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CYTOTOXIC ACTIVITY OF *LUVUNGA SCANDENS* AGAINST HUMAN CANCER CELL LINES

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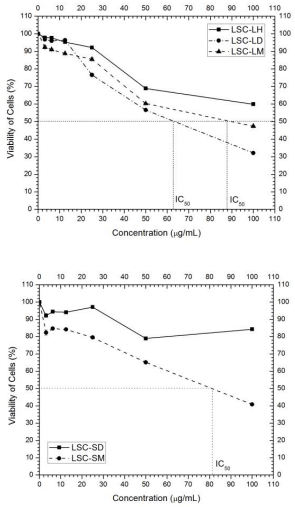
Graphical abstract



Abstract

Luvunga scandens belongs to the family of Rutaceae which usually inhabit tropical and moist environment. This plant is known as 'Mengkurat Jakun' among locals and used traditionally to treat fever and fatigue via decoction. The aim of this study was to investigate the cytotoxic activity of the leaves and stems extracts of *L. scandens* extract. Extracts of the leaves and stems were obtained from sequential extraction procedures by various organic solvents. All extracts were subjected to cytotoxic study by 3-(4, 5-dimethylthaizol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. In *in vitro* cytotoxicity assay, all *L. scandens* extracts exhibited cytotoxicity against human breast adenocarcinoma (MCF-7) and human lung adenocarcinoma (A549) cell lines. The IC_{50} values of dichloromethane and methanol extracts from the leaves of *L. scandens* against MCF-7 cell line were 62.5 µg/mL and 88.0 µg/mL, respectively, whereas IC_{50} of methanol extract from stem was 81.0 µg/mL. All extracts were less active against A549 cell line where IC_{50} value were not be determined. The present findings revealed the potential of *L. scandens* as a cytotoxic agent against MCF-7 cell line. However, further studies should be planned to evaluate role of the plant in cytotoxic activity.

Keywords: *Luvunga scandens*, cytotoxic activity, MTT, MCF-7, A549

Abstrak

Luvunga scandens adalah species tumbuhan tergolong dalam keluarga Rutaceae yang kebiasaannya mendiami persekitaran tropika dan lembap. Pokok ini dikenali sebagai 'Mengkurat Jakun' di kalangan penduduk tempatan dan digunakan secara tradisional untuk mengubati demam dan keletihan dengan meminum air rebusan. Kajian ini adalah bertujuan untuk menyiasat aktiviti ketoksikan ekstrak daripada daun dan batang pokok *L. scandens*. Ekstrak daripada daun dan batang telah diperoleh daripada prosedur pengekstrakan berurutan oleh pelbagai pelarut organik. Kesemua ekstrak telah diuji untuk pemeriksaan aktiviti ketoksikan menggunakan 3-(4, 5-dimethylthaizol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Dalam ujian ketoksikan, kesemua ekstrak dari *L. scandens* menunjukan ketoksikan terhadap sel payudara adenokarsinoma (MCF-7) dan sel epitelium paru-paru adenocarcinoma (A549). Nilai IC_{50} dari ekstrak diklorometana dan metanol daripada daun *L. scandens* terhadap MCF-7 sel adalah masing-masing 62.5 µg/mL dan 88.0 µg/mL, manakala ekstrak methanol daripada batang adalah 81.0 µg/mL. Semua ekstrak kurang aktif terhadap sel A549, dimana nilai IC_{50} tidak ditentukan. Kajian mendapati bahawa, *L. scandens* mempunyai potensi sebagai agen sitotoksik terhadap sel MCF-7.

Walaubagaimanapun, kajian selanjutnya telah dirancang untuk menilai peranan tumbuhan dalam aktiviti sitotoksik.

Kata kunci: *Luvunga scandens*, kesan sitotosik, MTT, MCF-7, A549

1.0 INTRODUCTION

Plants have become important source to discover new drug for the treatment of various diseases. Plants have a long history for cancer treatment and known as effective anticancer agents [1]. Taxol is an example of plant-based drug that is effective to fight different kinds of cancers. Taxol had been originally isolated from the bark of *Taxus brevifolia* which was later approved by FDA as an anti-cancer drug for breast, ovarian and non-small lung carcinomas treatments [2].

