Research Article

www.ijrap.net

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES IN EXTRACTS OF DIFFERENT RHIZOME PARTS FROM *CURCUMA AERUGINOSA* ROXB.

Nurcholis Waras^{1,2,3}, Khumaida Nurul^{2,3}*, Syukur Muhamad², Bintang Maria¹, Ardyani I.D.A.A.C¹

¹Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor-West Java, Indonesia

²Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor-West Java, Indonesia

³Laboratory of Biopharmaca Research Center, Bogor Agricultural University, Bogor-West Java, Indonesia

Received on: 27/06/15 Revised on: 28/07/15 Accepted on: 22/08/15

*Corresponding author

Dr. Ir. Nurul Khumaida, MSc, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti Kampus IPB, Bogor-West Java, 16680 – Indonesia E-mail: nkhumaida@yahoo.com

DOI: 10.7897/2277-4343.065118

ABSTRACT

Crude aqueous, 70% ethanol and 96% ethanol extracts from different part of rhizome of *Curcuma aeruginosa* Roxb., namely primary rhizome (PR), secondary rhizome (SR) and tertiary rhizome (TR) were screened for phytochemical compounds using qualitative methods, antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and cytotoxicity using brine shrimp lethality test (BSLT). The crude 70% ethanol extract of PR (IC₅₀, 437.07 µg/ ml) had stronger DPPH scavenging activity compared to the other crude extracts of different part of rhizomes of *C. aeruginosa*. None of the extracts from different rhizome parts of *C. aeruginosa* were found to be more active than standard (ascorbic acid, IC₅₀, 32.91 µg/ ml) for DPPH scavenging activity. In the BSLT, we found that the crude 96% ethanol and 70% ethanol extracts of PR exhibited most activity, which had LC₅₀ values of 90.40 and 265.17 µg/ml, respectively. Phytochemical screening revealed the presence of tannins and triterpenoids in all extracts, and saponins in the 70% ethanol and pure aqueous extracts, but alkaloids, flavonoids, and steroids were absent in all the extracts of different part of rhizome of *C. aeruginosa*.

Keywords: *Curcuma aeruginosa* RoxB., Temu hitam, Different parts of rhizome, Phytochemical, Antioxidant, Cytotoxic

INTRODUCTION

Curcuma aeruginosa Roxb. also known as “temu hitam” in Indonesia, is a perennial herbaceous plant from the family Zingiberaceae distributed throughout Indonesia. Traditionally, its rhizomes have been used medically to treat colic, obesity and rheumatism, asthma and coughs, scurvy and mental derangement¹. This plant has been reported to have various pharmacological activities like antioxidant², anti-inflammatory³, inhibitory of HIV-1⁴, anti-cancer⁵, and antifungal⁶. The *C. aeruginosa* rhizome has three levels of different parts, namely the primary rhizome (PR), the secondary rhizome (SR) and tertiary rhizome (TR). The PR and SR of *C. aeruginosa* were the most frequently utilized plant part for medicinal and pharmacological properties. Up to now, comparisons of pharmacological activities and phytochemical constituents among different parts of the rhizome of *C. aeruginosa* and effects of various solvents have not been reported. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) and BSLT (brine shrimp lethality test) are general bioassays for pharmacological activities of active phytochemical components of medicinal plants. The DPPH test is a well established assay for the *In vitro* determination of antioxidant activity in medicinal plant extracts⁷. BSLT is a general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts of medicinal plants⁸. Therefore, the objective of the present study was to assess crude extracts (aqueous, 70% ethanol

and 96% ethanol) from different part of rhizome of *C. aeruginosa* for phytochemical analysis and antioxidant and cytotoxic activities.

MATERIALS AND METHODS

Materials

The rhizomes of *C. aeruginosa* were collected from Cirebon, West Java, Indonesia, in September 2014. A voucher specimen (BMK0064032015) was deposited at Biopharmaca Research Center, Bogor Agricultural University, Indonesia. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma-Aldrich (St Louis, USA). The *Artemia salina* eggs was obtained from aquarium shops in Bogor, Indonesia, while, the analytical grade ethanol and DMSO for solvent purposes were obtained from Merck (Darmstadt, Germany).

