Journal of Functional Food and Nutraceutical

EFFECT OF ETHANOL CONCENTRATION AND EXTRACTION TIME WITH MICROWAVE ASSISTED EXTRACTION ON ANTIOXIDANT ACTIVITY OF TEMULAWAK-EXTRACT *(Curcuma xanthorrhiza.Roxb)*

Aisyah Tri Septiana*
Erminawati Wuryatmo

Department of Food Technology, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia.

ABSTRACT

Temulawak (*Curcuma xanthorrhiza* Roxb) is commonly used as traditional medicine. This study aimed at determining the effect of ethanol concentration and of the duration of microwave-assisted extraction (MAE) on antioxidant activity of *C. xanthorrhiza* ethanol extract. *C. xanthorrhiza* rhizomes were extracted using 70%, 80% and 90% (v/v) ethanol for 5, 7 and 9 minutes. Compared to control (without microwave extraction) and analyzed for curcumin content, total phenolic content (TPC), and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (RSA). Increasing the ethanol concentration from 70% to 90% as solvent and increasing the extraction time from 0 to 7 minutes at 240 watt caused an increase in curcumin content, TPC, and DPPH RSA. Curcumin, TPC, and DPPH RSA of *C. xahorrhiza* extract using 90% ethanol solvent for 7 minutes were the largest compared to other treatments, namely 184 mg/g, 319.5 mg/g and 71.08%; respectively.

Keywords: antioxidant; ethanol extract; microwave assisted extraction

ABSTRAK

Temulawak (*Curcuma xanthorrhiza* Roxb) biasa digunakan sebagai obat tradisional. Penelitian ini bertujuan untuk mengetahui pengaruh konsentrasi etanol dan lama ekstraksi berbantu gelombang mikro (MAE) terhadap aktivitas antioksidan ekstrak etanol *C. xanthorrhiza*. Rimpang *C. xanthorrhiza* diekstrak menggunakan etanol 70%, 80% dan 90% selama 5, 7 dan 9 menit dibandingkan kontrol (tanpa ekstraksi gelombang mikro) dan dilakukan analisis kurkumin, total fenol, dan kapasitas penangkapan radikal DPPH. Peningkatan konsentrasi etanol dari 70% sampai 90% sebagai pelarut dan peningkatan lama ekstraksi 0 sampai 7 menit daya 240 watt menyebabkan peningkatan kadar kurkumin, total fenol, dan kapasitas penangkapan radikal DPPH. Kurkumin, total fenol, dan kapasitas penangkapan radikal bebas dari ekstrak *C. xanthorrhiza* menggunakan pelarut etanol 90% selama 7 menit adalah paling besar dibandingkan perlakuan yang lain yaitu masing-masing 184 mg/g, 319,5 mg/g dan 71,08%.

Kata kunci: antioksidan; ekstrak etanol; ekstraksi berbantu gelombang mikro

Article Information

Article Type: Short Communication And Notes
Journal Type: Open Access
Volume: 3 Issue 2

Manuscript ID
V3n2896-1

Received Date
15 September 2021

Accepted Date
04 February 2022

Published Date
28 February 2022

DOI: 10.33555/jffn.v3i2.86

Corresponding author:
Aisyah Tri Septiana
Purwokerto, Indonesia, 53123
Email:
aisyah.septiana@yahoo.com

Citation:
Septiana, A.T., Wuryatmo, E. 2022. Effect of ethanol concentration and extraction time with microwave assisted extraction on antioxidant activity of temulawak-extract *(Curcuma xanthorrhiza.roxb)*. J. Functional Food & Nutraceutical, 3(2), pp.63-69

INTRODUCTION

Lipid oxidation can occur in food products and in the human body. Oxidation of food causes rancidity and damages to vitamins, pigments and proteins. Lipid oxidation can also lead to the emergence of degenerative diseases, such as coronary heart disease, stroke and diabetes mellitus. Antioxidants can inhibit lipid oxidation.

