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Optimization of Ultrasound-Assisted Extraction of Alkaloids from *Acalypha indica*: Solvent and Extraction Time Variation

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Abstract. Alkaloids is one of the largest group of natural product which has an abundance of pharmacological activities. This study objected to optimize the extraction of alkaloids compound of *Acalypha indica* using the ultrasonic method with several parameters: solvents (ethanol, ethyl acetate, and methanol) and extraction time (10, 20, and 30 min). The crude extract was determined by profile separation, toxicity, and total alkaloids. Alkaloid isolate from TLC separation was characterized by FTIR. Preliminary phytochemical analysis, all crude extract exhibited the presence of alkaloids compounds. TLC separation showed a different number of alkaloid spots in each solvent (ethanol 3 spots, methanol 3 spots, and ethyl acetate 4 spots). Based on the highest total alkaloid contained, the best condition for ultrasound-assisted extraction method was using ethyl acetate for 20 min. The total alkaloid in optimized condition was 0.286 mg/g and its toxicity level was LC_{50} 35.3600 ppm. The specific functional groups of the purified extract were N-H, C-H, C=O, C=C, C-N, and –N-C=O.

INTRODUCTION

Acalypha indica is a common annual herb belong to Euphorbiaceae family, found mostly in tropic countries including Indonesia [1]. This plant has many pharmacological activities such as antidiarrheal, antidiabetic and antimalarial, and antibacterial agents [2-3]. The activities are related to bioactive compound such as alkaloids, flavonoids, triterpenoids, steroids, and saponins [4]. Alkaloids is one of the largest natural product with a highly diverse group of chemical entities and bioactivities [5].

The extraction of bioactive compound from plant materials using various solvent is an important step to the obtained phytochemical-rich product. The bioactive compound from various plants was commonly isolated by extraction methods such as soxhlet extraction and maceration. The conventional extractions were generally time-consuming and low efficiency. New extraction methods have been recently increased with shortened extraction time and more efficient [6].

Ultrasonic-assisted extraction (UAE) is recently has been widely used in plant material extraction. UAE is an ideal extraction method which is capable of producing high bioactive compounds quantities with shorter extraction time [6]. This method uses the ultrasonic wave to break up the cell wall and all the substances were soluble into the solvent [7]. The efficiency of UAE is affected by several factors such as type of solvent and extraction time [8].

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Previous researches showed solvent affects the activity of bioactive extraction. *A. indica* activity of ethanol, chloroform, and n-hexane extract produced 73.45, 149.37, and 57.09 ppm of LC50 value [9], while the activity of ethyl acetate, dichloromethane and petroleum ether extract produced 21.01, 17.65, and 11.85 ppm of LC50 [10]. There are some studies showed the various solvents were able to extract alkaloid in a certain concentration. The methanol extract of *Mentha longifolia* yielded 0.0081 mg/g [11], ethanol of *Dipsacus asperoides* yielded 0.059 mg/g [12], and ethyl acetate extract of *Hibiscus tillaceus* wood yielded 66.01 mg/g in a total of alkaloid content [13].

Another parameter in this study is the extraction time. Winata and Yunianta [14] studied the extraction of anthocyanin from mulberry leaves (*Morus alba*) and produced the highest yield of 45.26% for 30 min. According to Handayani *et al.* [15], the highest yield to extract soursop leaves by the ultrasonic method was 11.72% for 20 min. Sari *et al.* [16] stated the best extraction time to extract *Kappahycus alvarezzi* using methanol was 10 min. The researchers showed each plant material have their own maximal extraction time. This study attempts to optimize UAE of alkaloid form *A. indica* with parameter solvents and extraction time variation.

EXPERIMENTAL DETAILS

Sample Preparation

A. indica was collected from Singosari, Malang, Indonesia. The collected leaves were washed and dried at 60°C. The dried leaves were powdered and shifted with 60 mesh.

Ultrasonic Extraction

Powdered leaves were extracted by an ultrasonic method with various solvents (ethanol, ethyl acetate, and methanol) and extraction time (10, 20 and 30 min). One gram of sample powder was mixed with 10 mL solvents and the mixture was placed into the ultrasonic bath (Branson 3510-DT Ultrasonic Cleaner, USA) at 42 KHz frequency in room temperature. The ultrasonic extract was separated and the filtrate was concentrated. The crude extract was filtrate containing alkaloids [17].

Identification of Thin Layer Chromatography

Identification of *A. indica* crude extract was carried out on GF254 plate 4 x 10 cm in size. The plates were elucidated by a mixture of cyclohexane, toluene and diethylamine (75:15:10) in an unsaturated chamber prior to the application of the sample. The plates were sprayed and the spots were observed under UV at 366 nm of wavelength.