Extraction of different parts of the rhizome

Fresh rhizomes of *C. aeruginosa* were immediately separated into three parts, namely primary rhizome (PR), secondary rhizome (SR) and tertiary rhizome (TR) (Figure 1A). The raw materials were washed with water, cut into small pieces and dried for 5 days in the sun (to give a moisture content of <10%). They were then ground in a grinder to give a powder (particle size, 100 mesh). Extraction of the powder (PR, SR, and TR) was carried out using a solvent maceration method with aqueous, 70%

ethanol, and 96% ethanol. The PR, SR and TR (25 g each) of *C. aeruginosa* were separately macerated (250 ml each) in a tightly closed round bottom flask at room temperature for a period of 24 h and then filtered with Whatman filter paper (type 4). The whole process was repeated once (1 x 24 h). The filtrate of the aqueous, the 70% ethanol, and the 96% ethanol of different parts of the rhizome were then concentrated under reduce pressure on a rotavapor (BUCHI, R-250, Switzerland) at $\pm 50^{\circ}\text{C}$. These extracts (aqueous extracts: 4.09 g (PR), 4.56 g (SR), 6.06 g (TR); 70% ethanol extracts: 1.31 g (PR), 2.02 g (SR), 3.33 g (TR); 96% ethanol extracts: 1.05 g (PR), 1.28 g (SR), 0.71 g (TR)) were then used to conduct the experiments reported here.

Phytochemical screening

The crude aqueous, 70% ethanol and 96% ethanol extracts of different part of rhizome (PR, SR, and TR) of *C. aeruginosa* were screened for alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids by standard phytochemical methods as described by Harborne⁹.

DPPH radical scavenging assay

The free radical scavenging activity of the crude aqueous, 70% ethanol and 96% ethanol extracts of different rhizome parts (PR, SR, TR) of *C. aeruginosa* was determined by DPPH assay method¹⁰, with modification. In a well of a 96-well plate, 50 μL of different concentrations of extracts (25-800 $\mu\text{g}/\text{ml}$) and ascorbic acid, as standard (0.25-20 $\mu\text{g}/\text{ml}$), was added to 150 μL of ethanolic DPPH solution (300 μM) and then incubated in the dark for 30 min at 37°C . The decrease in absorbance was determined at 515 nm using a microplate reader (Epoch BioTek, USA). The percentage was calculated as follows: % inhibition = $((A_c - A_{cb}) - (A_s - A_{sb})) / (A_c - A_{cb}) \times 100$. Where A_c is the absorbance of DMSO plus DPPH (in ethanol), A_{cb} is the absorbance of the blank (DMSO plus ethanol without DPPH), A_s is the absorbance of the sample plus DPPH (in ethanol), and A_{sb} is the absorbance of the sample plus ethanol without DPPH. All experiments were conducted in triplicate ($n = 3$). IC_{50} values were derived from the inhibition curves obtained.

Cytotoxic activity by BSLT

The brine shrimp lethality test (BSLT) was used to explore the potential biochemical activity of the extracts of different parts of the rhizome (PR, SR, TR) of *C. aeruginosa*¹¹. Briefly, 2 x 24 h were given to hatch the shrimp larvae in seawater and to mature as nauplii. The test extracts were prepared by dissolving them in dimethyl sulfoxide (DMSO) added to the seawater to make final concentration of 10, 50, 100, 500, and 1000 $\mu\text{g}/\text{mL}$. A vial containing DMSO was used as the control. The nauplii of *A. salina* ($n = 10$) were applied to each experimental and control vials. After 24 hours of exposure, the dead nauplii were counted as percent of mortality. From these data, the percent of lethality of the brine shrimp nauplii and the concentration variations of

extracts were plotted on a graph, and LC_{50} values was calculated.

Statistical analysis

The experimental results were expressed as the mean $\pm$ standard deviation (SD). Analysis of variance was determined by completely random design using Statistical Tool for Agricultural Research (STAR) 2.0.1 program. Significant differences between the means were calculated according to Tukeys's multiple range tests. Differences at $p < 0.05$ were considered statistically significant.