Temulawak (*Curcuma xanthorrhiza Roxb*) is an Indonesian native plant with important medicinal value (Dharma 1985). *C. xanthorrhiza* as a medicinal plant has properties as hepatoprotection, anti-inflammatory, anticancer, antidiabetic, antimicrobial, preventing cholera, and antihyperlipidemia (Hwang et al., 2006). Traditionally, this plant is used as a health supplement known as "herbal medicine" or to treat particular health disorders including hepatitis, liver complaints, diabetes, rheumatism, anticancer, hypertension, and heart disorders (Salleh et al., 2016).

The efficacy of *C. xanthorrhiza* may be due to its activity as an antioxidant. According to Gordon (1990), antioxidants are substances with the ability to prolong or prevent the lipid oxidation process. *C. xanthorrhiza* and turmeric acetone extract from sliced rhizomes dried using a cabinet dryer for 14 h have a higher inhibitory activity of peroxide and malonaldehyde formations from linoleic acid than ginger (Septiana et al., 2006a). The antioxidant activity of decoction of dried *C. xanthorrhiza* is greater than that of dry turmeric (Samsudin and Panigoro, 2013). *C. xanthorrhiza* extract is potential to be used as antiatherosclerosis because it has inhibitory activity of LDL oxidation and macrophage cholesterol accumulation (Septiana et al., 2006b). The bioactive components of *C. xanthorrhiza*, such as curcuminoids (Menon and Sudheer, 2007) and xanthorrhizol (Jantan et al., 2012) can function as antioxidants. *C. xanthorrhiza* also contains bioactive components in the form of alkaloids, saponins, quinones and triterpenoids (Panigoro et al., 2013).

Various extraction methods have been developed to extract bioactive components from natural sources, including several innovative technologies such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction, enzyme-assisted extraction, and several conventional methods such as maceration and Soxhlet extraction. Compared to the maceration and soxhlet methods, MAE method has some advantages, such as shorter extraction time and lower temperature, which leads to less degradation of thermally labile compounds (Li et al., 2017). In addition, MAE is also compatible with water as an extraction solvent so that the use of organic solvents is reduced or eliminated, and more non-polar organic compounds from plant materials could also be extracted by MAE (Zghaibi et al., 2019). The use of a solvent mixture of ethanol and water is widely used for extraction because of the difference in polarity of the two, so that the polarity can be adjusted based on the proportion of ethanol with water, and the solvent mixture is safe for humans. This study was conducted with the aim of determining the optimum proportion of ethanol with water as a solvent in the extraction of *C. xanthorrhiza* and the optimum duration of microwave-assisted extraction (MAE) on the antioxidant activity of the resulting extract.

MATERIALS AND METHOD

Materials

The main materials used in this study were *C. xanthorrhiza* from farmers in Purbalingga, Central Java, while the chemicals used in this study were methanol, ethanol, standard curcumin, gallic acid, Na_2CO_3, folin ciocalteau, and DPPH or 1,1-diphenyl -2-picrylhydrazyl (Merck, Germany).

The tools used in this study were scales (Ohaus, United States), erlenmeyer, beaker glass, test tubes (Pyrex, Germany), vortex, microwave (Electrolux), blender (Philip, Neetherlands), UV-Vis spectrophotometer (Shimadzu 1240, Japan), and cabinet dryer.

Method

Preparation of *C. xanthorrhiza* ethanol extract

First, the rhizomes of *C. xanthorrhiza* were washed, drained and dried and powderized. The *C.*

xanthorrhiza powder was obtained from fresh *C. xanthorrhiza* rhizomes sliced 4 mm, dried at 57^0C for 14 h using a cabinet dryer, crushed and filtered using a 60 mesh filter. A total of 5 g of *C. xanthorrhiza* powder was dissolved in 75 ml 70%, 80% or 90% ethanol solvent (1:15 g/ml), allowed to stand for 20 min. (Handayani *et al*., 2014), and put in a 240 Watt microwave for 0, 5, 7 and 9 min. Every one minute, the radiation will be paused for one to two minutes, which was carried out to avoid the temperature from increasing to above the boiling point of the solvent (Li et al., 2009). The extract was then cooled down to room temperature, and filterred using a filter cloth, then filterred again using Whatman No.1 Filter Paper to produce the filtrate. Next, the filtrate was concentrated using a rotary evaporator, at a temperature of 60°C.

