www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.3, pp 1064-1069, July-Aug 2014

Cytotoxic and Antioxidant activity of Petroleum Extract of Andaliman Fruits (*Zanthoxylum acanthopodium* DC.)

Ruth Elenora Kristanty[1*], Junie Suriawati[2].

[1,2] Department of Pharmacy and Food Analysis, Health Polytechnic Health Ministry Jakarta II, Jakarta 12540.

***Corres. author: ruth.elenora@yahoo.com**
Fax: (+6221-78831642).

Abstract: Cancer is a growing health problem coming next to the stress oxidative diseases. The objectives of this study were to investigate potential *in vitro* cytotoxic effect on breast cancer cell line T47D and antioxidant activity of the petroleum extract of *Zanthoxylum acanthopodium* (Andaliman) fruits. Andaliman is a wild spices well known in North Sumatera, Indonesia for Batak's traditional cuisine. The activity of this extract to inhibit the growth of breast cancer T47D cells was determined by MTT assay with doxorubicin HCl as standard. Free radical scavenging potential was determined by *in vitro* evaluation against DPPH and xanthine oxidase. The results revealed that petroleum extract of andaliman fruits was active as xanthine oxidase inhibitor with IC_{50} 9,9 µg/mL and possessed cytotoxic activity on T47D cell line with IC_{50} 149,4 µg/mL.
Keywords: Antioxidant, cytotoxic, xanthine oxidase, *Zanthoxylum acanthopodium* DC.

Introduction

Free radicals can start chain reactions that damage cells which ultimately lead to pathogenesis of many human diseases such as cancer. Cancer is the leading cause of death worldwide. Finding potential natural products as a cure for this disease is always an important objective for human health benefits[1]. Inhibition of free radical formation via the mechanism of xanthine oxidase inhibition can reduce the amount of free radicals and protect the body from tissue damage[2].

Antioxidant activity of *Zanthoxylum* species, mainly extracts, have been studied from fruits and seeds. *Zanthoxylum* species are also potential sources for finding new antitumor agents, because diverse substances obtained from some of this species have showed strong citotoxic activity against different tumor cell lines[3].

Andaliman fruits (*Zanthoxylum acanthopodium* DC.), known as the spice in Batak society to eliminate the smell of fish and raw meat, had been reported to have anti-inflammatory activity[4] and antioxidant activity[5]. The essence of the fruits comes from essential oils which are mostly composed of terpenoids and the composition varies between species. Essential oils of Zanthoxylum exhibit a strong inhibition of the growth of fungal mycelium and has *in vitro* and *in vivo* antitumor activity[3]. These activities were thought to be related to its activity as an antioxidant by inhibiting the formation of oxygen free radicals (ROS) that can damage cells and initiate cancer[6]. The types of cell line observed in this test was the T47D breast cancer cells.

Materials and Methods

Materials

Petroleum ether technical (Brataco Chemika, Indonesia) that had been distilled, methanol p.a (Merck, Germany), demineralized distilled water (Brataco Chemika, Indonesia), dimethyl sulfoxide (Merck, Germany),

potassium dihydrogen orthophosphoric (Merck, Germany), hydrochloric acid (Merck, Germany), sodium hydroxide (Univar, USA), Allopurinol (Pyridam Farma, Indonesia), Doxorubicin Hydrochloride (Sigma Aldrich, Singapore), DPPH (Sigma Aldrich, Singapore), BHT (Sigma Aldrich, Singapore), quercetin (Sigma Aldrich, Singapore), Xantin (Sigma Aldrich, Singapore), xanthine oxidase (Sigma Aldrich, Singapore), T47D cell lines (BPPT, Indonesia), MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Invitrogen, USA), dimethyl sulfoxide/DMSO (Biobasic, Canada), and T47D cell lines (ATCC, USA), micro 96 well plates (Nunc, Denmark).

This research was conducted at Phytochemistry Laboratory of University of Indonesia for extract preparation and antioxidant activity assay, meanwhile *in vitro* cytotoxic assay was studied in the Cell Culture Laboratory at BPPT Serpong.