Cancer is a leading cause of serious disease and cancer death worldwide. It is becoming a major public health concern in both developed and developing countries due increased cancer incidences every year. According to the International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization (WHO), in the GLOBOCAN project 2012 estimated, about 14.1 million new cancer cases and 8.2 million cancer-related deaths were estimated to have occurred in 2012 compared with 12.7 million and 7.6 million, respectively in 2008 [3]. Breast and lung cancers are among the most frequently diagnosed cancers and the leading cause of cancer death in the developing and developed countries of the world [4]. Sharp rise in the breast and lung cancers incidences prompted us to evaluate *in vitro* cytotoxic effect of *L. scandens* against human breast adenocarcinoma (MCF-7) and human lung adenocarcinoma epithelial (A549) cell lines in order to search new potential anticancer agents.

In this study, plant from the genus Luvunga (Rutaceae) was used to investigate cytotoxic activity. *L. scandens* is one of the species which is considered heavy woody climber with recurved spines and evergreen shrub in the temperate zones. It mainly inhabits tropical and moist environment such as China, Cambodia, India, Laos, Indonesia, Philippines, Malaysia, Myanmar, Thailand and Vietnam. The leaves are simple which consist of 3-foliolate and long-petiole. In Malaysia, *L. scandens* is known as 'Mengkurat Jakun' [5]. From literature studies, only alkaloids and coumarins have been reported from this species. As for coumarins, luvangetin has been isolated from *L. scandens* [6]. In term of bioactivity study, only a few studies on antifungal and insecticidal activities of *L. scandens* have been reported [7, 8]. So far, no study has been carried out to investigate the cytotoxic potential of this plant. The traditional use of *L. scandens* in in the form of decoction that is taken orally to treat malaria fever and tiredness [5,9]. Then, this paper describes the cytotoxic activity of the stems and leaves of *L. scandens*.

2.0 METHODOLOGY

2.1 General Experimental Procedure

All organic solvents were purchased from Merck, Germany. Dulbecco's modified Eagle medium (DMEM), Fetal Bovine Serum (FBS), Penicillin-Streptomycin and TripLE Express were purchased from GIBCO (USA). 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Invitrogen (USA). Doxorubicine hydrochloride and Phosphate Buffer Saline (PBS) were obtained from Sigma Aldrich (USA).

2.2 Plant Materials

The stems and leaves of *L. scandens* were collected from Kuantan, Pahang, Malaysia, in September, 2012. Plant specimen was identified by Dr. Shamsul Khamis (Institute of Bioscience, Universiti Putra Malaysia). The voucher specimen (No. PIIUM 0204) was deposited in the Herbarium of Faculty of Pharmacy, International Islamic University Malaysia.

2.3 Extraction

The dried and powdered stems and leaves of *L. scandens* were extracted separately by maceration method in series of organic solvents namely *n*-hexane, dichloromethane (DCM) and methanol (MeOH) for overnight at room temperature. This process was repeated for three times. The extracts were combined and filtered through Whatman filter paper no. 1. The solvents were removed by vacuum rotary evaporator (BUCHI Rotavapor R-200) at 60 °C and the concentrated crude extracts were weighed and placed at 4°C refrigerator for further chemical and biological analysis.

2.4 Cell Lines

MCF-7 and A549 cell lines were provided by Prof. Masa-Aki Ikeda of Tokyo Medical and Dental University, Tokyo. These cells were maintained in

sterile complete growth media containing DMEM, supplemented with 10% FBS and 1% Penicillin-Streptomycin in 5% $C0_2$ at 37°C.

