Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(12): 51-57

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination of Total Curcuminoid Content and Free Radical Scavenging Activity of Turmeric (curcuma longa linn.) Ethanol Extract with Different Soil type Sing dpph (1,1- diphenyl-2-picrylhydrazyl)

Any Guntarti* and Romadhina Nurficahyanti

Fakultas Farmasi Universitas Ahmad Dahlan Yogyakarta, Jl. Dr. Soepomo SH, Janturan, Warungboto, Yogyakarta, Indonesia

---

ABSTRACT

Natural antioxidants such as turmeric rhizome (Curcuma longa Linn.) Are known to have antioxidant activity against free radicals because they contain bioactive compounds in the form of curcumin. This study aims to determine the total levels of curcuminoids expressed as equivalent to curcumin (EK) and antioxidant activity using the DPPH method (1,1 diphenyl 2 picrylhydrazyl).

Turmeric extract was obtained by using Soxhlet using ethanol solvent p.a. Curcuminoid identification in extracts and antioxidant activity by Thin Layer Chromatography (TLC) test. While the total levels of curcuminoids and antioxidant activity were determined by visible spectrophotometry. Quantitative analysis of total levels of curcuminoids and antioxidant activity was carried out by determining the correlation coefficient value of the linear regression equation obtained.

The qualitative test results showed that ethanol extract of turmeric from Alfisol and Inceptisol soil types positively contained curcuminoid compounds and had antioxidant activity. The total curcuminoid content in C.longa L. ethanol extract from Alfisol soil was 11.24 ± 0.409% (EK) while from Inceptisol soil type was 5.87 ± 0.174% (EK). Standard antioxidant activity of curcumin, ethanol extract of C.longa L. from Alfisol and Inceptisol soil types ES50 values of were 6.17 µg / mL, 29.56 µg / mL, and 39.08 µg / mL, respectively.

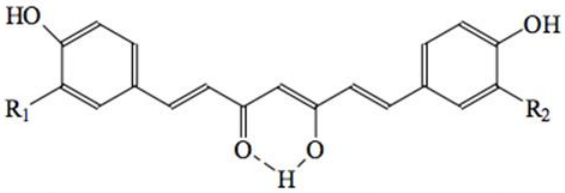
Based on the results of the determination of total levels of curcuminoid and antioxidant activity it can be concluded that the extract of ethanol of turmeric from Alfisol soil has a total level of curcuminoid and higher antioxidant activity compared to ethanol extract of turmeric from Inceptisol soil type.

Keywords: Turmeric rhizome; Antioxidant; DPPH; Ethanol; Soil type

---

INTRODUCTION

Turmeric is one of the superiors plants from director general POM that has a lot of benefits for medicinal ingredients [1]. Curcuminoid or curcumin which is isolation coumpond of turmeric, have a lot of wide activities, such as for antioxidant that can catch free radicals existence. Turmeric is kind of Famili Zingiberaceae and its fitochemicals is most often analyzed. The main compound in the turmeric rhizome is curcuminoid and essential oil. Curcuminoid in the turmeric contains around 3,0–5,0% which consists of curcuminoid and its derivative are demetoxicurcumin and bisdimetoxicurcumin [2]. The structure of curcumin can be seen in Figure 1.



Senyawa	R1	R2
Kurkumin (1)	OMe	OMe
Demetoksikurkumin (2)	H	OMe
Bisdemetoksikurkumin (3)	H	H

Figure 1. Curcuminoid Structure

The interesting chemical property of curcumin is the change of color that caused by environment pH. When it turns acidic, curcumin is yellow or yellowish orange, while in alkalic it turns red. The other uniqueness of curcumin in alkalic circumstance is curcumin degradation turns into ferulic acid ferulloilmetan. This can happen when curcumin in the pH state around 8,5-10,0 and in the long period of time. One of the degradation result is feruloilmetan which is yellowish-brown that will affect the red color that should appear [3].