RESULTS

Phytochemical screening

The phytochemical screening of crude aqueous, 70% ethanol and 96% ethanol extracts of different rhizome parts (PR, SR, TR) of *C. aeruginosa* revealed the presence of some secondary metabolites such as tannins and triterpenoids in all extracts in all parts of the rhizome as shown in Table 1. Saponins were detected in the 70% ethanol and the aqueous extracts, but not detected in the 96% ethanol extracts in all different parts of the rhizome. Alkaloids, flavonoids, and steroids were absent in all the extracts from different parts of the rhizome of *C. aeruginosa*.

DPPH radical scavenging assay

The result for the DPPH free radical scavenging effect of the crude aqueous, the 70% ethanol and the 96% ethanol extracts of different rhizome parts (PR, SR, TR) of *C. aeruginosa* are shown in Figure 1 and Table 2. The percentage of scavenging of DPPH free radicals was found to rise with increasing concentrations of the different parts of the rhizome. Extracts with the highest scavenging were the 70% ethanol ones of the primary rhizome of *C. aeruginosa* (Figure 1B). The IC_{50} values of different extracts of different parts of the rhizome of *C. aeruginosa* are shown in Table 2 and compared with those of the standard antioxidant ascorbic acid. The crude 70% ethanol extract of PR (IC_{50} , 437.07 $\mu\text{g}/\text{ml}$) had better DPPH scavenging activity compared to the other crude extracts of different rhizome parts. None of the extracts of *C. aeruginosa* were found to be more active than the standard (ascorbic acid, IC_{50} , 32.91 $\mu\text{g}/\text{ml}$) for DPPH scavenging activity.

Cytotoxic activity by BSLT

The results of BSLT of the crude aqueous, the 70% ethanol and the 96% ethanol extracts of different parts of the rhizome (PR, SR, TR) of *C. aeruginosa* (% mortality at different concentrations and LC_{50} values) are shown in Table 3. The percentage mortality increased with an increase in extracts concentration. In the BSLT for LC_{50} values, we found that the crude 96% ethanol and 70% ethanol extracts of PR exhibited most active, which gave LC_{50} values of 90.40 and 265.17 $\mu\text{g}/\text{ml}$, respectively.

Table 1: Results of phytochemical screening of the different extracts of different parts of the rhizome of *C. aeruginosa*

Phytochemical	Result (Extracts: different part of rhizome)								
	Aqueous			70% Ethanol			96% Ethanol		
	PR	SR	TR	PR	SR	TR	PR	SR	TR
Alkaloids	-	-	-	-	-	-	-	-	-
Dragendroff's test	-	-	-	-	-	-	-	-	-
Mayer's test	-	-	-	-	-	-	-	-	-
Wagner's test	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-
Tanins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	-	-	-
Triterpenoids	+	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-

The different part of rhizome: PR = primary rhizome; SR = secondary rhizome; TR = tertiary rhizome. + = showed positive result; and - = showed negative result.

Table 2: IC₅₀ values of the crude aqueous, 70% ethanol and 96% ethanol extracts from different part of rhizome of *C.aeruginosa*

Extracts	IC ₅₀ *, µg/ mL							
	Different rhizome parts						Standard	
	Primary rhizome		Secondary rhizome		Tertiary rhizome		Ascorbic acid	
Aqueous	1141.45 ±	218.83 ^a	898.14 ±	63.64 ^{abc}	1061.42 ±	72.89 ^{ab}	32.91 ±	1.62 ^e
70% Ethanol	437.07 ±	36.35 ^d	791.19 ±	63.19 ^{bc}	986.59 ±	98.74 ^{abc}		
96% Ethanol	681.39 ±	16.17 ^{cd}	849.36 ±	31.58 ^{abc}	1002.45 ±	266.22 ^{ab}		

*Data are expressed as the mean of triplicates ± SD. Means with different superscript indicates significant differences (p < 0.05) among different extract samples and standard.

