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Anti-leukemia Activity of Methanolic Extracts of *Lantana camara*

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ABSTRACT

Anticancer effect of *Lantana camara*'s root and leaf extracts against Jurkat leukemia cell line was investigated by MTT assay. These extracts had statistically similar antineoplastic property (root IC50, 328.36 ± 53.08 µg/ml; leaf, 394.41 ± 99.73 µg/ml; $p > 0.1$, $n = 3$), averagely 1/10 times as activity as carboplatin (IC50 34.83 ± 3.60 µg/ml; $p < 0.05$, $n = 3$). Decreasing cytotoxicity at higher concentrations implied the existence of cytoprotective compounds. Morphological examinations indicated apoptosis induction as the mechanism of activity on Jurkat cells. In conclusion, *L. camara*'s root and leaf extracts might be subjects for further fractionation and identification to find new anticancer agents.

Keywords: Anticancer, Cell Culture, Jurkat Cell Line, *Lantana camara*, MTT Assay.

INTRODUCTION

Cancer is the second leading cause of death in economically developed countries (following heart diseases) and the third leading cause of death in developing countries (following heart diseases and diarrheal diseases). The estimates for total cancer deaths in 2007 are 7.6 million (about 20,000 cancer deaths a day), 2.9 million in economically developed countries and 4.7 million in economically developing countries. Worldwide statistics for leukemia leading to death in 2007 is 245871 cases (1).

The lush tropical rainforests have long been a source of promise in the fight against cancer and other diseases. Between 1940 and 2002, 40% of all anticancer drugs entering the market were natural products or derivatives thereof, with a further 8% consisting of natural product analogues (2). However, these compounds haven't produced the utmost efficacious anticancer agents that many would have hoped for. Basically, finding new anticancer drugs needs a huge screening process. In the first group of extracts studied from 1960 to 1982 that resulted in two anticancer agents, Taxol and camptothecin, over 114,000 extracts were investigated (3).

In 1991, Herbert J.M. *et al.* (4) reported that verbascoside isolated from *Lantana camara* L (Verbenaceae) possesses antitumor activity in vitro. This might be due at least in part to inhibition of protein kinase C. Also based on Shashi B.M *et al.* (5) finding, *L. camara*'s extracts displayed antitumor effect. Lantadenes and related triterpenoids from *L. camara* inhibited Epstein-Barr virus activation, making hope to build antitumor promoters by few changes in chemical structure (the substitutions on the carboxylic acid through an ester bond, 6). In addition, some terpenoids of *L. camara*, such as 22 beta-acetoxylantic acid and 22 beta-dimethylacryloyloxy lantanolic acid has been reported to have antimutagenic effects (7). A recent structure-activity study on lantadenes and their esters by Sharma M. *et al.* (8) revealed the importance of the groups

attached to C-22 and C-17 in relation to the antitumor activity of these compounds.

The overall objective of the current research was to discover new drug progenitors from *L. camara*'s root and leaf extracts for the treatment of leukemia. Consequently, the Jurkat cells were treated by plant extracts through MTT assay. MTT (3 - (4,5 - dimethylthiazol - 2 - yl) - 5 - diphenyltetrazolium bromide) assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which are largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is directly proportional to the level of the formazan product created (9). The Jurkat cell line was established from the peripheral blood of a 14 year old boy and was originally designated JM (10). This cell line is an immortalized line of T lymphocyte cells that their primary use is to determine the mechanism of differential susceptibility of cancers to drugs and radiation. They grow in lymphoblast suspension form (11).

MATERIALS AND METHODS

Extraction

Mature *L. camara* was collected in November 2008 from Sungai Petani, Kedah, Malaysia. The identity of plant was confirmed by Dr. S. Sudhakaran, associate professor in Faculty of Applied Sciences, AIMST University, Kedah, Malaysia. A voucher specimen with number 11008 was deposited in the Biology School herbarium, Universiti Sains Malaysia, Penang, Malaysia. Separated root and leaf parts of plant were extracted via maceration in methanol. The percentages of crude extract yield for root and leaf extracts were 7.40% and 17.75% of dried plant material, respectively.