Plant metabolism result, is influenced by the plant existence and growth in the growing place. Soil is the complex environment for plants to grow. Root is directly connected to the ground, which is the vital part that has important role for plant to grow and live by absorbing water and nutrients. Soil types can affect the forming of fenolic compounds that is functioned as antioxidant substance [4].

Antioxidant is a very important substance for body because it is related to the body immune also functioned to prevent damage caused by free radicals towards normal cell, protein, and fat. Antioxidant works by transfering one electron to the free radical existence, then the chain reaction of the compounds forming can be inhibited. Besides, there is also anxiety of the possibilities in the unknown side effects of syntetic antioxidant that caused natural antioxidant become the most needed alternative [5].

This research uses two different types of soil which are Gunungkidul (Alfisol) and Sanden (Inceptisol), according to Bureau of Region Establishment Planning in Gunungkidul Regency (2012) also Government Communication and Informatic Service of Bantul Regency (2018). By those types of soil, there are signs of different physical characteristic that is reviewed by the soil color, soil structure, and soil composer components. This is based on the sample collection in the research, because the turmeric sample that collected by different physical soil type will influence the content of active compounds as antioxidant potency [6].

RESEARCH METHODS

Ingredients and Tool

Ingredients: turmeric rhizome (*Curcuma longa* L.*)* from Kudu Village Tanjungsari Sub-district and Pucunganom Village aquadest (Brataco), methanol p.a, ethanol p.a, DPPH p.a (Sigma Aldrich), Silikagel GF_{254} (E-Merck), toluene (E.Merck).

Tools: Soxhlet rotary evaporator (Buchi R-200), glassware, sieve 40/50 mesh, waterbath (Memmert), distillation tool (PYREX), Halogen Moisture Analyzer (Mettle Toledo type HB-43), spectrophotometer UV-Vis (Shimadzu type SP. UV-1800).

The Research Course

Plants collection: Turmeric rhizome that would be used came from Tanjungsari Sub-distric, Gunungkidul (Alfisol) and Sanden sub-district, Bantul (Inceptisol).

Creating simplicia : Turmeric rhizome was dried under the sun radiation which is covered with black cloth for 5 days. When it is dried, turmeric rhizome simplicia rimpang kunyit was smoothed with blender and sifted used mesh 40/50 sieve that would produce powder [7].

Shrinking dried determination: The simplicia dried determination is by Halogen Moisturizer Analyzer [8].

Creating turmeric ethanol extract : The extraction used Soxhlet. Turmeric rhizome simplicia powder with the weight of 50 grams, is added with ethanol p.a for about 400 mL (1:8). The extraction was done for 6 hours. The collected filtrate was evaporated with rotary evaporator in temperature of 50ºC in the velocity of 250 rpm to get condensed extract [9].

Determining water extract content : At least 5 grams of extract was filled in the pumpkin, the water content determination was with toluene distillation. The water content was calculated in v/b [10].
Curcumin Qualitative Test: with the method of KLT silika GF254 as stationary phase and diclorometan solvent: ethanol : glacial acetic acid (94 : 5 : 1) as mobile phase [11].
Curcuminoid Content Determination
Creating curcumin standard curve: Create a series of standard curcumin solvent concentration of 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, and 5 µg/mL with methanol p.a. as the solvent which was measured in the wavelength of 420 nm [12].
Curcuminoid content determination: Turmeric powder of 10,0 mg was filled in the container, then added 10 mL of methanol and extracted by sonication for 20 minutes. Turmeric sample or standard was measured by the absorption with spectrophotometer in the maximum wavelength result [13].

Antioxidant Activity Test with DPPH
Antioxidant activity test: some solvent of ethanol extract sample of rhizome C.longa L. from Alfisol and Inceptisol type with 6 series of concentration, which was filled in measuring pumpkin of 5,0 mL. Then it was added 1,0 mL DPPH 0,15 mM and methanol until the sign.