Table 3: Results of brine shrimp lethality on crude aqueous, 70% ethanol and 96% ethanol extracts from different parts of the rhizome of *C.aeruginosa*

Extracts		% Mortality at different concentration*														LC ₅₀ ^a , µg/ mL			
		10 µg/ mL			50 µg/ mL			100 µg/ mL			500 µg/ mL			1000 µg/ mL					
Aqueous	PR	30.0	±	0.0	33.3	±	5.8	40.0	±	0.0	53.3	±	5.8	56.7	±	5.8	387.1 ^{abc}	±	58.0
	SR	20.0	±	10.0	16.7	±	5.8	30.0	±	10.0	46.7	±	5.8	53.3	±	5.8	1131.1 ^{ab}	±	639.4
	TR	13.3	±	5.8	16.7	±	5.8	33.3	±	5.8	43.3	±	5.8	50.0	±	10.0	1380.4 ^a	±	812.2
70% Ethanol	PR	16.7	±	5.8	30.0	±	0.0	40.0	±	0.0	83.3	±	5.8	93.3	±	5.8	103.6 ^{bc}	±	6.5
	SR	13.3	±	5.8	26.7	±	5.8	33.3	±	5.8	56.7	±	5.8	73.3	±	5.8	249.4 ^{bc}	±	35.2
	TR	16.7	±	5.8	26.7	±	5.8	33.3	±	5.8	53.3	±	5.8	63.3	±	5.8	361.9 ^{abc}	±	26.0
96% Ethanol	PR	26.7	±	5.8	40.0	±	10.0	63.3	±	11.5	90.0	±	10.0	100.0	±	0.0	53.5 ^c	±	21.8
	SR	10.0	±	0.0	23.3	±	5.8	20.0	±	0.0	36.7	±	5.8	76.7	±	5.8	510.6 ^{abc}	±	228.0
	TR	10.0	±	0.0	23.3	±	5.8	26.7	±	5.8	46.7	±	11.5	63.3	±	5.8	510.6 ^{abc}	±	228.0

The different parts of rhizome: PR = primary rhizome; SR = secondary rhizome; TR = tertiary rhizome. *Data are expressed as the mean of triplicates ± SD. In LC₅₀ values, different superscript indicates significant differences (p < 0.05) among different extract samples.

Figure 1: (A) The different part of rhizome of *C.aeruginosa*, namely primary rhizome (PR), secondary rhizome (SR), and tertiary rhizome (TR); (B) DPPH radical scavenging activity of crude aqueous, 70% ethanol (EtOH) and 96% ethanol (EtOH) extracts from different parts of rhizome of *C.aeruginosa*.

DISCUSSION

The antioxidant (DPPH radical scavenging) and cytotoxic activities are associated with phytochemical compounds. Our results indicates that a 70% ethanol extract of primary rhizome of *C.aeruginosa* was the most active as a radical scavenger than other extracts of different rhizome parts. However when compared to our positive control (ascorbic acid) it showed very weak activity (significant at $P < 0.05$). For the cytotoxic activity according to Meyer *et al.*¹¹, when assessing the toxicity of plant extracts with BSLT, a LC_{50} value of less than 1000 $\mu\text{g/ml}$ is considered to be bioactive. Therefore, we found that the samples analyzed in this study, were most active for biochemical and pharmacological activities, except for our aqueous extracts of SR and TR ($LC_{50} > 1000 \mu\text{g/ml}$) of *C. aeruginosa*. The crude 96% ethanol and the 70% ethanol extracts of PR showed good activity in the BSLT, which had LC_{50} values of 90.40 and 265.17 $\mu\text{g/ml}$, respectively. Preliminary phytochemical screening of the different extracts of different rhizome parts from *C. aeruginosa* revealed the presence of tannins, triterpenoids, and saponins. The phytochemical compounds detected are known to have medicinal importance for antioxidant and cytotoxic activities. Tannins, naturally occurring plant polyphenols, have been reported to have multiple biochemical and pharmacological effects, including as antioxidant^{12, 13}, and cytotoxic activity as antitumor¹⁴. Triterpenoids and saponins are also reported to show antioxidant and cytotoxic activities^{15, 16}.

CONCLUSION

Based on the results of the present study, it can be concluded that the 70% ethanol extracts of primary rhizome of *C. aeruginosa* have antioxidant and cytotoxic activities. The 70% ethanol extracts of primary rhizome of *C. aeruginosa* contains secondary metabolites such as tannins, triterpenoids, and saponins.