MTT Assay

Antiproliferative activity of leaf and root extract of *L. camara* was estimated through MTT assay described by Su *et al.* (12) with some adaptations. To make the complete growth medium, fetal bovine serum was added to a final concentration of 10% to the RPMI (Roswell Park Memorial Institute, PAA Laboratories GmbH, Austria) medium. Antibiotic was inserted to a final concentration of 5%; L-glutamine, 2%. After first passage under cell culture procedures for suspension cell lines, leukemic cells were diluted with complete RPMI medium to 1 x 10^5 cells/ml and aliquots (5000 cells/50 µl) were placed in individual wells in 96-well microplate. Each well in addition received 50 µl of either leaf or root extract which had been serially diluted 2-fold in methanol (final concentration 50% v/v), ranged from 7.8 to 500 µg/ml in final solution. The first column of microplate was kept empty as blank; 11th, remained untreated to act as negative control and 12th one, treated with Triton 100X 1% as process control. Cells were incubated at CO_2 5%, 37.0°C for 24, 48 or 72 h and then their viability was determined by MTT color. The MTT (amResco, Ohio, USA) solution (5 mg/ml in PBS, 10 µl) was added to each well and following 5 min shaking in 150 rpm, the plates were incubated for 3 h. Acidified isopropanol (100 µl) was put in each well to dissolve the formazan crystals and the plates were shaken for 20 min in 150 rpm. The absorbance of wells was read at 570 nm and at background (630 nm) on a microplate reader. Appearance of cells was monitored by inverted phase contrast microscope. Carboplatin (final concentrations from 0.3 to 30 µg/ml) was applied as positive control. The test was performed in triplicate. Cytotoxicity index percentage was calculated based on the following equation:

$$\%CI = [1 - (OD_{570-630} \text{ Treatment} / OD_{570-630} \text{ Control})] \times 100$$

where the $OD_{570-630}$ is absorbance at 570 nm minus absorbance at 630 nm (13). Cytotoxicity percentage figures were applied to determine the mean value of IC50 (50% inhibitory concentration) through curve estimation by SPSS 16.00 (SPSS Inc, TEAM EQX). Significances were evaluated by one way ANOVA.

RESULTS AND DISCUSSION

Games-Howell statistical test showed that leaf and root extracts of *L. camara* had similar antiproliferative activity ($p > 0.1$, $n = 3$) after 24 h and 72 h that was contrastable with carboplatin one ($p < 0.05$, $n = 3$). On the basis of Table I, leaf extract had about 1/11.5 times as antileukemia activity as carboplatin after 72 h (leaf extract IC50, 394.41 ± 99.73 µg/ml; carboplatin, 34.83 ± 3.60 µg/ml) and root extract, around 1/9.5 times as effect as this drug at the same interval (root extract IC50, 328.36 ± 53.08 µg/ml).

One way ANOVA analysis exhibited that except root extract, there was no significant divergence between IC50 values after different durations of treatment ($p > 0.1$, $n = 3$). Therefore, it can be concluded that the effect of leaf extract of *L. camara* and carboplatin on Jurkat cells is not time dependant. 1 illustrates an erratic pattern of action for leaf and root extract, particularly obvious at the first 24 h of treatment. While toxicity increased to reach a peak at approximately 300 µg/ml, it declined at the higher concentrations rapidly (leaf extract in the first period) or gradually (leaf and root extracts in the

Table I. IC50 Mean Values for Jurkat Leukemia Cell Line Treated by *L. camara* Leaf and Root Extract as well as Carboplatin (Positive Control) incubated in Different Time Intervals based on MTT Cell Viability Assay. The quantities are mean ± standard deviation.

L. camara Part and Carboplatin	Time	IC50 (µg/ml)
Leaf	24 h	455.81 ± 43.81
	48 h	377.53 ± 168.24
	72 h	394.41 ± 99.73
Root	24 h	270.19 ± 105.91
	48 h	506.49 ± 60.92
	72 h	328.36 ± 53.08
Carboplatin	24 h	38.58 ± 14.71
	48 h	35.60 ± 4.66
	72 h	34.83 ± 3.60

third period). Carboplatin had a comparatively converse model of chart at the same conditions. It may reflect the diversity of phytochemicals available in leaf and root extract of *L. camara* so that some of them protect cells against antineoplastic components at the enhanced levels of concentrations. Then, isolating mentioned possible compounds may lead to more potent anticancer effect for these extracts. The reason for fluctuations in lower concentrations of extracts or carboplatin is not clear.

Morphology contrasting among negative control and treated cells demonstrates that the magnitude of most of cells has decreased in extract treated cells (Figures 2 and 3). As the cells shrink in apoptosis and swell while necrosing (14), it may imply that *L. camara* extracts inhibited proliferation through apoptosis induction. Meanwhile, Triton treated cells have been dramatically disintegrated, mirroring the disruption of membrane by this detergent.