Preparation of Extract

Fresh fruits of *Zanthoxylum acanthopodium* were collected from Dairi, North Sumatera, Indonesia and determined at Indonesian Institute of Sciences, Biological Research Center, Cibinong. The green fruits were sorted, washed, and dried in a cabinet dryer at 40º C, then homogenized to fine powder and stored in airtight bottles.

The powdered dried fruits were extracted by stirring at room temperature for 6 hours with petroleum ether. Mixture was allowed to stand 24 hours, filtered, and concentrated by using vacum evaporator at 50-60° C to yield a crude extract and then weighed.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay[7]

The mixture contained 1,0 mL of sample at concentration ranging from 10, 20, 50, 100, and 200 ppm, and 1.0 mL of DPPH solution of 100 ppm and 2.0 mL of methanol p.a then homogenized. Absorbance of the solution was measured at 517 nm after incubation for 30 minutes at 37°C in the dark tube. Measurements were carried out in duplicate. The same procedure was also done to BHT as positive controls at concentrations of 1, 2, 4, 10, and 16 ppm. The percentage of the DPPH inhibition can be calculated by the formula:

$$Q = \frac{A_0 - A_1}{A_0} x\ 100\%$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample. The IC_{50} values were calculated by using inhibition curve in liniear equation.

Xanthine Oxidase Assay[8,9]

Measurements were carried out in duplicate.

Solution prepared for the assay included solution of 0.15 mM xanthine as a substrate, a solution of enzyme (xanthine oxidase) 0.1 unit/mL, and test solutions. Test solution was obtained by weighing 10 mg of samples, dissolved in DMSO and phosphate buffer and then diluted with phosphate buffer to obtain a final concentration of sample solution at 100, 50, 20, 10, 5 and 1 ppm.

Each 1.0 mL sample was put in a separate reaction tubes and added with 2.9 mL phosphate buffer solution and 2.0 ml of xanthine and then praincubated at a temperature of 30° C for 10 minutes. Xanthine oxidase 0.1 units / mL total of 0.1 mL was added and incubated at a temperature of 30° C for 30 minutes. After the incubation period, 1.0 mL hydrochloric acid 1N was added immediately into the mixture to stop the reaction and homogenized. Measurement was carried out spectrophotometrically at 284 nm to see the formation of uric acid that occurs in the test solution and then determined percentage of the tested barriers against xanthine oxidase. Assay was carried out on the blank solution, controls of blank, and controls of samples.

The percentage of xanthine oxidase barriers (XO) was calculated by the formula[8]:

$$\%\ \text{inhibition} = 1 - \frac{B}{A} x100\%$$

where IC_{50} values were obtained by linear regression analysis[10].

***In vitro* cytotoxicity assay**[11]

T47D cells were seeded into 96-well plates at a density of $5x10^4$ cells/well and left to attach to the plates for 48 hr. Different concentrations of petroleum extract (100µL) were added to the T47D cancer cells,seeded in 96-well microtiter & incubated at 37º C for 24 hours. At the end of the treatment, 100 µl of MTT [(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well & the microtiter plate were incubated for 4hrs at 37º C. Finally, SDS (100 µl) was added to each well, after which optical absorbance was read at 570nm on ELISA microplate reader.

Phytocemical Screening[12]

Phytochemical screening with some chemical reagents for alkaloids, flavonoids, triterpenoids/steroids, glycosides, saponins, and tannins has been done on petroleum extract.

Results and Discussion

The results of antioxidant assay by using DPPH showed IC_{50} values of petroleum extract was 220 ug / mL (Table 1). Extract with IC_{50} values between 100-250 µg/mL has weak antioxidant activity[13]. It was expected that free radicals can be suppressed by antioxidant compounds with smallest concentration. In this test, two compounds were used as standard, quercetin as natural antioxidant with IC_{50} value of 2,43 µg/mL and BHT as antioxidants synthetic with IC_{50} values of 5,5 µg/mL which indicates that both standard compounds had powerful antioxidant activity (<10µg/mL).