RESULT AND DISCUSSION

This research uses two rhizome samples which is taken by different soil types with different physical criteria, they are Gunungkidul (Alfisol) with red soil and Sanden (Inceptisol) with black soil.
Creating Simplicia
Pollination is done to get the degree of certain smoothness. Powder creation is meant to increase the particle surface width that contacted with the solvent that makes extraction process more effective, the pollination is expected to damage the cells then it makes easy to get the active substance directly by the solvent in the cell [14].
Powder Dried Shrinking Result
With Halogen Moizturizer Analyzer, the determination result of turmeric rhizome powder dried shrinking of alfisol is in the percentage of 6,51% and inceptisol in the percentage of 7,25%. Then it can be concluded that turmeric rhizome simplicia from alfisol and inceptisol fulfill the requirement of FHI below 10% [10].
Extraction Water Content Determination
The result of ethanol extraction water content *C.longa* L. for Alfisol is in the amount of 6,99 % and for Inceptisol around 6,08 %, this is corresponding to the required water content of Indonesia Farmakope Herbal which is test sample is considered good to be used when its water content is below 10% [10].
Qualitative Test
The thin-layered chromatography (KLT) test is chosen as qualitative test method because it is specific, by using comparison compounds until it can be known which classified compound that contained in the sample [15,16] (Figure 2).

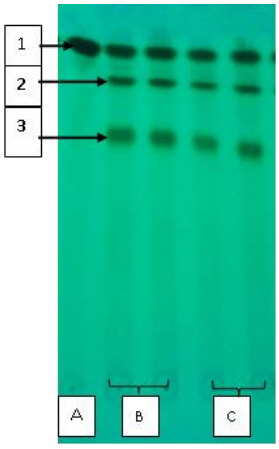


Figure 2. Curcumin Identification Result which is used KLT with (A. *curcumin standard; B. Alfisol* ethanol extraction; C. inceptisol ethanol extraction) (1. Curcumin), (2. Desmetoxicurcumin), (3. Bisdesmetoxicurcumin)

Curcuminoid Content Determination

Curcuminoid content of ethanolextraction *C. longa* L. uses the visible spectrophotometri. The measuring is done in the maximum absorption wavelength. The wavelength is 419 nm for Alfisol dan Inceptisol turmeric wavelength. Based on the calculation of the standard curve concentration vs absorbance of the standard curcumin solution obtained by the linear regression equation y = 0.0972x + 0.0965 with R count 0.997. The turmeric ethanol extraction concentration is shown in Table 1.

Table 1. Curcumin content of ethanol extraction of *C. longa* L

Sample	Sample weight (mg)	Absorbance	Content (% EK)	± LE (%)	CV (%)
Extraction Ethanol Turmeric Alfisol	25,25	0,327	11,94	11,24±0,41	4,698
	24,95	0,303	10,82		
	25,20	0,316	11,39		
	24,98	0,304	10,86		
	25,04	0,310	11,17		
Extraction Ethanol Turmeric Inceptisol	25,20	0,212	5,96	5,88 ± 0,17	3,170
	25,25	0,216	6,16		
	24,98	0,209	5,86		
	24,90	0,206	5,72		
	24,95	0,205	5,68		

LE: Limit of Error; CV: Coefisien Corelation

The result shows that Alfisol and Inceptisol type has in order curcuminoid content which are 11,24 ± 0,53 % (EK) dan 5,87 ± 0,19 % (EK), as Thai Herbal Pharmacopoeia volume 1 year 2009 then it can be concluded that curcuminoid content fulfill the requirement which is below 5,0 % b/b. The CV value of each samples are below 5 % which required the data homogenity [17].

Antioxidant Activity Test

The activity is determined by the compound ability in the extration to decrease purple radical intensity of DPPH in the maximum wavelenght. Spectrum wavelength of curcumin and the sample is shown in Figure 3.