Analysis of curcumin content (Delfiya et al., 2014)

Curcumin content of ethanolic extract of *C. xanthorrhiza* tested using curcumin as a standard. The standard curve made by making a series of dilutions of curcumin in ethanol with various concentrations (0.2; 0.4; 0.6; 0.8 and 1.0 µg/mL). Analysis of curcumin from various ethanolic extracts of *C. xanthorrhiza* with different MAE durations and standard curcumin carried out by placing each sample in a spectrophotometer cuvette and the absorbance measured at a 425 nm. Absorbance vs curcumin standard concentration curve was made by connecting a line at these points and the correlation equation was made. Curcumin concentrations were calculated using a curcumin standard absorbance vs concentration graph. Tests on aqueous extracts were carried out in the same method as the standard based on the curcumin standard concentration.

Analysis of total phenolic content (Hammerschmidt and Pratt, 1978)

Total phenolic content (TPC) of *C. xanthorrhiza* extract was tested using gallic acid as a standard. The standard curve was made by making a series of dilutions of gallic acid in ethanol. Each gallic acid and 0.1 mL ethanol extract of *C. xanthorrhiza* were added with 2 mL of Na_2CO_3, then mixed and allowed to stand for 2 minutes, added 0.1 mL of folin ciocalteau and mixed using a vortex. The mixture was then kept in the dark for 30 minutes and the absorbance was measured at a wavelength of 750 nm using spectrophotometry.

Free radical scavenging capacity (Sheikh et al., 2009)

The extract solution was prepared by dissolving the sample of ethanol extract of *C. xanthorrhiza* in methanol. Then, 2 ml of ethanol extract was mixed with 2 ml of 0.16 mM DPPH solution in methanol. The mixture shaken for 1 minutes, left for 30 minutes in the dark and the absorbance was measured at 517 nm. The ability to capture DPPH radicals is calculated by the following equation:

Radical scavenging capacity (%)

$$\frac{Abs.Control - Abs.Sample}{Abs.\ Control} \times 100\%$$

Abs: Absorbance

Statistical analysis

DPPH radical scavenging capacity, curcumin content, and total phenolic content analysed using Analysis of variance (ANOVA) at the 5% level. If there was a significant effect on the treatment, the analysis continued with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results in Table 1 show that the increase in ethanol concentrations of 70% to 90% increased TPC of *C. xanthorrhiza* extract. The results of this study are in line with the study conducted by Do et al (2014), stating that the use of ethanol concentrations of 50% to 100% increased total phenolic content of *Limnophita aromatica* extract. The increase in ethanol concentration does not necessarily increase total phenolic content depending on the type of sample. The highest total phenol content of *Vermonia amygdalian* ethanol extract using microwave assisted extraction (MAE) was obtained using 60% ethanol solvent compared to 40% and 80% ethanol solvent (Alara et al,, 2020).

Total phenolic content test used to test compound

content belonging to the phenolic compounds group. Phenolic compounds are characterized by the presence of a phenyl ring containing one or more hydroxyl substitutes. Thus, total phenolic content has a very diverse structure identified as phenolic monomer, dimer, and polymeric. Some phenolic compounds include simple phenolics, benzoquinones, phenolic acids, flavonoids, and condensed tannins (Lattanzio, 2013). Thus, phenolic compounds have different polarities depending on the material.

Table 1. Curcumin content, total phenolic content and DPPH radical scavenging capacity of *C. xanthorrhiza* ethanol extract

Treatment	Curcumin content (mg/g)	Total phenolic content (mg/g)	DPPH scavenging at 4000 ppm
Ethanol concentration			
70% ethanol	100.5^{c}	170.75^{c}	55.67^{b}
80% ethanol	124.5^{b}	232^{b}	58.51^{b}
90% ethanol	153.25^{a}	259.5^{a}	64.96^{a}
Duration of MAE			
0 minutes	106^{c}	202.5^{c}	53.40^{c}
5 minutes	128.25^{b}	229^{b}	62.57^{b}
7 minutes	151^{a}	271.5^{a}	65.47^{a}
9 minutes	119^{b}	182.75^{c}	57.40^{b}

Curcumin belongs to a group of phenolic compounds that are commonly found in *C. xanthorrhiza*. The increase in ethanol solvent concentration of 70% to 90% increased curcumin content of ethanolic extract of *C. xanthorrhiza*. The results of this study are in line with study on the curcumin content of turmeric extract (Paulucci et al., 2013) and multistage extraction of *C. xanthorrhiza* (Anggoro et al., 2015) extracted using 96% ethanol which was higher than that using 70% ethanol.