Table 1. Antioxidant Measurement of Sample and Standards

Sample	Concentration (µg/mL)	% inhibition	IC_{50} (µg/mL)
Petroleum Extract	200	14,92	220,67
	100	14,61	
	50	8,19	
	20	6,83	
	10	6,29	
BHT	16	37.09	5.52
	10	26.65	
	4	15.22	
	2	9.23	
	1	6.87	
Quercetin	16	69.15	2.43
	10	50.33	
	4	33.17	
	2	22.75	
	1	19.80	

Inhibition of xanthine oxidase activity measurements performed at the optimum conditions at 30º C, 0.15 mM substrate concentration, pH 7.8 and a maximum wavelength of 284 nm. The results showed that the petroleum extract had strong inhibitory activity on xanthine oxidase with IC_{50} 9.9 µg/mL and Allopurinol had IC_{50} 0.02 µg/mL (Table 2). The cytotoxic effect of plant extract in comparison to doxorubicin on breast carcinoma T47D cell line was determined by MTT method and produced a concentration and inhibition percentage of the cell growth (Figures 1-2; Table 3-4). Results showed that the extract had IC_{50} 149.4 µg/mL, while Doxorubicin HCl had IC_{50} value of 0,103 µg/mL and Gallic Acid 1,26. Our phytochemical screening revealed the presence of terpenoid in the petroleum extract of *Zanthoxylum acanthopodium* DC, which could be responsible for these activities (Table 5).

Table 2. Xanthine Oxidase Inhibition by Samples dan Allopurinol

Sample	Concentration (µg/mL)	% inhibition	IC_{50} (µg/mL)
Petroleum extract	1	31,1	9,9
	5	37,8	
	10	53,6	
	20	57,1	
	50	48,5	
	100	49,9	
Allopurinol	0,1	45,11	0,02
	0,25	55,42	
	0,5	74,6	
	1	87,56	

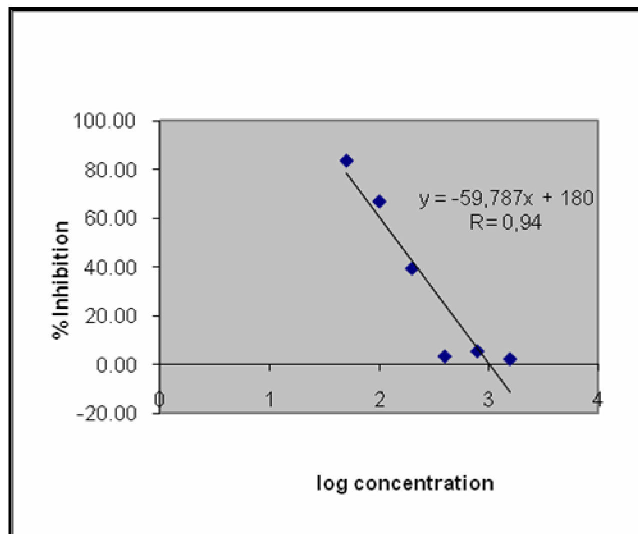

Figure 1. Relationship Between Log Concentration of Extract on Inhibition Percentage of T47D Cells

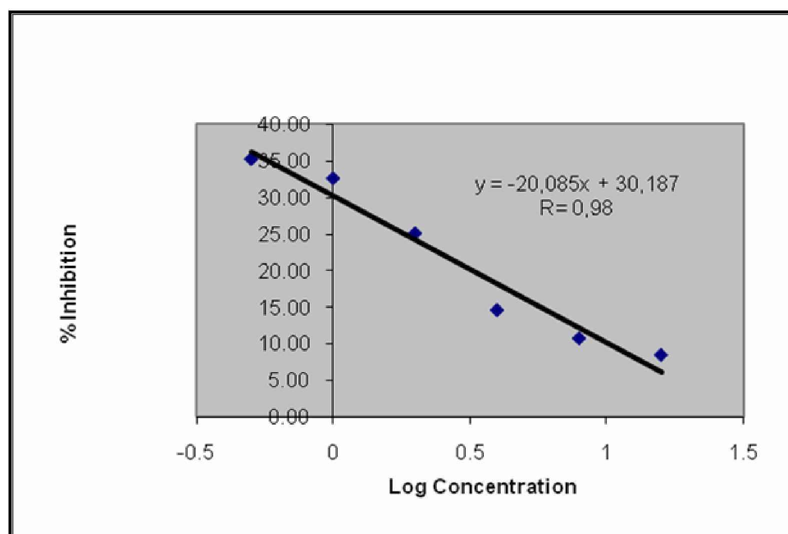

Figure 2. Relationship Between Log Concentration of Doxorubicin HCl on Inhibition Percentage of T47D Cells