2.5 MTT Assay

Cytotoxic activity was determined by MTT assay according to Mosmann with a slight modification [10]. 100 µL of suspension cells with density of 2 x 10^5 cells/mL were plated in 96-well plate for overnight. The cells were treated in triplicates with different concentrations of extract in medium (3.125 to 100 µg/mL). After 24 h of incubation, the media was discarded and the cells were washed with PBS once. Cell proliferation was assayed by the addition of 30 µL of MTT solution at concentration of 5 mg/mL in PBS. After 4 h incubation, the formazan crystal was dissolved in 120 µL DMSO and left in dark at room temperature for an additional 1 h. The absorbance was measured at 570 nm and 630 nm, as reference wavelength [11] using microplate reader (TECAN infinite M200). The wells with untreated cells were taken as the control, medium with 0.1% (*v*/*v*) of ethanol was used as solvent control and doxorubicin was used as positive control. Results were expressed as a percentage of average absorbance of treated cells with respect to untreated ones.

The half maximal inhibitory concentration (IC_{50}) was defined as the concentration of extract that caused a 50 % reduction in cell viability against MCF-7 and A549 cell lines. Cell viability (%) was calculated using the following equation [12]:

$$\text{Cell viability (\%)} = [(A_{Sample} - A_{Blank}) / (A_{Untreated} - A_{Blank})] \times 100$$

The IC_{50} values were determined from the plotted graph of viability of cells versus concentration by OriginPro 8 software.

2.6 Statistical Analysis

The data were analyzed statistically by comparison of experimental samples of the same treatment conditions as a group with untreated cells (negative control) and presented as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA; 95 % confidence interval), followed by Dunnet post hoc test for the determination of level of significance and results were considered significant, if $p < 0.05$.

3.0 RESULTS AND DISCUSSION

In this study, all five extracts from the stem and leaves of *L. scandens* were tested for cytotoxic activity against MCF-7 and A549 cell lines.

Percentage of MCF-7 cell viability against different concentration of *L. scandens* leaves extracts showed the results in a dose-dependent manner at a range of 3.125-100 µg/mL (Figure 1). The IC_{50} values of dichloromethane (LSC-LD) and methanol (LSC-LM) extracts from leaves of *L. scandens* were found to be 62.5 µg/mL and 88.0 µg/mL, respectively. Whereas, IC_{50} values of *n*-hexane (LSC-LH) extract could not be extrapolated since none of the concentration of this extract was able to reduce the proliferation activity to 50 %. Figure 2 displayed the IC_{50} values of methanol (LSC-SM) extract from stem of *L. scandens* which were found to be 81.0 µg/mL. Whereas, IC_{50} values of dichloromethane (LSC-SD) extract could not be extrapolated since none of the concentration of this extract was able to reduce the proliferation activity to 50 %. The IC_{50} value of doxorubicin against MCF-7 cell line was found to be 3.55 µg/mL (Figure 3).

Figures 4 and 5 show the percentage of cell viability of A549 treated with different concentrations of extracts. All extracts exhibited dose-response inhibition but were not able to reduce the percentage of cell viability to 50 %. Thus, the IC_{50} could not be extrapolated. The IC_{50} value of doxorubicin against A549 cell line was obtained at 2.05 µg/mL (Figure 3). From the results obtained, the percentage of EtOH (0.1 %) used in the experiment did not find to affect the growth of the cells. The IC_{50} values of all the extracts are presented in Table 1.

Three different solvents from non-polar, semi-polar and polar were used for samples' extraction namely *n*-hexane, DCM and MeOH. The selection of solvent systems largely depends on the specific nature of the bioactive compound being targeted. Many researchers reported that different extraction solvents could influence the effect on the results of the tested assay [13]. It is because when the extracts are well matched in polarity with the solvent, they will be easily extracted [14]. Therefore, the extracts from different solvents used were active and inactive towards the tested cell lines.

Table 1 IC_{50} value of L. scandens extracts on MCF-7 and A549 cell lines

No	Extracts of *L. scandens*	IC50 (µg/mL)	
		MCF-7	A549
1	LSC-LH	Not detected*	Not detected*
2	LSC-LD	62.5	Not detected*
3	LSC-LM	88.0	Not detected*
4	LSC-SD	Not detected*	Not detected*
5	LSC-SM	81.0	Not detected*

