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Extraction of Curcumin Bioactive Compounds from Rimpang Temulawak (*Curcuma xanthorihiza Roxb*.) Using Supercritical Carbon Dioxide (CO_2) and Ethanol

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Abstract

Separation of curcumin bioactive compounds from temulawak rhizome was done using a supercritical CO2 fluid extraction method. This method of extraction is done by varying the time of extraction and the volume of the ethanol solvent. The yield of curcumin obtained was compared with the result of the maceration extraction method. Qualitative and quantitative analyzes of curcumin were characterized by thin layer chromatography (TLC), FTIR and UV-visible spectrophotometers. The TLC results show that curcumin was successfully extracted based on the obtained Rf value. The FTIR results show the specific functional groups in the standard of curcumin, maceration, and supercritical fluid extraction of CO_2, ie at 3436, 3328, and 3361 cm^{-1} (-OH) and 1622, 1620, and 1620 cm^{-1} (C = O). The UV-visible spectrophotometer result showed curcumin level of 2.29% with 30 mL ethanol volume for 30 min and curcumin level using maceration of 1.67% for 3 × 24 hours. This is evidenced by the short time and small volume of ethanol obtained greater levels. Supercritical fluid extraction was performed at a pressure of 72.8 atm, a temperature of 31.1 °C adjusted to the critical point of CO_2 solvent.

Keywords: supercritical fluid extraction of CO_2, curcumin, maceration, temulawak rhizome

Introduction

Indonesia is one country where many people who cultivate medicinal plants to be used as traditional medicine / herbal. Traditional medicinal plants are part of the largest biodiversity and in industrial scale has been widely used as raw materials in the manufacture of herbal medicine. One of the herbal medicinal plants that are abundant in Indonesia is temulawak (*Curcuma xanthorhiza Roxb*.). The main part of temulawak plant commonly used as raw material of rhizomes.

The ginger rhizome contains curcumin which can be separated by extraction method. The methode used to extraction is supercritical CO_2 fluid with the addition of ethanol to separate the curcumin compound from the ginger rhizome. The use of this method aims to determine the separation of curcumin bioactive compounds whether to be more effective and efficient. This is because in the

processing of herbal medicine in Indonesia is still found many extraction process without considering the factors that affect the process efficiency and quality of extraction.

Supercritical fluid extraction is an extraction method which uses a supercritical liquid which is above its critical point, showing the strength of the solvent and ignoring the surface tension in a liquid-gas phase (Capuzzo *et al*. 2013). The solvent used in this study is CO2, because carbon dioxide is a solvent which considered ideal in the process of supercritical fluid extraction. Some of the advantages in this process are fast and short extraction processes, using eco-friendly solvents, unnecessarily toxic, non-flammable, and can use relatively low temperatures (Lang, *et al*. 2000). In addition, the use of supercritical fluid extraction may also provide a solvent which can be varied by controlling the pressure and in the separation process only by reducing the pressure. In contrast to the usual method of extraction, which varies by changing the temperature and solvent composition, there is a need for a vaporization process to remove the solvent, and it takes a long time, and the use of a toxic solvent. (Schantz, 1986)

This researchs aim to extraction with supercritical fluid using CO_2 and ethanol to obtain curcumin biocative compounds from the ginger rhizomes (*Curcuma xanthorrhiza Roxb*.), and it is expected to be more effective and efficient. In addition, we studied the effect of the condition of the variation in the duration of extraction time, and the ratio of the ethanol solvent volume used.

Materials and Methods

Rimpang temulawak obtained from Center for Tropical Biopharmaca Study, IPB. 5 kg of ginger rhizome dried at 40 ^{o}C.Then, it was smoothed to be 60 mesh.

The maceration extraction was done by dissolving the sample in ethanol which was then soaked for 24 hours then filtered to obtain the extract. The process is repeated as much as 3 × 24 hours. For supercritical fluid extraction is done by inserting the sample on the filter paper, then inserted in a reactor containing dry ice CO_2 and added with ethanol. they are grounded in a waterbath with a temperature of 31.1 °C and a pressure of 72.8 atm. The parameters used are variation of time and volume of ethanol. The extraction results were characterized

by TLC and FTIR. Amounts of curcumin were obtained by absorbance analysis using a UV-visible spectrophotometer.