Table 3. Absorbance of Samples

Concentration (µg/mL)	Absorbance
50	0,551
100	0,462
200	0,315
400	0,123
800	0,134
1600	0,117

Table 4. Absorbance of Doxorubicin HCl

Concentration (µg/mL)	Absorbance
0,5	0,301
1	0,286
2	0,243
4	0,183
8	0,161
16	0,148

Table 5. Phytocemical Screening of Petroleum Extract of *Zanthoxylum acanthopodium* Fruits

No.	Chemical Groups	Results
1.	Alkaloid	-
2.	Flavonoid	-
3.	Glikosida	-
4.	Antrakuinon	-
5.	Steroid/triterpenoid	+
6.	Saponin	-
7.	Polifenol	-

Conclusion

Petroleum extract of andaliman fruits was weak as antioxidant but potentially has cytotoxic activity on T47D cell line and active as xanthine oxidase inhibitor.

Acknowledgement

This study was carried out in Faculty of Pharmacy, University of Indonesia, and Cell Culture Laboratory of BPPT Serpong.

References

1. Chou Z-T., Chan H-H., Peng H-Y., Liou M-J. and Wu, T-S., Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of *Zanthoxylum ailanthoides* Sieb. & Zucc, Phytomedicine., 2011, 18, 344-348.
2. Lin C.N., Huang A.M., Lin K.W., Hour T.C., Ko H.H., Yang S.C. and Pu Y.S., Xanthine oxidase inhibitory terpenoids of *Amentotaxus formosana* protect cisplatin-induced cell death by reducing reactive oxygen species (ROS) in normal human urothelial and bladder cancer cells, Phytochem., 2010, 71(17–18), 2140–2146.
3. Negi J.S, Bish V.K., Bhandari A.K., Singh P. and Sundriyah R.C., Chemical constituents and biological activities of the genus Zanthoxylum*:* A review, African J. of Pure and Appl. Chem., 2011, 5,12, 412-416.
4. Yanti., Pramudito T.E., Nuriasari N. and Juliana, K., Lemon pepper fruit extract (*Zanthoxylum acanthopodium* DC.) suppresses the expression of inflammatory mediators in lipopolysaccharide-induced macrophages in vitro, American J. of Biochem. and Biotec., 2011, 7,4, 176-186.
5. Suryanto E., Sastrohamidjojo H., Raharjo S. and Tranggono., Antiradical activity of andaliman (*Zanthoxylum acanthopodium* DC.) fruit extract, Indonesian Food and Nutrition Progress., 2004, 2,1, 15-19.
6. Ding H., Chin YW Kinghorn A.D. and D'Ambrosio S.M., Chemopreventive characteristics of avocado fruit, Seminars in Cancer Biology., 2007, 17, 386–394.
7. Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181,1199-1200.
8. Owen P. and Johns T., Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout, J. of Ethnopharm., 1999, 64, 149-160.

9. Tamta H., Sukirti K. and Anup KM., Biochemical characterization of some pyrazolopyrimidinebased inhibitors of xanthine oxidase, Biochem., 2006, 71,1, S49-S54.
10. Apaya K.L. and Chichioco-Hernandez C.L., Xanthine oxidase inhibition of selected Philippine medicinal plants, J. of Medicinal Plants Research., 2011, 5,2, 289-292.
11. Cancer Chemoprevention Research Center Fakultas Farmasi UGM., Prosedur Tetap Uji Sitotoksik dengan Metode MTT, 2012, Jogjakarta.
12. Tiwari P., Kumar B., Kaur M., Kaur G. and Kaur H., Phytochemical screening and Extraction: A Review, Internationale Pharmaceuticasciencia., 2011, 1,1.
13. Phongpaichit S., Nikom J., Rungjindamai N., Sakayaroj J., Hutadilok- Towatana N., Rukachaisirikul V. andKirtikara, K., Biological activities of extracts from endophytic fungi isolated from garcinia plants, FEMS Immunology and Medical Microbiology., 2007, 51, 517-525.

*****