Determination of Total Alkaloids

About 10 mg of crude extract was dissolved in 2 mL hydrochloric acid 2 N and was added 2 mL sodium hydroxide 0.1 N. Three mL of the solution were transferred into a separatory funnel then the solution was added 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green (BCG) solution ($1x10^{-4}$ M). The mixture was extracted with 5 mL chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance was measured at a maximum wavelength and stabilized time. A standard curve was made of berberine chloride (0, 5, 10, 15, 20, 25, and 30 ppm) [18].

Toxicity Test using BSLT Method

Toxicity of extract was conducted by Meyer et al. [19] using Brine Shrimp Lethality Test (BSLT) and *Artemia salina* as an animal test. Ten mg of the extract was diluted with the solvents then the solution was added 10 µL DMSO, 5 µL bread yeast and seawater up to 1 mL of volume. The final concentration of the solution was 5, 10, 15, 20 and 25 ppm. Ten shrimp larvae were place into the vial and incubated for 24 h, hereafter, the larvae were observed the mortality. Seawater was used as a negative control, while DMSO was used as medium control. The procedure was performed five repetitions.

FTIR Identification

FTIR identification was carried out to alkaloid-contained filtrates with the best separation. The filtrates were concentrated by nitrogen gas. The concentrated extract was analyzed FTIR spectrophotometer at 400-4000 cm^{-1} of wavenumber.

RESULT AND DISCUSSION

Identification of Thin Layer Chromatography

TLC analysis result was assembled in Table 1. The result showed the variation of solvent affected the alkaloid extraction. It can be identified from the Rf (resolution factor) value. Especially for ethyl acetate extract, the number of the spot (4 spots) was more than other solvents (3 spots). Besides, the ethyl acetate extract had a better resolution. Otherwise, the extraction time insignificantly affected the extraction. Even there was a different Rf value or resolution but it was influenced by the environment condition such as interday analysis, humidity, uncontrolled temperature and light absorbed. The best resolution value that is greater than 1.25 [20]. Hence, it can be concluded that the best extraction time of UAE was ethanol extraction for 20 min, methanol extraction for 10 min, and ethyl acetate extraction for 20 min.

TABLE 1. Rf Value and Resolution of *A. indica* Crude Extract

Treatment	Spot number	Average Rf ± SD	Average Resolution ± SD
Ethanol 10 min	1	0.281 ± 0.000	1.23 ± 0.043 1.04 ± 0.039
	2	0.463 ± 0.000	
	3	0.600 ± 0.000	
Ethanol 20 min	1	0.269 ± 0.000	1.67 ± 0.163 1.40 ± 0.059
	2	0.444 ± 0.000	
	3	0.606 ± 0.000	
Ethanol 30 min	1	0,219 ± 0.000	1.20 ± 0.044 1.58 ± 0.062
	2	0,375 ± 0.000	
	3	0,506 ± 0.000	
Methanol 10 min	1	0.294 ± 0.000	1.56 ± 0.130 0.95 ± 0.068
	2	0.475 ± 0.000	
	3	0.600 ± 0.000	
Methanol 20 min	1	0.288 ± 0.000	1.25 ± 0.000 0.98 ± 0.032
	2	0.469 ± 0.000	
	3	0.613 ± 0.000	
Methanol 30 min	1	0.333 ± 0.010	1.18 ± 0.102 0.80 ± 0.047
	2	0.502 ± 0.020	
	3	0.615 ± 0.010	
Ethyl acetate 10 min	1	0.317 ± 0.004	1.15 ± 0.159 1.04 ± 0.081 0.60 ± 0.053
	2	0.488 ± 0.000	
	3	0.656 ± 0.000	
	4	0.763 ± 0.000	
Ethyl acetate 20 min	1	0.356 ± 0.000	1.25 ± 0.017 0.83 ± 0.025 0.66 ± 0.042
	2	0.563 ± 0.000	
	3	0.700 ± 0.000	
	4	0.813 ± 0.000	
Ethyl acetate 30 min	1	0.327 ± 0.003	1.26 ± 0.095 1.01 ± 0.082 0.72 ± 0.025
	2	0.508 ± 0.004	
	3	0.656 ± 0.000	
	4	0.769 ± 0.000	

Determination of Total Alkaloids

The total alkaloids identification was conducted at λ 609 nm and color complex formed between alkaloid and BCG was stable from 15-35 min after it was mixed. The total alkaloid of each variation was determined (Table 2). Statistical method Two Way ANOVA (95%) obtained the significance <0.05. The result showed that different solvent and extraction time affected towards total alkaloids concentration of anting-anting extract. The highest total alkaloid obtained by extracting using ethyl acetate for 20 min. Less than 20 min, the wall of the cell still upheld and alkaloid was not extracted completely. On the contrary, due to it heat irresistance, after 20 min, the number of total alkaloid was decrease. For instance, phenolic will be degraded due to the influence of the length of ultrasonic waves and rising of temperatures exposure, resulting in a reduction in total phenolic content which was extracted [21].