Results snd Discussion

The process of extraction of temulawak simplicia was done by maceration method and also extraction of supercritical fluid. In the supercritical fuid extraction process was carried out with the sample inserted into the extraction reactor and mixed with the CO2 solvent. CO2 solvent was used because it has ideal pressure and temperature that was at temperature around 31,1 °C and pressure 72,8 atm, so it can withstand extracted components from thermal degradation (Harimurti,2010).

Supercritical CO_2 extraction fluid can be used to extract secondary metabolite compounds present in plants. In this research, extraction of curcumin compound from temulawak rhizome. Extraction of polar compounds was done by adding co-solvets that can extract polar compounds to improve the extraction process. Curcumin can be extracted using ethanol as a co-solvent. (Sidik, 1995). *Co-solvent* ethanol was used to increase the polarity of a supercritical CO2 solvent fluid so that the extraction of curcumin compounds can occur.

The content of curcumin with super critical fluid extraction can be identified with TLC and FTIR. The analysis with TLC was performed using an ethyl acetate eluent: n-hexane (1: 1). Fig. 1. is a chromatogram of TLC extract of ginger rhizomes after being exposed to UV light at a wavelength of 366 nm. TLC plates appear bluish-green and yellow spots appear, except for spots that can be observed in orange.

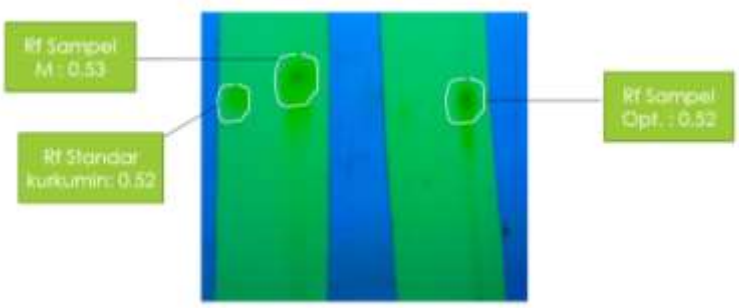

Fig. 1. TLC from Standard Curcumin, Maseration, and Supercritical Fluid Extraction.

In extracts of maceration and supercritical fluid extraction have adjacent Rf values, this indicates that the extract contains curcumin. Single spot on TLC indicates a good level of purity (Gritter, et al., 1991), which may mean that in the sample extract there is only curcumin. If it shows a lot of spots indicating that the presence of compounds other than curcumin extracted which can interfere with the results of purity levels at the level of curcumin.

The FTIR spectrophotometer works based on the interaction between samples by light (electromagnetic radiation) so that chemical bonds at certain wavelengths will absorb this ray and will vibrate, both vibration and bending vibration. The vibration is connected to a single bond or functional group of molecules for identification of unknown compounds. The wave number used to analyze the extract is at the wavelength of 4000-400 cm^{-1}. According to Colthup et al (1975), for the standard curcumin band consisting of the -OH functional group located at the wavelength number 3700-3100 cm^{-1}, the C-H is stretched at 3000-2700 cm-1, C = O at 1900-1550 cm^{-1} , C = C at 1700-1550 cm^{-1}, C-C at 1500-1430 cm^{-1}, C-O at 1300-1000 cm^{-1}, and CH bending at 880-750 cm^{-1}. FTIR results are shown in Table 1.

Curcumin can be measured using the UV-VIS spectrophotometer method with a maximum wavelength of 424 nm based on a wavelength scan on standard curcumin. Quantitative analysis is done by external standards by making calibration curves (Fig. 2) and line equations are used to determine the level of curcumin..

