminoids, such as curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) inturmeric products (ZangJet al,2009),[21].Comparison of system performance UPLC with conventional HPLC was made with respect to analysis time, efficiency and sensitivity. The proposed method was found to be reproducible and convenient for quantitative analysis of three curcuminoids in C. longa. This work provided some references for quality control of C. longa.(Cheng et al,2010)),[22].

4. CONCLUSION

Medicinal plant species that produce raw material for pharmaceuticals and phyto-chemicals for manufacturing drugs.In the commercial market,medicinal herbs are used as raw drugs,extracts or tinctures. Turmeric is used as traditional medicine in many countries because of the antibiotic and antiseptic effects of curcumin ,an important constituent of turmeric .A yellow-pigmented fraction isolated from the rhizemos of Curcuma contains curcuminoids.In this study, a local potential of curcuma domestica val contain curcumin 16.1% followed by demetoxycurcumin 3.2%and the least

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