TABLE 2. Total alkaloids concentration content

Treatment	Average Total Alkaloids Concentration (mg/g) ± SD
Methanol 10 min	0.078 ± 0.0047 ^{c}
Methanol 20 min	0.045 ± 0.0017 ^{ab}
Methanol 30 min	0.066 ± 0.0016 ^{c}
Ethanol 10 min	0.044 ± 0.0031 ^{ab}
Ethanol 20 min	0.061 ± 0.0018 ^{bc}
Ethanol 30 min	0.040 ± 0.0015 ^{a}
Ethyl Acetate 10 min	0.193 ± 0.0022 ^{e}
Ethyl Acetate 20 min	0.286 ± 0.0009 ^{f}
Ethyl Acetate 30 min	0.134 ± 0.0017 ^{d}

Note: Notation of *a*, *b*, *c*, *ab* and *bc* showing no significant different bases on LSD test

Toxicity Test BLST Method

After examined the separation by using TLC method and measured the total alkaloid. This research compared the capability of extract from varied solvent and extraction time based on its toxicity test. After 5 replication and LSD test, the LC_{50} test was insignificantly different but in each solvent variation, in 30 min will show the highest LC_{50}. So, it was deducted because of the lower the total alkaloid the lower toxicity properties.

TABLE 3. Toxicity test using BSLT for extraction period differentiation

Treatment	LC_{50} (ppm) ± SD
Methanol 10 min	35.3660 ± 0^{a}
Methanol 20 min	33.6686 ± 0^{a}
Methanol 30 min	39.0629 ± 0^{b}
Ethanol 10 min	35.2547 ± 0^{a}
Ethanol 20 min	39.0629 ± 0^{a}
Ethanol 30 min	41.0490 ± 0^{b}
Ethyl Acetate 10 min	39.0629 ± 0^{a}
Ethyl Acetate 20 min	35.3660 ± 0^{a}
Ethyl Acetate 30 min	42.9557 ± 0^{b}

Note: 5 replications, the letter *a* and *b* showed least significanc (α<0,05)

Identification of Alkaloids Compound with FTIR

The FTIR identification was conducted to determine the functional group of alkaloid compound found in *A. indica*. The crude extract alkaloid spectra are shown in Figure 1. The interpretation of the presence of alkaloids showed specific functional groups such as NH, CH, C = O, C = C, CN, and -NC = O and based on the transmittance at 3400 cm^{-1} and 3500 cm^{-1} indicated an aromatic compound with a primary amine bond.