Key: * = IC50 value higher than 50 % viability of cells

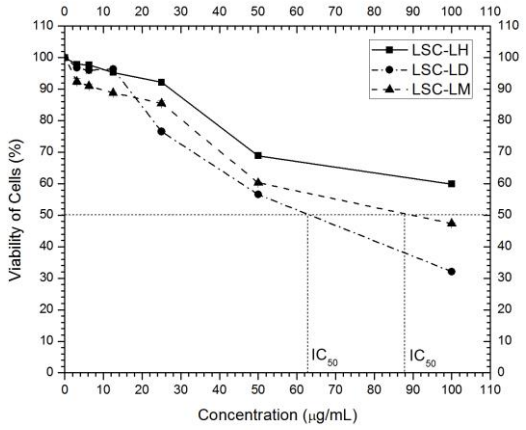


Figure 1 Percentage of cell viability of MCF-7 cell line against different concentrations of *L. scandens* leave extracts

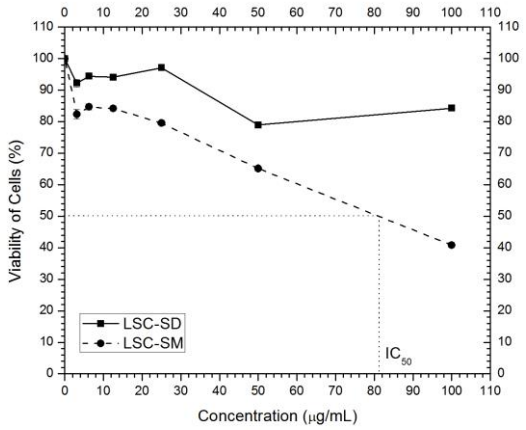


Figure 2 Percentage of cell viability of MCF-7 cell line against different concentrations of *L. scandens* stem extracts

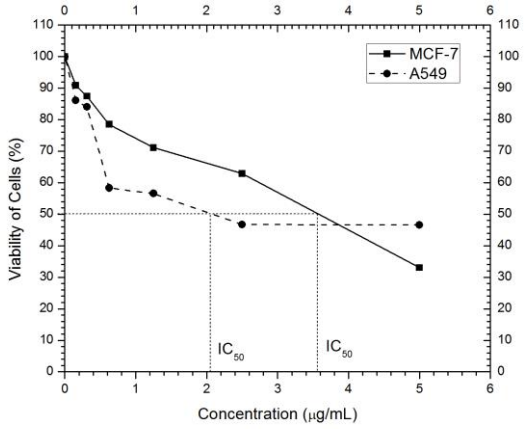


Figure 3 Percentage of cell viability of MCF-7 and A549 cell line against different concentrations of doxorubicin

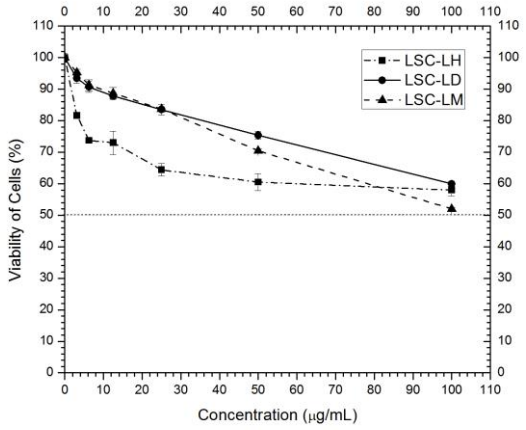


Figure 4 Percentage of cell viability of A549 cell line against different concentrations of *L. scandens* stem extracts

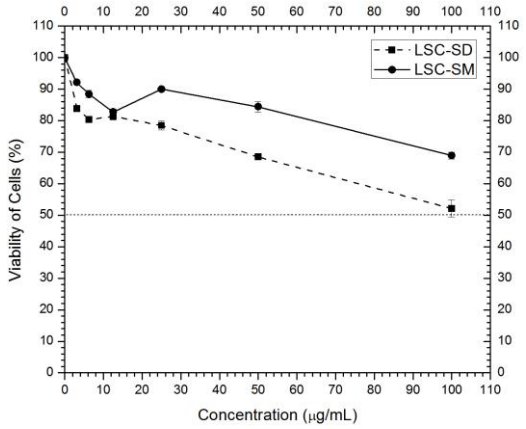


Figure 5 Percentage of cell viability of A549 cell line against different concentrations of *L. scandens* stem extracts

