ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antiproliferative Activity of *Lilium candidum* Alkaloid Extract on Human Breast Cancer Cell Line

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Abstract

Cancer is an uncontrolled cell division that grows and spreads all over the human body. Chemotherapy of cancer can cause further damage of the patients' health. Therefore, there is a need on using plant extracts as an alternative treatment against cancer

The leaves of the plants were extracted with methanol (80%), chloroform at pH 2 and pH 10 and the chloroform portion was dried to obtain the total alkaloid extracts. The total alkaloids were detected qualitatively by Mayer's and Dragendorff's reagents and estimated quantitatively using bromocresol green, depending on the Quinine calibration curve. Its antiproliferative activity was measured using the MTT assay.

The alkaloids extract of *Lilium candidum* showed cytotoxic activity against breast cancer cell line (MCF-7) with IC_{50} of 244.8μg/ml.

This study indicates that *Lilium candidum* alkaloids extract can be used as an efficient traditional anticancer alternative medicine at moderatly low dosage.

Keywords: *Lilium candidum*, alkaloids, MTT, MCF-7, Breast cancer

INTRODUCTION

Cancer, as a second killer, is an uncontrolled cell division that might invade other tissues and spread (metastasize). It is responsible for the death of millions of people worldwide [1,2].

Chemotherapy of cancer can cause further damage of the patients' health Therefore; there is a need on using plant extracts as an alternative treatment against cancer**.** Natural antiseptic properties of plants have been used in traditional medicine for decades. Thus, investigating their potential properties has taken so much concern in research to be used as an alternative treatments for many diseases including cancer with less or no dangerous side effects [3]. From a long period of time, many medically important secondary metabolites are produced by plants and are playing an important role as microbicides, pesticides, antitumor and many other pharmaceutical drugs [4]. Today, bioactive components derived from plants representing more than 40% of all medicines and are spread worldwide for its safety especially those with antioxidant, antimicrobial, antimutagenic and anticancer activities [5-6].

L. candidum L. (white lily) is a herbaceous perennial plant belongs to Liliaceae family. It is marketed for its fragrance, flowers and its medical usage of its bulb, this plant is successfully cultivated in Europe, USA and many other countries including Iraq for medical, ornamental and in perfumes purposes [7-9]. *L. candidum* extract is well known as an anti-inflammatory remedy for burns, ulcers andhealing wounds. It has been reported that it can modulate the clastogenic activity of drugs like Zeocin [10]. However, alkaloids like pyrroline and pyrrolidine may be enrolled in inducing significant oxidative stress and DNA damage, which lead to cell apoptosis or necrosis [11].

This study aim to investigate the cytotoxic activity of *L. candidum* L. total alkaloid extract against breast cancer cell line (MCF-7) in comparison with non-tumorigenic fetal hepatic cell line (WRL-68).

MATERIALS AND METHODS

Plant collection

Lilium candidum areal part were collected from a nursery in Babylon city. The plant parts were washed in tap water to remove dust and then in distilled water (DW), and dried in shade for 10 days at ambient temperature. The dried parts were ground and stored in airtight container and then refrigerated at ….. °C until use.

Total alkaloid extraction

Total alkaloids were extracted according to Harborne [12]. Briefly, 20g of plant powder was extracted with 80% methanol for 24h with continuous extraction by Soxhlet apparatus then filtered. The filtrate was concentrated by a rotary evaporator under vacuum at 45°C until the solution reached to 10ml. Subsequently, the concentrated extract was transferred to a separating funnel and 2N HCl was added gradually to adjust the pH to 2, after that the extract was washed with 10ml chloroform three times. Then, the pH of the extract was adjusted to 10 using NH_4OH, and partitioned with 10ml chloroform three times. The chloroform portion was dried to obtain the total alkaloid extract. The dried extract was weighed, and preserved in a strile container at 4°C for further investigation.

Qualitative detection of alkaloids

To detect the presence of alkaloids in the plant extract, some qualitative tests were performed using Mayer's and Dragendorff's reagents .Mayer's reagent used to screen all types of alkaloids, prepared by dissolving 13.5g of mercuric chloride and 5g of KI in 1000ml distilled water. The test was done by adding 1-2ml of the reagent to 5 ml of the plant extract. The formation of white or creamy precipitate indicates a positive result [13]. Furthermore, Dragendorff's reagent was used to investigate alkaloids in the plant extract. The reagent was prepared by dissolving 20g of bismuth nitrate in 40ml distilled water and 16g of sodium iodide in 40 ml distilled water, then, the two solutions were mixed. The test was performed by adding 1-2 ml of Dragendorff's reagent in 5 ml of the plant extract, the formation of a prominent orange color indicated that the test was positive [14].

Total alkaloid content estimation [15, 16]

Total alkaloid was estimated using bromocresol green (BCG) (Thomas Baker, India) spectrophotometry method. The BCG reagent was prepared by mixing 69.8mg of BCG with 3ml of (2N) NaOH and (5ml) D.W. with heating until completely dissolved then, the volume was adjusted to 1000ml.