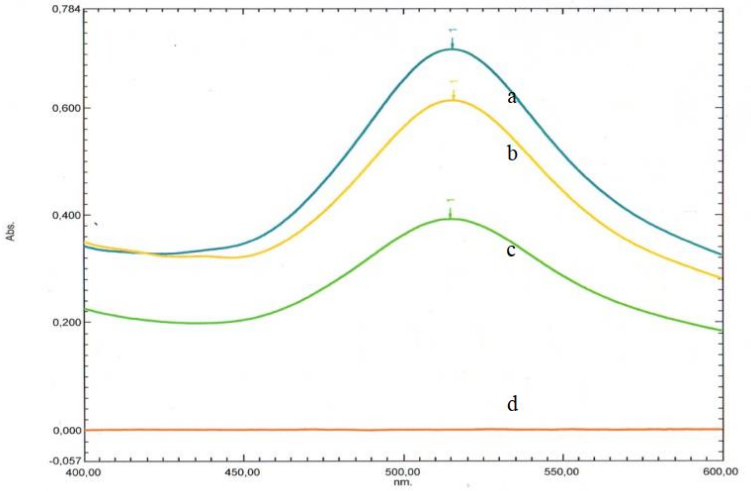


Figure 3. *Overlay* Spectrum of Inceptisol turmeric ethanol extraction (a), Alfisol turmeric ethanol extraction (b), Curcumin Standard (c), and Form (d) (with added reactor of DPPH)

From the results of the study showed that the potential for capturing free radicals from the largest to the smallest is curcumin> Alfisol turmeric ethanol extract> etisol Alfisol ethanol extract. The ES50 value is inversely proportional to the percentage of free radical capture [18]. The greater the ES50 value the greater the concentration needed to produce free radical capture activity as much as 50% so that the smaller the potential antioxidant activity of the test compound. The ES50 histogram is presented in Figure 4.

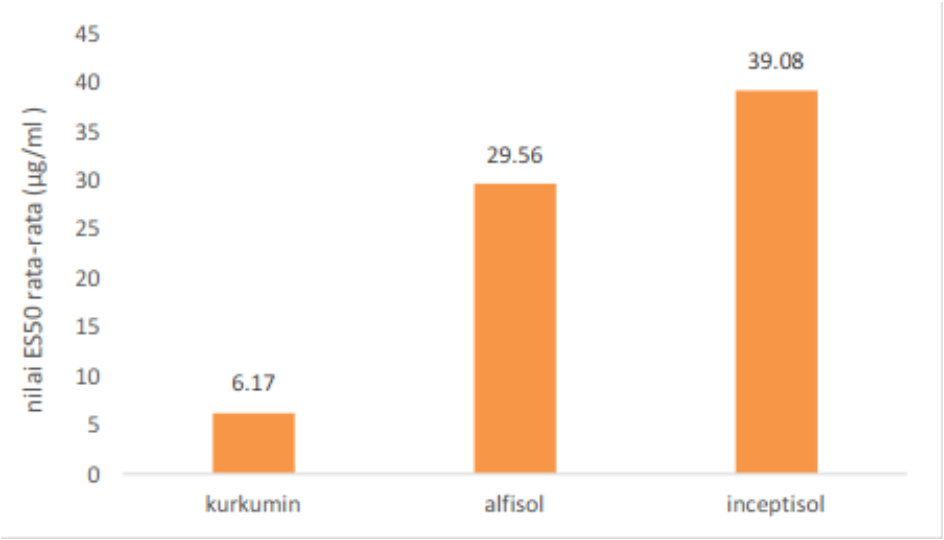


Figure 4. Graphic of average ES50 values between curcumin, ethanol extract of turmeric from the type of alfisol soil, and Inceptisol

Curcuminoids as secondary metabolites that have activity as antioxidants in their formation are strongly influenced by environmental conditions. Phenyl alanine amonialiase enzyme (PAL) which serves as a catalyst in the formation of curcumin is very sensitive to environmental factors including PAL activity will increase in extreme environmental conditions, including low nutrient levels and low soil moisture content [19].