4.0 CONCLUSION

The present study demonstrates that the stem and leaves extracts from *L. scandens* were found to be responsible for the cytotoxic effects due to their ability to reduce the half maximal inhibitory concentration (IC_{50}) against breast adenocarcinoma (MCF 7) cell lines compared with lung adenocarcinoma epithelial (A549) cell line. Further study still required to identify and purify the active compounds of *L. scandens* for subsequent *in vitro* and *in vivo* evaluation for their role as cytotoxic agents.

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References

[1] Shoeb, M. 2007. Anticancer Agents From Medicinal Plants. *Bangladesh Journal of Pharmacology*. 1(2): 35-41.

[2] Peteros, N. P. and Uy, M. M. 2010. Antioxidant And Cytotoxic Activities And Phytochemical Screening Of Four Philippine Medicinal Plants. *Journal of Medicinal Plants Research*. 4(5): 407-414.

[3] Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D. and Bray, F. 2013. Cancer Incidence And Mortality Worldwide: Sources, Methods And Major Patterns in GLOBOCAN 2012. *International Journal of Cancer*. 136(5): E359-386.

[4] Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., Parkin, D. M. 2010. Estimates Of Worldwide Burden Of Cancer In 2008: GLOBOCAN 2008. *International Journal of Cancer*. 127(7): 2893-2917

[5] Ong, H., Chua, S. and Milow, P. 2011. Ethno-Medicinal Plants Used By The Temuan Villagers In Kampung Jeram Kedah, Negeri Sembilan, Malaysia. *Ethno Med*. 5(2): 95-100.

[6] Chopra, R. N. 2006. *Indigenous Drugs Of India*. Kolkata: Academic Publishers.

[7] Garg, S., Jain, R. 1999. Antifungal Activity Of Luvunga Scandens Against Some Keratinophilic Fungi. *Indian Journal of Pharmaceutical Science*. 61(4): 248.

[8] Eswani, N., Kudus, K.A., Nazre, M., Noor, A., Ali, M. 2010. Medicinal Plant Diversity And Vegetation Analysis Of Logged Over Hill Forest Of Tekai Tembeling Forest Reserve, Jerantut, Pahang. *Journal of Agricultural Science*. 2(3): 189-210.

[9] Singh, G. and Maurya, S. 2005. Antimicrobial, Antifungal And Insecticidal Investigations On Essential Oils: An Overview. *Natural Product Radiance*. 4: 179-192.

[10] Mosmann, T. 1983. Rapid Colorimetric Assay For Cellular Growth And Survival: Application To Proliferation And Cytotoxicity Assays. *Journal of Immunology Methods*. 65(1-2): 55-63.

[11] Taher, M., Susanti, D., Rezali, M. F., Zohri, F. S. A., Ichwan, S. J. A., Alkhamaiseh, S. I. and Ahmad, F. 2012. Apoptosis, Antimicrobial And Antioxidant Activities Of Phytochemicals from *Garcinia malaccensis* Hk. f. *Asian Pacific Journal of Tropical Medicine*. 5(2): 136-141.

[12] Al-Zikri, P. N. H., Taher, M., Susanti, D., Rezali, M. F., Read, R. W., Sohrab, M. H., Hasan, C. M. and Ahmad, F. 2014. Cytotoxic Tirucallane Triterpenes From The Stem Of Luvunga Scandens. *Revista Brasileira de Farmacognosia. 24*(5): 561-564.

[13] Tomsone, L., Kruma, Z., and Galoburda, R. 2012. Comparison Of Different Solvents And Extraction Methods For Isolation Of Phenolic Compounds From Horseradish Roots (Armoracia rusticana). *World Academy of Science, Engineering and Technology*. 64: 903-908.

[14] Tan, M. C., Tan, C. P., and Ho, C. 2013. Effects Of Extraction Solvent System, Time And Temperature On Total Phenolic Content Of Henna (*Lawsonia inermis*) Stems. *International Food Research Journal.* 20(6): 3117-3123.