As far as our knowledge, there is no report on anti-Jurkat activity of *L. camara* extracts. An antiproliferative study on *E. officinalis, A. marmelos, M. oleifera, T. arjuna* and *O. indicum* using a model ZBI Coulter Counter determined IC50 values between 4 to more than 500 µg/ml (15). In addition, Amirghofran Z. *et al* (16) found that *Euphorbia cheiradenia* inhibited Jurkat cells growth at IC50 12.5 µg/ml

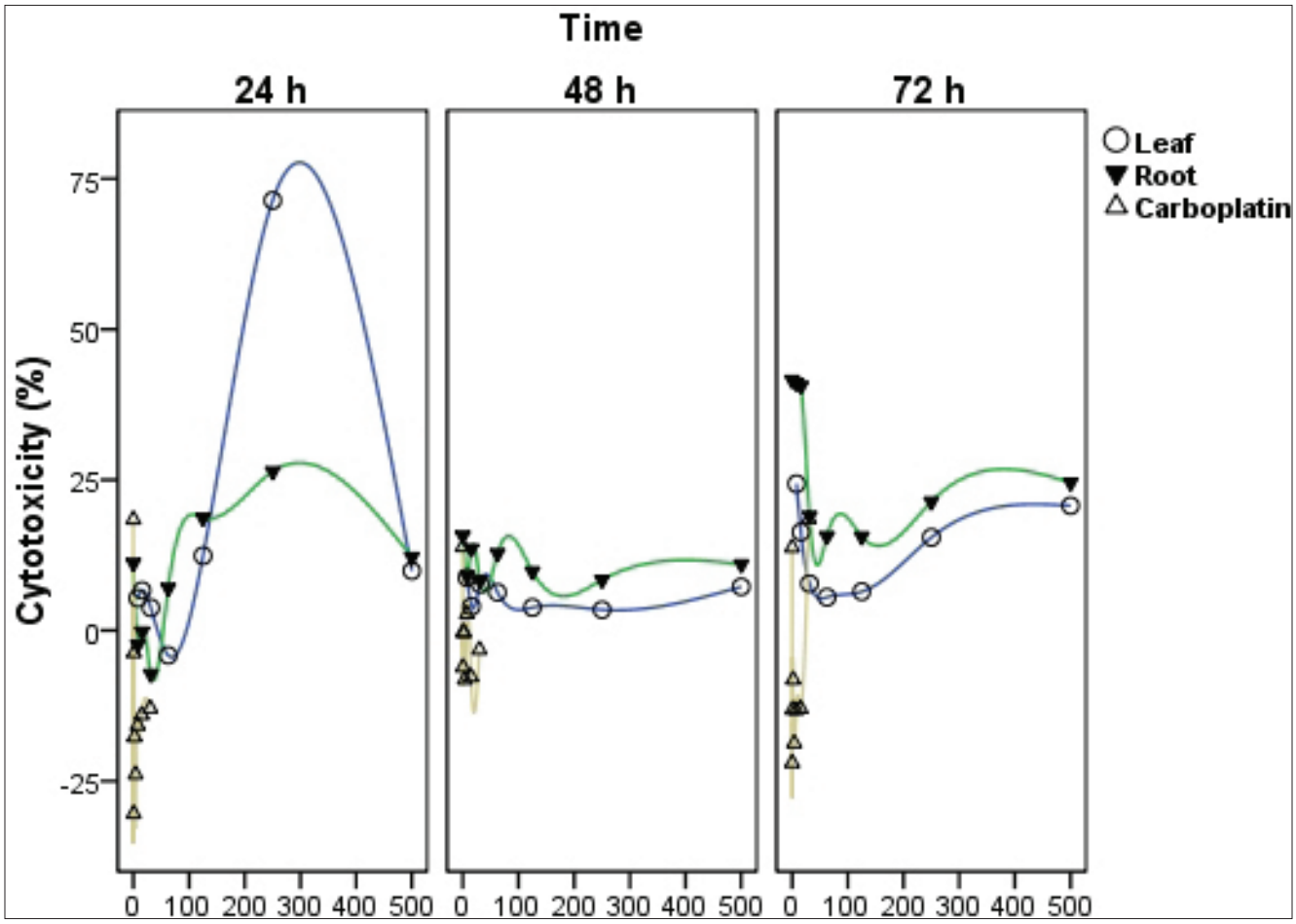

Figure 1. Effects of Concentration and Exposure Time on Cytotoxicity of *L. camara*'s Leaf and Root Extracts and Anticancer Drug Carboplatin on Jurkat Leukemic Cell line Tested by MTT Assay.