The research of Hossain and Ishimine, [20], states that in soil samples of red soil have a higher Fe content than gray soil and show the level of curcumin of the red soil can be higher and cause the soil to be alkaline. Alfisol or red Mediterranean soil originating from Gunungkidul is a red soil group caused by high iron content with low humus content, Alfisol soil usually has high Fe with low humus content which causes this soil to be red [21]. The red color is formed due to high levels of iron in the soil that is oxidized. Fe levels that are classified as very high will inhibit the development of roots and interfere with nutrient uptake by plants [22].

The highest level of curcumin and the more concentrated color of the rhizome is obtained when turmeric is grown in dark red soil rather than ash soil [20]. Good aeration ensures continuous exchange of oxygen and carbon dioxide through the soil pores. Very wet soil will paralyze the roots but very dry soil will dry the roots [23]. In sandy soils, soil porosity is dominated by macro pores that function as water traffic so that infiltration increases [24]. A comparison of the results of curcuminoid levels and activity tests is presented in Figure 5.

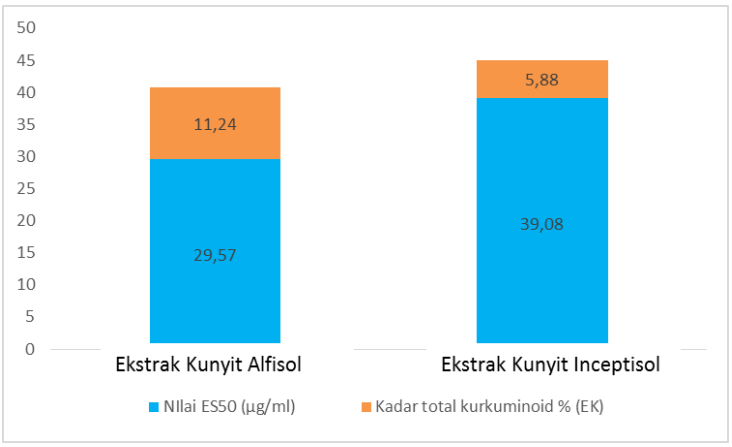


Figure 5. Histogram of Average ES50 Value between Turmeric Ethanol Extract from Alfisol Soil Type, and Inceptisol with Curcuminoid Total Level

In Figure 5, it can be concluded that between ES50 values and total curcuminoid levels are positively correlated, in the sense that the higher the total level of curcuminoids the antioxidant activity will increase. From the results of this study indicate that the ethanol extract of the rhizome from Alfisol and Inceptisol soil types proved to contain compounds that have activity as free radical catchers namely curcuminoids [6]. The higher the total level of curcuminoids, the increased activity of free radical catchers. There is a positive correlation between antioxidant activity and total levels of curcuminoids in ethanol extract of turmeric from Alfisol and Inceptisol soil types. The antioxidant activity of the second ethanol extract of the sample was contributed by curcuminoid compounds.

CONCLUSION

There was a significant difference between the total levels of curcuminoid ethanol extract of C.longa L. from Alfisol soil type (Kudu Village, Tanjungsari Subdistrict, Gunungkidul) with Inceptisol soil type (Pucunganom Village, Sanden Subdistrict, Bantul). In the Alfisol soil type, total curcuminoid levels obtained were 11.24 ± 0.409% (equivalent of curcumin) and Inceptisol soil type was 5.88 ± 0.174% (equivalent of curcumin). Ethanol extract of C.longa L. from Alfisol soil has more potent antioxidant activity than ethanol extract of C.longa L. from Inceptisol soil type. With ES50 values in a sequence of 29.56 µg / mL and 39.08 µg/mL. Both samples are included in the strong antioxidant class.