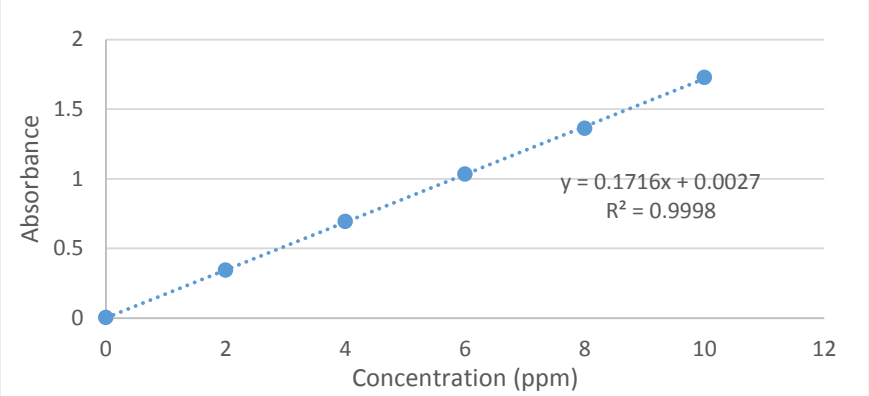

Fig. 2. Curcumin standard calibration curve

The extraction time variation was carried out to determine the length of extraction process required to obtain the highest concentration of a compound. The result of the time variation obtained in supercritical fluid extraction can be seen in Fig. 3a, which shows that the longer the extraction time the more extracted curcumin. The longer the extraction time the longer the contact between the solvent and the raw material so that the process of solvent penetration with the raw material will be better resulting in the increasing number of compounds that diffuse out of the cell. In addition, the use of supercritical CO_2 solvents that can provide a real enough pressure, because in a short time able to extract significant levels of curcumin. Levels of curcumin with super critical fluid extraction compared with maceration estraction. The result shows that extraction of super critical fluid only takes 30 minutes of extraction with curcumin level of 1,71% while maserasi extraction takes 3 × 24 hours of extraction with curcumin level of 1.67%..

Variations in ethanol volume were performed to determine the amount of ethanol as the co-solvent needed to extract curcumin and produce the highest levels. The results obtained can be seen in Fig 3b and it was found that with the increasing amount of ethanol used then also increases the level of curcumin produced. The more solvent then the breaking of walls and cell membranes due to the pressure difference between inside and outside the cell runs optimally so that the levels of curcumin will increasingly dissolve in the solvent. The result of extraction of super critical fluid compared with maceration extraction and produced that extraction of super critical fluid only require ethanol volume as much as 30mL with curcumin level equal to 2,29% while maceration require volume equal to 36 mL with level of 1,67%.

The results of measurements of maceration curcumin levels and supercritical fluid extraction were compared with graphs of Fig. 4 which show that the use of supercritical fluid extraction processes can obtain higher levels of curcumin from the maceration extraction process. From the visible graph, in the process of maseration that was silenced for 3x24 hours obtained curcumin level of 1.67%, and from the extraction process by using the optimal supercritical fluid on voume 30 mL with time 30 minutes obtained curcumin level of 2.29%. It is therefore

seen that the use of a supercritical fluid extraction process can increase the rate of curcumin in a relatively short time. This is also caused by the addition of CO_2 that gives the effect of depressuation, so as to eliminate solvent in the solute so that the time required in the extraction takes a long time.

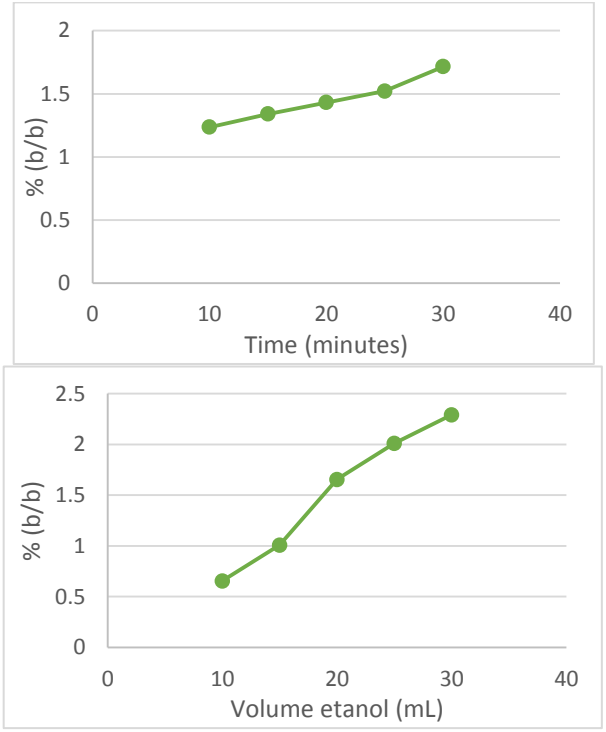

Fig. 3. (a) Graph of relation of curcumin level with time variation, (b) graph of relation of curcumin level with ethanol volume

