The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 29

1-1-2013

CYTOTOXIC EVALUATION OF A FRUIT EXTRACT FROM ZANTHOXYLUM LIMONELLA IN HUMAN DERMAL FIBROBLAST CELL LINE USING MTT ASSAY

Sarunva Laovitthayanggoon.

Buppachart Potduang

Natthachest Ketmanee

Chuleratana Banchonglikitkul

CYTOTOXIC EVALUATION OF A FRUIT EXTRACT FROM *ZANTHOXYLUM LIMONELLA* IN HUMAN DERMAL FIBROBLAST CELL LINE USING MTT ASSAY

Sarunya Laovitthayanggoon[1], Buppachart Potduang[1*], Natthachest Ketmanee[1] and Chuleratana Banchonglikitkul[1]

[1]*Pharmaceuticals and Natural Products Department, Thailand Institute of Scientific and Technological Research, Technopolis, 35 Mu 3, Thanon Liab Klong 5, Klong Luang, Pathumthani 12120, Thailand. E-mail: Sarunya@tistr.or.th*

KEYWORDS: *Zanthoxylum limonella*, Cytotoxicity, Human dermal fibroblast, MTT assay

INTRODUCTION

Zanthoxylum limonella Alston (RUTACEAE), locally called Ma-khwaen, is a deciduous tree up to 18 m tall. It is widely distributed in the northern part of Thailand and has been used in folk medicines for different medical purposes. Dry fruits are sold in local markets and traditionally used as spice. The bark, root-bark and fruit contained highly effective antibacterial substances. The essential oil from *Z. limonella* fruits exhibits the anti-oxidative potential. The oil contains Sabinene which is a potent bactericidal against the multi-drug resistant bacteria [1, 2, 3]. We have reported that the 60% ethanol fruit extract of *Z. limonella* is effective against tested microbial including *C. albicans* (MIC 2.5-10 mg/ml). It is anti-oxidant (EC_{50} 5.94 µg/ml) and its total flavonoids is 3.61 mg rutin/g extract[4]. We have later found that the extract exhibited potent anti-tyrosinase (IC_{50} 0.33 mg/ml) activity. The extract was proved to be a potent anti-inflammatory active extract which was more potent than Std. Diclofenac[5]. The anti-inflammatory, anti-tyrosinase and antioxidant activities make the plant valuable in cosmetics. This study was performed to evaluate cytotoxicity of the ethanol fruit extract from *Z. limonella* using MTT assay that was a part of scientific aspects.

MATERIALS AND METHODS

Preparation of plant extracts The dry fruit powder of *Z. limonella* was separately extracted using 60% ethanol at room temperature. The combined filtrates were evaporated under reduced pressure at 40-50°C to give crude ethanol extract.

Sample preparation The crude extract was weighed and dissolved in 100% dimethylsulfoxide making stock concentration of 1 mg/ml. Filtration through a 0.2 µm filter was done before serially diluted the sample in the culture medium of cells at a ratio of 1:2 giving 8 concentrations of 500, 400, 300, 200, 100, 50, 25 and 12.5 µg/ml.

Cell culture The human dermal fibroblast (ATCC CRL-1474 :NHFF) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine and 100 unit/ml penicillin and streptomycin. The cells were incubated for 72 h. at 37°C in a fully humidified, 5% CO_2: air atmosphere.

MTT cytotoxicity test[6,7] MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolism competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cells were seeded in a 96-well plate at a density of 10,000 cells/well, and incubated for 24 hours. The sample at various concentrations were added to the cells and incubated for 24 hours. The test samples were removed from the cell cultures and the cells were reincubated for a further 24 hours in fresh medium and then tested with MTT assay. Briefly, 50 µl of MTT in PBS at 5 mg/ml was added to the medium in each well and the cells were incubated for 4 hours. Medium and MTT were then aspirated from the wells, and formazan solubilized with 200 µL of DMSO and 25 µl of Sorensen's Glycine buffer, pH 10.5. The optical density was read with a microplate reader (Molecular Devices) at a wavelength of 570 nm. The average of 4 wells was used to determine the mean of each point. The data were analyzed with the SoftMax Program

(Molecular Devices) to determine the IC_{50} for each toxin sample. A dose-response curve was derived from 8 concentrations in the test range using 4 wells per concentration. Results of toxic compounds are expressed as the concentration of sample required to kill 50% (Inhibitory concentration 50; IC_{50}) of the cells compared to controls.