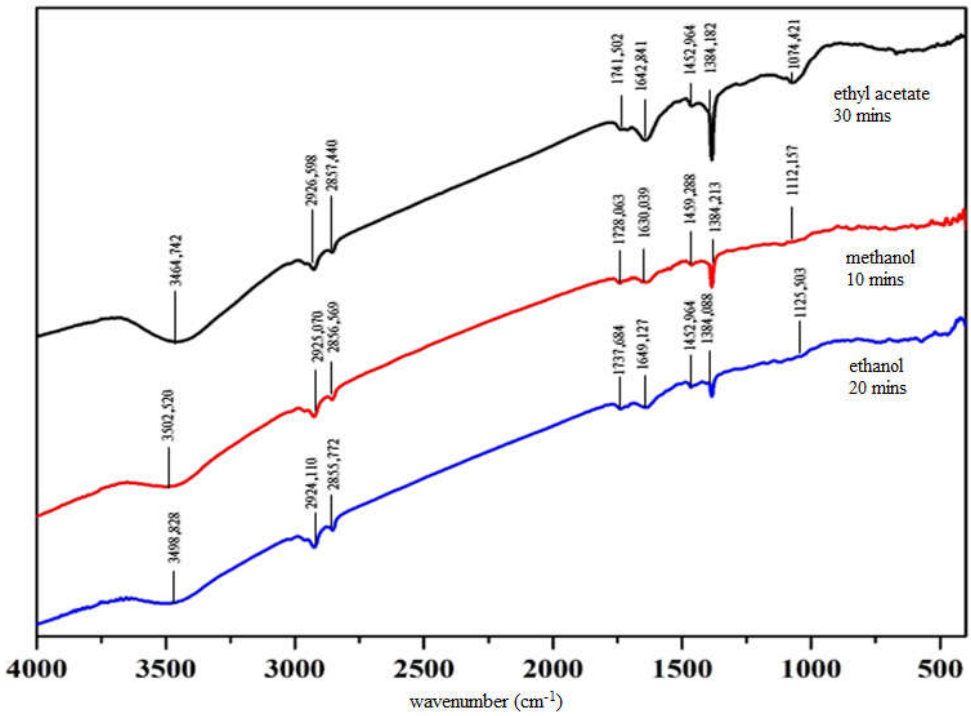

FIGURE 1. The IR Spectra of crude extract

SUMMARY

The toxicity test showed the whole variety of solvents and extraction period had a low LC_{50} value. The highest total alkaloid concentration which used ethyl acetate solvent for 20 min extraction was 0.286 mg/g. The TLC was obtained for ethanol formed 3 spots, methanol formed 3 spots, and ethyl acetate formed 4 spots, while the variation of time extraction did not affect the number of spots formed. The proper results of TLC and extraction periods were ethanol 20 min, methanol 10 min and ethyl acetate 20 min. The FTIR identification of the active compound showed that the proper result was using ethyl acetate for 20 min of extraction. The identification of the isolate alkaloids had primary amine which had property as aromatic with specific functional groups such as NH, CH, C = O, C = C, CN, and -NC = O.

REFERENCES

1. Tukiran, Suyatno and N. Hidayati, Jurnal FMIPA Jurusan Kimia UNESA, **2**(1), 1-6 (2014).
2. F. A. Carey, *Organic Chemistry,* 6th Ed. (McGraw Hill, New York, 2006).
3. M. Wink, *Ecological Roles of Alkaloids* (Wiley, Germany, 2008).
4. R. Ningrum, E. Purwanti and Sukarsono, Jurnal Pendidikan Biologi Indonesia **2**(3), 231-236 (2016).
5. N. Bribi, Asian J. Bot. **1**, 1-6 (2018).
6. J. P. Maran, S. Manikandan, C. V. Nivetha, and R. Dinesh, Arab J. Chem. **10**, S1145-S1157 (2017).
7. L. H. Thompson and L. K. Doraiswamy, Ind. Eng. Chem. Res. **38**(4), 1215–1249 (1999).
8. M. T. Savova, A. Kolusheva, Stourza and I. Seikova. JCTM **42**(3), 295-300 (2007).
9. E. K. Hayati, A. Jannah and R. Ningsih, Molekul **7**(1), 20-32 (2012).
10. I. Sriwahyuni, "Uji fitokimia ekstrak tanaman anting-anting (Acalypha indica L.) dengan variasi pelarut dan uji toksisitas dengan menggunakan brine shrimp" Bachelor Thesis, Universitas Islam Negeri Maulana Malik Ibrahim Malang, 2010.
11. A. Z. Adham, J Pharmacogn Phytochem **3**(6), 130-139 (2015).
12. J. Dai, H. Lin, S. Niu, X. Wu, Y. Wu and H. Zhang, Biomed. Res. 26(1), 37-42 (2015).
13. V. D. Tambe and R. S. Bhambar, J. Pharmacogn. Phytochem. **2**(4), 41-47, (2014).

14. E. W. Winata and Yunianta, JPA **3**(2),773-783, (2015).
15. H. Handayani, F. H. Sriherfyna, and Yunianta, JPA **4**(1), 262-272, (2016).
16. D. K. Sari, D. H. Wardhani, and A. Prasetyaningrum, Prosiding SNST ke-3, (2012).
17. A. Ardianti and K. Joni, JPA **2**(2), 28-35 (2014).
18. R. K. Patel, J. B. Patel, and P. D. Trivedi, J. Pharm. Pharm. Sci. **7**(10), (2015).
19. B. N. Meyer, N. R. Ferrigini, J. E. Putman, L. B. Jacbsen, D. E. Nicols, and J. L. McLaughlin, Planta Med. **45**(1), 31-34, (1982).
20. S. Wonorahardjo, *Metode - Metode Pemisahan Kimia*. (Indeks, Malang, 2013).
21. K. S. Denni, H. W. Dyah, and P. Aji, jtk **3**(19), 38-43, (2012).