REFERENCES

[1] Utomo; Muhajir; Sudarsono; Rusman; Bujang; Sabrina; Tengku; L Jamalam; Wawan. Ilmu Tanah Dasar-dasar dan Pengelolaan, Edisi Pertama, Prenademedia Grup, Jakarta, **2016**.
[2] C Chang; M Yang; H Wen; J Chern. *J Food Drug Analaysis*. **2002**, 10, 178-182.
[3] YJ Kim; HJ Lee; Y Shin. *J Agric Food Chem*. **2013,** 6(1), 10911-10918.
[4] L Jin; Y Zhang; L Yan; Y Guo; L Niu. *Molecules.* **2012,** 17(8), 9361-9378.
[5] MS Lin; RY Zer; JW Be; CW Chieng; MW Yih; K Malcolm. *Sains Malaysiana*, **2015**, 44(12), 1685-1691
[6] E Nihayati; T Wardiyati; Sumarno; R Retnowati. *Agrivita*, **2013**, 3(2), 218-226.
[7] R Gandhimathi; S Vijayaraj; MP Jyothirmaie. *Int J Pharm Res Anal*. **2012,** 2(2), 72-78.
[8] DN Permana; N Lajis; F Abas; AG Othman; R Ahmad; M Kitajama; H Takayama; N Aimi. *Natural Product Sci*. **2003**, **9**, 7-9.
[9] W Pothitirat; SD Nuryanti; P Jansook; C Pummangura; W Gritsanapan. *The Journal*. **2013.**
[10] Anonim. Farmakope Herbal Indonesia, Edisi I, Departemen Kesehatan Republik Indonesia, Jakarta; **2008,** 75-77.
[11] Sethi; G Sung; BB Anggarwal. *Herbal Drug to Modern Med*, *Springer*. **2009**, 114-121.
[12] VS Neergheen; T Bahorun; P Pugo-Gusnam; LD Ng Foong; D Ramful; OI Aruoma. *Int J Pharma Phytochem Res*. **2010**, 2(3), 44-52.
[13] SEP Wahyuningtyas; IDG Permana; M Wiadnyani; AAI Sri; *Jurnal ITEPA*, **2017**, 6(2), 61.

[14] TJ Mabry; KR Markham; MB Thomas. *The Sys Identification of Flavonoid, Springer-Verlag, Berlin*. **1970**, 50-52.
[15] Anonim. Farmakope Herbal Indonesia, Edisi I, Departemen Kesehatan Republik Indonesia, Jakarta; **2008,** 75-77.
[16] B Cahyono; MD Huda; L Limantara. *Reaktor*, **2011**, *13*, 165-171.
[17] P Molyneux. *J Sci Technol*. **2004.**
[18] D Huang; B Ou; RL Prior. *J.Agric Food Chem*. **2005,** 53, 841-1856.
[19] L Taiz; E Zeiger. Plant Physiology, Springer-Verlag, Berlin, Heidelberg, **2007**, 468-478.
[20] AM Hossain; Y Ishimine. *Plant Production Sci*. **2005**, 8(4), 482 - 486.
[21] SEP Wahyuningtyas; IDG Permana; M Wiadnyani; AAI Sri; *Jurnal ITEPA*, **2017**, 6(2), 61.
[22] J Mann; RS Davidson; JB Hobbs; DV Banthorpe; JB Harborne. Natural products: Their Chemistry and Biological Significance, Longman Group, United Kingdom; **1994**.
[23] Utomo; Muhajir; Sudarsono; Rusman; Bujang; Sabrina; Tengku; L Jamalam; Wawan. Ilmu Tanah Dasar-dasar dan Pengelolaan, Edisi Pertama, Prenademedia Grup, Jakarta, **2016**.
[24] G Soepardi. Sifat dan Ciri Tanah. Fakultas Pertanian Institut Pertanian Bogor, Bogor, **1983.**