RESULTS AND DISCUSSION

The cytotoxicity test was shown in Table 1 and Figure 1. The treatment of NHFF cells for 24 hr with various concentrations of *Z. limonella* extract at 500, 400, 300, 200, 100, 50, 25 and 12.5µg/ml. indicated that the IC_{50} value was 391.8 ± 0.01µg/ml for treatment times.

Table 1: Viability of NHFF following exposure to *Z. limonella* extract

Sample	Concentration (µg/ml)	% Viability	IC50(µg/ml)
Z. limonella	500.00	23.45 ± 0.02	391.8 ± 0.01
	400.00	55.66 ± 0.05	
	300.00	73.70 ± 0.13	
	200.00	103.04 ± 0.15	
	100.00	101.00 ± 0.02	
	50.00	102.00 ± 0.06	
	25.00	101.00 ± 0.05	
	12.50	102.00 ± 0.07	

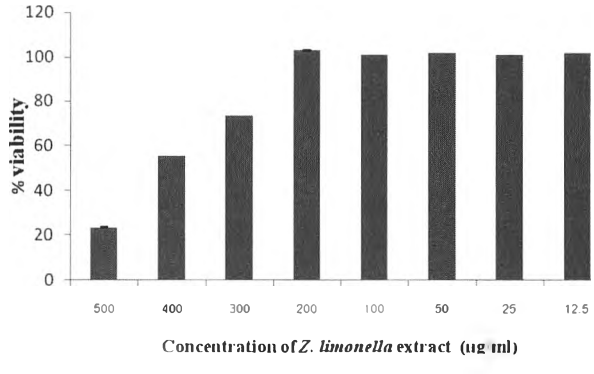

Figure 1 The Viability of NHFF following exposure to *Z. limonella* (µg/ml) extract

CONCLUSION

This assay was a modified version of conventional direct and indirect contact tests conformed to the published standard methods (BS-EN30993-5 and ISO10993-5). The MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolism competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cytotoxicity results showed the % survival of NHFF cell line, at each concentration compared to control and IC_{50} values, over the test concentrations of 500-12.5 µg/ml. The results showed that The IC_{50} values of *Z. limonella* extract was 391.8 ± 0.01 µg/ml .

ACKNOWLEDGEMENT

The author would like to gratitude thanks to Thailand Institute of Scientific and Technological Research (TISTR) for the financial support.

REFERENCES

1. Mallikarjuna P, Uma Maheswara Rao V and Satyanarayana T. 1999. Antimicrobial activity of *Zanthoxylum limonella*. Indian Drugs 36 (7): 476-478.
2. Tangjitjaroenkun J, Supabphol R and Chavasiri W. 2012. Antioxidant effect of *Zanthoxylum limonella Alston*. J Medicinal Plants Res. 1407-1414.
3. Charoenying P, Laosinwattana C, Phuwiwat W, and Lomratsiri J. 2008. Biological activities of *Zanthoxylum limonella Alston* fruit extracts. KMITL Sci. J. 8(1): 12-15.
4. Ngamnon Y, Potduang B*, Fungsin B, Phasuk S, Takolpuckdee P, Rerk-am U, Kaewduang M and Tanpanich S. 2012. Antimicrobial, antioxidant activities and total flavonoids of fruit extracts from Ma-Khwaen (*Zanthoxylum limonella*). Thai J Pharm Sciences 35 (Suppl.): 36-37.
5. Khayungarnnawee A, Soradech S, Potduang B*, Sematong T, Laovitthayanggoon S. Keeta I, Niwaspragrit C.and Tantrawong A. (2012). Anti-inflammatory activity of fruit extracts from *Phyllanthus emblica* and *Zanthoxylum limonella. Thai J. Pharm. Sci. 36 (Suppl.)* Dec., p.38-40
6. Freshney RI. 2005. Culture of Animal Cell: A manual of Basic Technique. Wiley-Less, NewYork, Chapter 21: pp.287-307.
7. Plumb JA, Milroy R, Kaye SB. Effects of the pH dependence of 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium based assay. Cancer Res. 1989 49:4435-4440.
8. BS-EN30993-5. 1994. Biological evaluation of medical devices, Part 5. Tests for cytotoxicity, in vitro methods: British Standard1994
9. ISO10993-5. 2009. Biological evaluation of medical devices, Part 5. Tests for in vitro cytotoxicity: International Standard 2009
10. Guidance Document on using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity, 2001 NIH Publication No. 01-4500 available under (http://iccvam.niehs.nih.gov/docs/acutetox_docs/guildance0801/iv_guild.pdf)