Curcumin content and total phenolic content of *C. xanthorrhiza* extract were also affected by the duration of Microwave-Assisted Extraction (MAE) from 0 to 9 minutes. The increase in the extraction time from 0 to 7 minutes at 240 watt increased curcumin content and total phenolic content of *C. xanthorrhiza* extract. Extraction using MAE method utilizes microwave radiation to accelerate selective extraction through heating the solvent quickly and efficiently, it generates tremendous pressure on cell wall due to swelling of plant cells and the pressure developed pushes cell wall from inside, stretch the cell wall and ruptures them (Jain et al., 2009) thereby increasing the contact between the sample and the solvent. The increase in the extraction time up to 7 minutes increased the contact time between the sample and the solvent, so that the amount of compounds extracted increased. The increase in the extraction time from 7 to 9 minutes reduced curcumin content and total phenolic content of *C. xanthorrhiza* extract because it was suspected that prolonged extraction cause the degradation of some antioxidants. Similar results were reported in studies on the extraction of total phenolic from blackthorn flowers (Lovric et al., 2017).

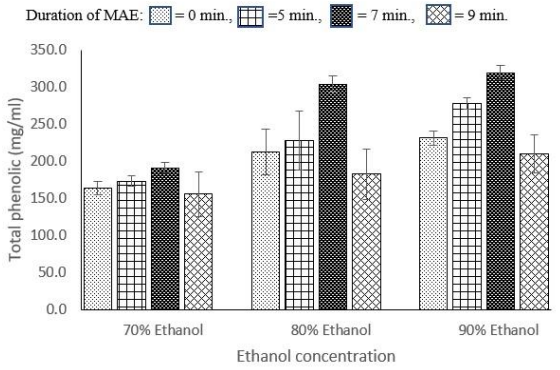

Figure 1. Effect of solvent type and duration of microwave-assisted extraction on total phenolic content of *C. xanthorrhiza* ethanol extract

The results of this study in Figure 1 show that at a concentration of 70%, the addition of extraction time does not play a role in the changes in the total phenolic content when compared to the concentrations of 80% and 90%. The increase in duration of MAE from 0 min. to 7 min. further increased total phenolic content of *C. xanthorrhiza* extract with 90% ethanol compared to 70% ethanol. The results of this study in line with the study conducted by Lee et al. (2011) on the ethanol extract of eleuthero. Higher water concentration causes more non-phenolic

compounds, such as carbohydrates to be extracted. Carbohydrates do not only cause phenolic content extracted to be lower, but also protect phenolic compounds so that at radiation for more than 7 min., the decrease in phenolic content of 70% ethanol extract was lower than that of 80% and 90% ethanol extract. In addition, it was suspected that more phenolic compounds in *C. xanthorrhiza* were non-polar than polar compounds. One type of phenolic compound commonly found in rhizomes of *C. xanthorrhiza* is curcumin.

The increase in total phenolic content and curcumin content through the increase in ethanol concentration of 70% to 90% and the increase in duration of microwave-assisted extraction to 7 minutes increased DPPH free radical scavenging capacity. DPPH radical scavenging capacity can be used to determine the antioxidant activity of *C. xanthorrhiza* extract. The results of this study are in line with the study conducted by Do et al (2014), indicating that the use of 50% to 100% ethanol increased total phenolic content and antioxidant DPPH radical scavenging activity of *Limnophita aromatica* extract.