ACKNOWLEDGMENT

The authors acknowledge the excellent technical support received from Prof. Latifah K Darusman and Dr. Laksmi Ambarsari. Deep thanks to The Indonesian Directorate General of Higher Education (DIKTI) for the financial support via a doctoral scholarship program and research grants (Penelitian Unggulan Perguruan Tinggi BOPN IPB: 082/SP2H/PL/DIT.LITABMAS/II/2015).

REFERENCES

1. Perry LM. Medicinal plants of east and southeast asia: attributed properties and uses. Massachusetts (USA): The MIT Press; 1980.
2. Nurcholis W, Priosoeryanto BP, Purwakusumah ED, Katayama T, Suzuki T. Antioxidant, cytotoxic activities and total phenolic content of four Indonesian medicinal plants. Valensi 2012; 2: 501-510.
3. Reanmongkol W, Subhadhirasakul S, Khaisombat N, Fuengnawakit P, Jantasila S, Khamjun A. Investigation the antinociceptive, antipyretic and anti-inflammatory activities of *Curcuma aeruginosa* Roxb. extracts in experimental animals. Songklanakarin J Sci Technol 2006; 28:999-1008.
4. Otake T, Mori H, Morimoto M, Ueba N, Sutardjo S, Kusumoto IT, et al. Screening of Indonesian plant extracts for antihuman immunodeficiency virus-type 1 (HIV-1) activity. Phytother Res 1995; 9: 6-10. <http://dx.doi.org/10.1002/ptr.2650090103>
5. Jantan I, Rafi IAA, Jalil J. Platelet activating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants. Phytomed 2005; 12: 88-92. <http://dx.doi.org/10.1016/j.phymed.2003.06.006>
6. Banerjee A, Nigam SS. Antifungal activities of the essential oil of *Curcuma caesia* Roxb. Indian J Med Res 1976; 64:1318-1321.
7. Nicklisch SCT, Waite JH. Optimized DPPH assay in a detergent-based buffer system for measuring antioxidant activity of proteins. MethodsX 2014; 1: 233-238. <http://dx.doi.org/10.1016/j.mex.2014.10.004>
8. Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. J Med Plant Res 2010; 4: 407-414.
9. Harbone JB. Phytochemical methods. London: Chapman and Hall; 1998.
10. Sivasothy Y, Sulaiman SF, Ooi KL, Ibrahim H, Awang K. Antioxidant and antibacterial activities of flavonoids and curcuminoids from *Zingiber spectabile* Griff. Food Control 2013; 30:714-720. <http://dx.doi.org/10.1016/j.foodcont.2012.09.012>
11. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-34. <http://dx.doi.org/10.1055/s-2007-971236>
12. Wojdylo A, Oszmian'ski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 2007; 105: 940-949. <http://dx.doi.org/10.1016/j.foodchem.2007.04.038>
13. Gülçin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arabian J Chem 2010; 3: 43-53. <http://dx.doi.org/10.1016/j.arabjc.2009.12.008>
14. Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, et al. Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines--a possible mechanism. Phytomed 2000; 7: 39-47. [http://dx.doi.org/10.1016/S0944-7113\(00\)80020-3](http://dx.doi.org/10.1016/S0944-7113(00)80020-3)
15. Tapondjou LA, Nyaa LB, Tane P, Ricciutelli M, Quassinti L, Bramucci M, et al. 2011. Cytotoxic and antioxidant triterpene saponins from *Butyrospermum parkii* (Sapotaceae). Carbohydr Res 2011; 346:2699-704. <http://dx.doi.org/10.1016/j.carres.2011.09.014>
16. Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, et al. 2010. Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. Phytochem 2010; 71: 2108-15. <http://dx.doi.org/10.1016/j.phytochem.2010.08.020>

Cite this article as:

Nurcholis Waras, Khumaida Nurul, Syukur Muhamad, Bintang Maria, Ardyani I.D.A.A.C. Phytochemical screening, antioxidant and cytotoxic activities in extracts of different rhizome parts from *Curcuma aeruginosa* Roxb. Int. J. Res. Ayurveda Pharm. 2015;6(5):634-637 <http://dx.doi.org/10.7897/2277-4343.065118>

Source of support: Indonesian Directorate General of Higher Education, Conflict of interest: None Declared

Disclaimer: IJRAP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJRAP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IJRAP editor or editorial board members.