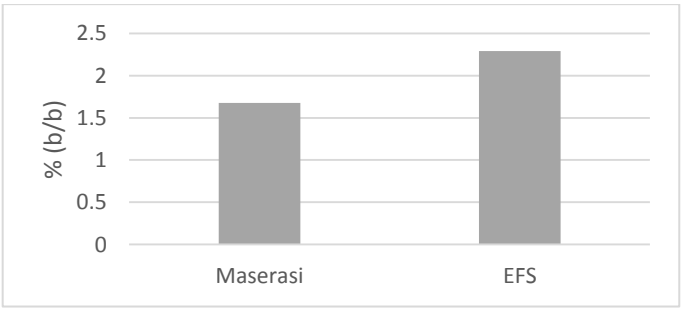

Fig. 4. Graph of Relationship Maseration with Supercritical Fluid Extraction

Conclusion

As the conclusion that extraction of super critical fluid with CO2 can be used for extraction of secondary metabolite compounds of ginger rhizome. The results were compared with the maceration method and it was found that super critical fluid extraction was an excellent method when used for the extraction process.

References

Capuzzo Andrea, Massimo E. Maffei, Andrea Occhipinti. (2013). *Supercritical Fluid Extraction of Plant Flavors Andfragrances.* Italy : University of Turin

Colthup NB, Daly LD, Wilbery SE. (1975). *Introduction to Infrared and Raman Spectroscopy. 2nd edition.*New york: Academic press

Gritter RJ, Bobbit JM, Schwarting AE. (1991). *Pengantar Kromatografi*. Ed ke-2. Padmawinata K, penerjemah. Bandung: ITB Pr. Terjemahan dari: *Introduction to Chromatography*.

Harimurti, N dan D. Sumangat. (2010). *Aplikasi fluida superkritis pada ekstraksiminyak astiri.*Balai Besar Penelitian dan Pengembangan Pascapanen Pertanian

Lang, Qingyang and Chien M. Wai. (2000). *Supercritical fluid extraction in herbal and natural product studies-a pratical review*. Department of chemistry, University of Idaho. ID 83844-2344, USA, 771-782

Schantz., M. Miller and S.N.,Chesler. (1986). Supercritical Fluid Extraction Procedure for the Removal Oftrace Organc Species from Solid Samples. Orgabic analitical research division, center for analitical chemistry. U.S.A. *Journal of Chromatography*.363,(1986)-397-401

Sidik, Moelyono MW dan Ahmad Muhtadi. 1995. *Temulawak (Curcumaxanthorrhiza).* Yayasan Pengembangan Obat Bahan Alam Phytomedica. Jakarta

Table 1. FTIR Measurement Results

Standard Curcumin + EtOH (cm^{-1})	Maseration (cm^{-1})	EFS (cm^{-1})	Explanation
3436	3328	3361	OH streching of phenol group,intra-molecular H bond
2931	2939	2918	Assymetric CH strech of CH_3
2851	2842	2853	CH strech of OCH_3
1622	1620	1620	C=O,C-C=C stretching
1610	1610	1610	Aromatic C=C stretching
1506	1512	1512	C=O streching, CCC, CC=O in plane bending
1426	1447	1447	In plane bending of aromatic (CCC, CCH), enolic COH, CH in plane bending due CH_2
1381	1372	1383	In plane bending of CH, enolic COH, skeletal CCC
1277	1275	1275	CH in plane bending of C=CH, aromatic CO streching
1128	1134	1112	In plane bending of aromatic CCH,skeletal CCH
1024	1026	1037	C-O-C streching, out of plane bending of CH_3, in plane bending of aromatic CCH
967	972	983	C=O streching, in plane bending of CCH
829	842	821	CH out of plane bending of armatic CCH and skeletal CCH
714	712	712	In plane bending of skeletal CCH and aromatic CCH, C=C streching