Curcumin and other phenolic compounds in *C. xanthorrhiza* are compounds with the potential as antioxidants. The correlation analysis between total phenolic content and curcumin content with antioxidant activity aims the determination the relationship between the compounds and antioxidant activity. The relationship between curcumin content and total phenolic content with antioxidant activity as DPPH scavenger has a correlation coefficient (R^2) of 0.92 and 0.80; respectively. The resulting value was positive, therefore the relationship between total phenolic content and curcumin content on antioxidant activity was directly proportional. Based on the correlation coefficient (R^2) value that greater than 0.8; both phenolic and curcumin compounds (as part of phenolic compounds) had a significant correlation or the bioactive compounds had a significant effect on antioxidant activity based on DPPH radical scavenging capacity. The results of this study in line with the study conducted by Septiana et al (2021).

The highest curcumin content, total phenolic content, and free radical scavenging capacity of *C. xanthorrhiza* extract were obtained through MAE using 90% ethanol solvent for 7 minutes these are 184 mg/g, 319.5 mg/g and 71.08%; respectively. DPPH free radical scavenging capacity was carried out at a fairly small concentration of 4000 ppm. DPPH radical scavenging capacity testing at large concentrations generaly higher. Lemongrass antioxidant activity testing at a concentration of 20,000 ppm was 79.98% (Suri et al., 2020).

CONCLUSIONS

The increase in concentration of ethanol solvent from 70% to 90% and the increase in extraction time from 0 to 7 minutes at 240 watt caused an increase in curcumin content, total phenolic content, and DPPH radical scavenging capacity, but an increase in extraction time from 7 to 9 minutes decreased the extract parameters. The highest curcumin content, total phenolic content, and free radical scavenging capacity of *C. xanthorrhiza* extract were using 90% ethanol for 7 minutes compared to other treatments, namely 184 mg/g, 319.5 mg/g and 71.08%; respectively.

ACKLOWLEDGEMENT

The author would like to thank the Ministry of Research and Technology for providing research funds through the Applied Research scheme based on Decree Number 1310/UN23/HK.02/2021.

REFERENCES

Alara, O.R., Abdurahman, N.H., and Olalere, O.A. 2020. Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University – Science*. 3, pp.7-16

Anggoro, D., Rezki, R.S., dan Siswarni MZ. 2015. Ekstraksi multi tahap kurkumin dari temulawak *Curcuma xanthorriza* Roxb.) menggunakan pelarut etanol. *Jurnal Teknik Kimia USU*. 4(2), p.39-45

Delfiya, A., Thangavel, K., Natarajan, N., Kasthuri, R., and Kailappan, R. 2014.

Microencapsulation of turmeric oleoresin by spray drying and *in vitro* release studies of microcapsules. *Journal of Food Process Engineering*. 38**,** pp.37–48.

Dharma, A.P. 1985. *Tanaman Obat Tradisional Indonesia*. Jakarta: PN Balai Pustaka.

Do, Q.D., Artik Angkawijaya, A.E., Tran-Nguyen, P.L, Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju. Y. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*. 22, pp.296-302

Gordon, M. H. 1990. The mechanism of antioxidants action *in vitro*. *In:* B.J.F Hudson (Ed), *Food Antioxidant.* London: Elseiver Applied Science.

Hammerschmidt, P.A. and Pratt, D.E. 1978. Phenolic antioxidants of dried soybeans. *Journal of Food Science.* 43(6723), pp.556-559.

Handayani, D., Mun'im, A., & Ranti, A. S. 2014. Optimation of green tea waste extraction using microwave assisted extraction to yield green tea extract. *Traditional Medicine Journal*. 19(1), pp.29–35.

Hwang, J.K., Shim, J.S. and Pyun. 2006. Antibacterial activity of xanthorrizol from *Curcuma xanthorrhiza* against oral pathogens. *Fitoterapia*. 71, pp.321-323.

Jantan I, Saputri FC, Qaisari MN, and Buang F. 2012. Correlation between chemical composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human low density lipoprotein oxidation. *Evidence Based Complementary and Alternative Medicine*. 2012 (438356), pp.1-10.

Handayani, D., Mun'im, A. and Ranti, A. S. 2014. Optimation of green tea waste extraction using microwave assisted extraction to yield green tea extract. *Traditional Medicine Journal*. 19(1), pp.29–35.