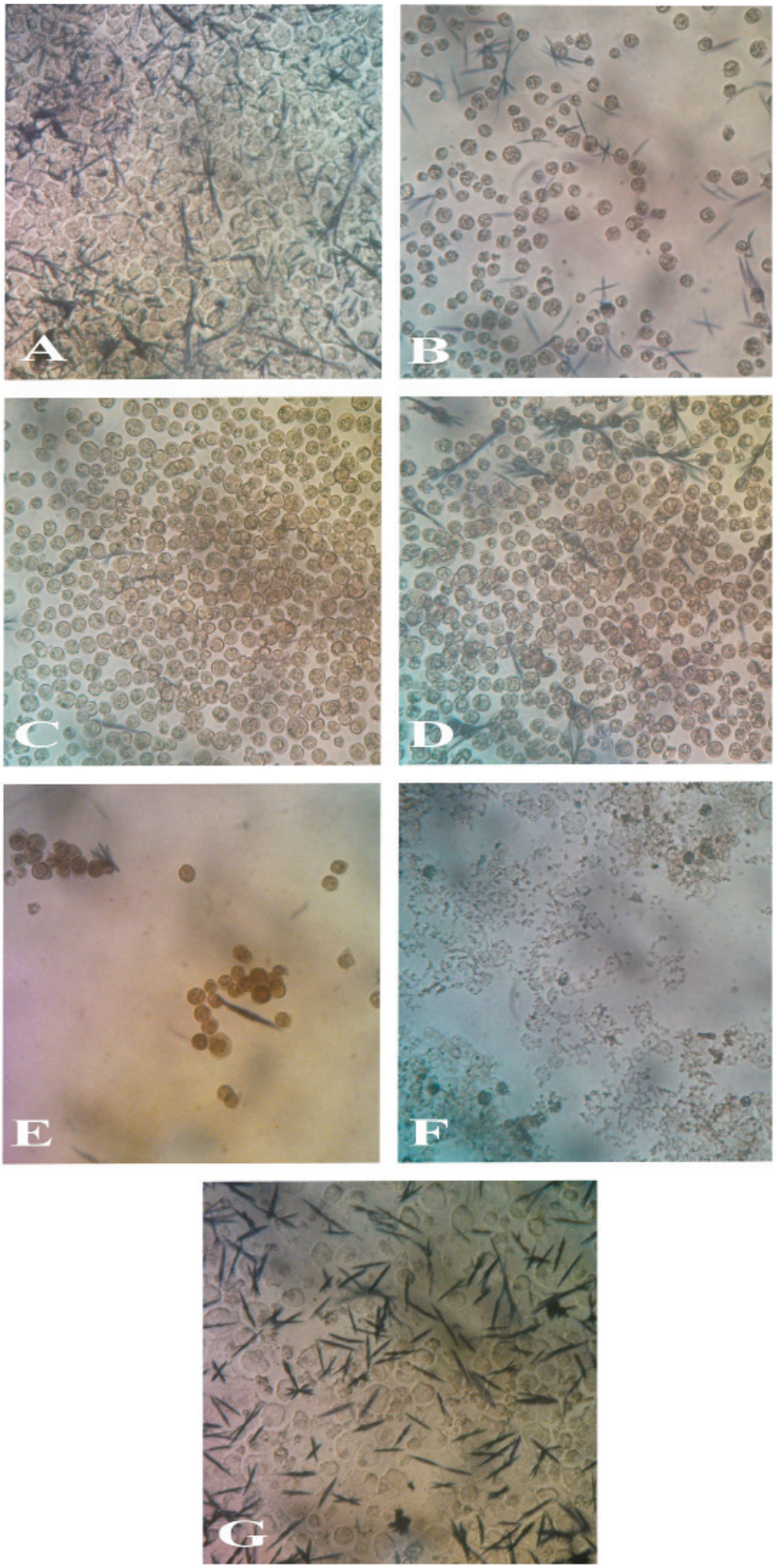

Figure 2. Morphology of Jurkat Leukemia Cells after 72 h Treating with Various Concentrations of Leaf Extract of *L. camara*. A: Solvent control, B: 15.6, C: 62.5, D: 250 and E: 500 µg/ml; F: Triton 100X 1%, G: Negative Control. Density of formazan crystals (blue color stick-like objects) is nearly straight linked to cell viability. Magnification is 400×.