BCG assay

Plant extract (10mg) was dissolved in 1ml of 2N HCl, then filtered. The solution was washed with about 10ml of chloroform, three times, in a separating funnel then, the pH of the extract was adjusted to 7 using 0.1N NaOH. About 5ml of BCG solution and 5ml of phosphate buffer (pH 4.7) were added with gentle mixing. The complex was extracted with 1, 2, 3, and 4ml chloroform with vigorous shaking. The extract was then collected and diluted with chloroform. The absorbance was measured at 470nm [16]. The total alkaloids were calculated according to the standard curve of Quinine. The standard curve was constructed using (0.4, 0.8, 1.2, 1.6, and 2ml) dilutions of Quinine standard solution (0.1mg/1ml) [15].

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay

Two kinds of cell lines including MCF-7 and normal fetal hepatocyte WRL-68 (control) were used to assay the cytotoxic activity using MTT dye (Sigma-Aldrich, USA).

Cultured cell were washed then trypsinized before being plated in a 96-well, plate, with about $5x10^3$ cells per well for MCF-7 cell line, while $5x10^4$ cells for WRL-68 cell line. After 24h of incubation at 37°C, diluted extract (50, 100, 200 and 400µg/ml) were added in triplicates in addition to control (untreated cultures).and incubated at 37°C for 4h in a humidified 5% CO_2 incubator. The supernatant was removed from the wells and cells were incubated for their respective doubling times (average 48h). After the incubation 10µl of MTT dye (5mg/ml in PBS) were added to each well and were incubated again at 37°C for 4h. About 50µl of dimethyl sulfoxide (DMSO) (SDFCL, India) was added into each well. After complete solubilization of the dye, the absorbance was read at 620nm. Cell viability was evaluated using the following formula [17, 18].

$$\%\text{Cell viability} = \frac{\text{Mean absorbance of treated sample}}{\text{Mean absorbance of non-treated sample}} \times 100$$

Statistical Analysis

SPSS software (one-way analysis) was used and data with $p \leq 0.05$ were considered significant.

Results And Discussion

Both the qualitative and quantitative analysis of all extracts showed high level of alkaloids by changing the color in each regent. The total alkaloids were calculated depending on the calibration curve of Quinine (Figure 1). Tables 1 and 2 show the total alkaloid contents, about 345.6 mg/100 g and this agree with Shamsa F.(2008) [15]. The results of cell viability assay based on the MTT assay using MCF-7 and WRL-68 cell lines which treated with total alkaloid extracts in different concentrations, showed high percentage of cytotoxicity in correspondence with concentration as shown in Table 3. The highest reduction percentage of viability was observed at the highest concentration (400µg/ml) was 52.25 ± 3.70 % for the breast cancer compared with normal cell lines (85±3.3%) in same concentration.(

The result agree with Tokgun *et al* [19] where the methanolic extract of *L. candidum* has strong anti-proliferative against MCF-7 cells but disagree with [11] who reported that *L. candidum* alkaloids might cause DNA damage that have negative effect on normal cells. Some other alkaloids might increase the proliferation of cancer cells at low concentration [20].

Table 1: Qualitative detection of alkaloids in *Lilium candidum* plant extract using different reagents.

Reagent	Result	Resulted color
Mayer's reagent	+	Creamy precipitate
Dragendorff's reagent	+	Orange color

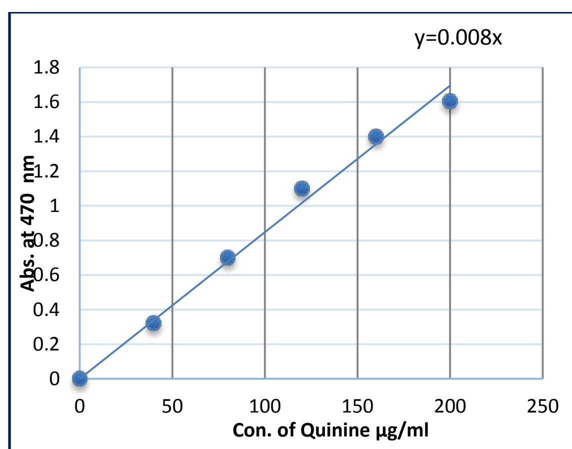

Figure 1: Standard curve of the Quinine alkaloid using BCG method at 470nm

Table 2: The total alkaloid contents of *Lilium candidum*

Plant	Part used	Amount in mg/ 100 g of plant DW ± SD
Lilium candidum	Leaves	345.6 ± 1.8

Table 3: Cytotoxic activity of the total alkaloids of *Lilium candidum L.* against MCF7 and WRL-68 cell lines.

Alkaloid extract conc. µg/ml	WRL-68 Mean%± SD	MC-7 Mean%± SD	IC_{50} of MCF-7 µg/ml
400	85±3.3	52.25 ± 3.70	244.8
200	91±2.01	74.75 ± 1.80	
100	92.9±5.2	77.45 ±4.00	
50	93.33 ±3	81.22 ± 2.20	

Conclusion

Lilium candidum leaves' extract have high alkaloids content. These alkaloids show high cytotoxicity against MCF-7 cells compared with normal cell line, which may find a potential application in the area of anticancer therapy.

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