Jain, T., V. Jain, R. Pandey, A. Vyas and S. S. Shukla. 2009. Microwave assisted extraction for phytoconstituents – An overview. *Asian Journal Research Chemistry*. 1(2), pp.19-25.

Lattanzio, V. 2013. Phenolic Compounds Introduction. In.: Ramawat, K.G., Me´rillon, J.M. (Eds). *Natural Products*. Berlin Heidelberg: Springer-Verlag, pp.1543-1580. (Accessed 15 September 2021). Available from: https://www.researchgate.net/publication/281611160_Phenolic_compounds

Li, Y., Li, S., Lin, S., Zhang, J., Zhao, C and Li, H. 2017. Microwave-assisted extraction of natural antioxidants from the exotic Gordonia axillaris fruit: Optimization and identification of phenolic compounds. *Molecules.* 22, pp.1481-1495.

Lovric, V., Predrag, P., Danijela B.K., Marijana, J. and Verica D. 2017. Effect of Microwave-assisted extraction on the phenolic compounds and antioxidant capacity of blackthorn flowers. *Food Technol. Biotechnol*. 55(2), pp.243–250

Menon, V.P. and Sudheer, A.R. 2007. Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology*. 595, pp.105-25.

Panigoro, R., Samsudin, S.and Dhianawaty. 2013. Comparison of curcumin level in fresh and decoction of dried *Curcuma xanthorrhiza* Roxb. rhizome. *International Journal* of *Research* in *Pharmaceutical Sciences*. 4(2), pp.256-259.

Paulucci, V.P., Couto, R.O., Teixeira, C.C.C., and Freitas, L.A.P. 2013. Optimization of the extraction of curcumin from *Curcuma longa* rhizomes. *Brazilian Journal of Pharmacognosy*. 23(1), pp.94-100

Salleh, N.A.M., Ismail, S. and Halim. M.R.A

2016. Effects of *Curcuma xanthorrhiza* extracts and their constituents on phase ii drug-metabolizing enzymes activity. *Pharmacognosy Research* 8(4), pp.309–315.

Samsudin S. and R. Panigoro. 2013. Comparison of antioxidant activity between decoction of dried *Curcuma longa*. L., and *Curcuma xanthorrhiza* Roxb. rhizomes. *International journal of research in phytochemistry and pharmacology*. 3(1), pp. 27-30.

Septiana, A.T. Mustaufik, Dwiyanti, H., and Ola, M.M. 2006a. Pengaruh spesies Zingiberaceae (jahe, temulawak, kunyit, dan kunyit putih) dan ketebalan irisan sebelum pengeringan terhadap kadar dan aktivitas antioksidan ekstrak aseton yang dihasilkan. *Agritech.* 26(2), pp.49-54.

Septiana, A.T., Dwiyanti, H., Muchtadi, D., and Zakaria, F. 2006b. Penghambatan oksidasi LDL dan akumulasi kolesterol pada makrofag oleh ekstrak temulawak (*Curcuma xanthorriza* Roxb). *Jurnal Teknologi dan Industri Pangan.* XVII(3), pp.221-226.

Septiana, A.T., Erminawati and Winarsi, H. 2021. The effect of basic ingredients form, cold storage and precipitate separation on bioactive components and antioxidant activity of *Curcuma xanthorrhiza* Roxb. *Food Research.* 5(4), pp.203 - 209

Sheikh, T. Z. B., Yong, C. L. and Lian, M. S. 2009. In vitro antioxidant activity of the hexane and methanolic extracts of *Sargassum baccularia* and *Cladophora patentiramea*. *Journal of Applied Sciences*. 13(9), pp.2490-2493.

Suri, A., Erminawati, and Naufalin, R. 2020. Antioxidant activities of lemongrass with solvent multi-step extraction microwave-assisted extraction as natural food. preservative. *Journal of Functional Food and Nutraceutical.* 2(2), pp.117-128

Zghaibi, N., Omar, R., Kamal, S.M.M., Biak, D.R.A. and Harun, R. 2019. Microwave-assisted brine extraction for enhancement of the quantity and quality of lipid production from microalgae *Nannochloropsis sp. Molecules. 24*, pp.3581-35101.