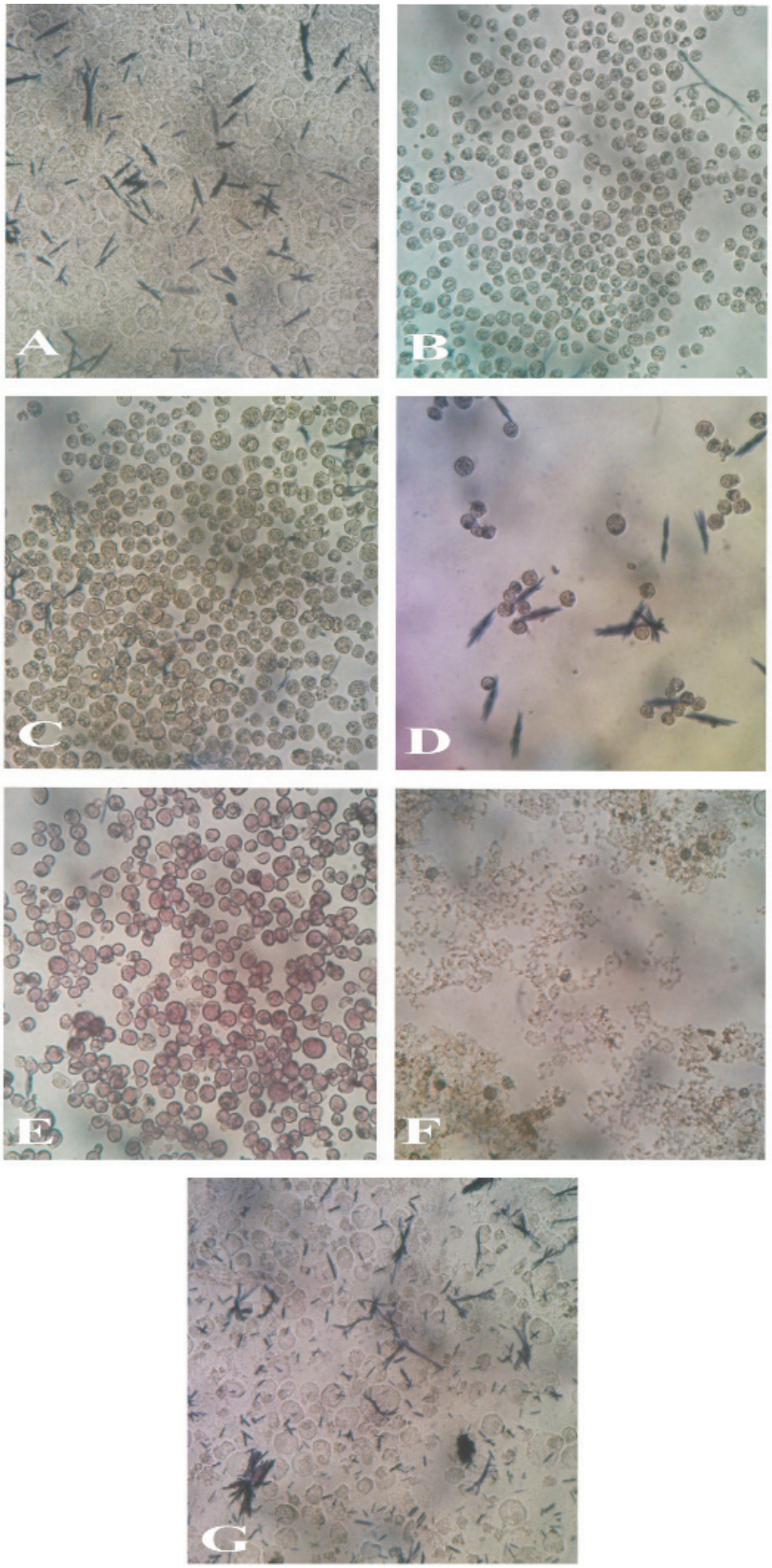

Figure 3. Morphology of Jurkat Leukemia Cells after 72 h Treating with Various Concentrations of Root Extract of *L. camara* as A: Solvent control, B: 15.6, C: 62.5, D: 250 and E: 500 µg/ml; F: Triton 100X 1%, G: Negative Control. Magnification is 400×.

based on MTT assay. Accordingly, leaf and root extracts of *L. camara* may be classified as moderate anti-Jurkat agents.

In summary *L. camara*'s leaf and root extract had roughly equal antiproliferative activity on human leukemia Jurkat cell line, but this activity was about 1/10 of carboplatin potency, a reference anticancer drug. Nevertheless, *L. camara* extracts may include both anticancer and cell protective compounds which apparently make a concentration-dependant pattern in their anti-reproductive effect. Possibly, the mechanism of anticancer action against Jurkat cells for leaf and root extracts is through apoptosis induction.

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