

Anti-Cancer Activity of *Opuntia polyacantha* Alkaloid Extract on Human Breast Cancer Cell Line

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Abstract

Cancer as one of the major killer disease is getting tremendous concern in research and demands a proactive strategy for protection and treatment. Research on plant secondary metabolites revealed a promising safe alternative anti-cancer agents. Alkaloids are naturally occurring organic nitrogen containing compounds that have many biological activities including anti-cancer. Alkaloids were extracted from *Opuntia polyacantha* plant by methanol (80%) and chloroform and then estimated quantitatively and qualitatively. The MTT viability assay used to determine alkaloids cytotoxicity by using MCF-7 and WRL-68 cell lines. The results showed that the MCF-7 cell line growth was significantly decreased by 52.7% at 400µg/ml total alkaloid concentration with 91.89% viability of WRL-68.

The extracted alkaloids found toxic to cancer cell line with negligible effect on normal cell line proliferation that make it a promising safe anti-cancer alternative drug.

Keywords: *Opuntia polyacantha*, Anti-cancer, alkaloids, MCF-7.

INTRODUCTION

Malignant tumors where abnormal cell proliferation, invasion and sometimes metastasis might be caused by different causes like radiation and carcinomas chemicals [1, 2]. Cancer as one of the major killer disease is getting tremendous concern in research and demands a proactive strategy for protection and treatment. Research on plant and microbial secondary metabolites revealed a promising safe alternative anti-cancer agents [3].

Using phytopharmaceutical substances, is an old practice in treating diseases that has led to the discovery of more than 50% of all modern medicine as complimentary or alternative drug either to prevent or ameliorate many diseases in recent years.[4]. Recently, many herbs have been screened for anti-cancer activity *In vitro* and *In vivo* as an alternative drug or in combination with chemotherapy [5]. The use of Polyphenolic compounds including alkaloids that are produced as secondary metabolites by plants and classified according to its biosynthetic pathways, have many bioactive properties including anti-cancer activities [6-8].

Opuntia (prickly pear cactus) which belongs to Cactaceae family is a xerophytic plant with 200–300 species. They are widely spread in nature and available all year long. It is mainly cultivated as an ornamental plant and for its delicious fruit. It is well known for health benefits since the first century. They have many bioactivities such as analgesic, anti-inflammatory, Anticancer, Antidiabetic, Anti-hyperlipidemic and Antiulcer (9). Few studies concerning the bioactivity or safety of cactus and most positive results disagree with other most recent reports especially those related with skin and hair treatments [10]. Cactus pear extract proved to act as chemoprotective through inhibiting tumor growth with unknown mechanism [11]. It was reported that the anti-cancer activity is mostly because of bioactive polysaccharides that induce cell apoptosis or cell cycle arrest and angiogenesis suppression with minor toxicity on normal cells [12]. Add to that, *Opuntia polyacantha* polysaccharides proved to act as immunotherapeutic adjuvant through activation of nuclear factor (κB) [13].

MATERIALS AND METHODS

Total alkaloid extraction

The aerial part of *Opuntia polyacantha* plants was cut and washed with distilled water (DW). The plant parts were let to dry in shade for ten days at room temperature. Alkaloids were extracted according to Harbone [14], 100g of dried plant parts were grounded and then extracted with methanol for 24 using Soxhlet apparatus. After filtration of the extract, methanol was evaporated

to dryness by rotary evaporator under vacuum at 45°C. About 10ml of 2N HCl was added to dissolve the extract then filtered. 1ml of this solution was washed three times with 10ml chloroform in a separation funnel. The pH was adjusted to 10 using NH₄OH, then partitioned three times with 10ml chloroform. The chloroform layer was collected and dried. The dried extract was weighed, and stored in a sterile container at 4°C.

Qualitative detection of alkaloids

Alkaloid presence was confirmed by Dragendroff's reagent [15]. Orange crystalline precipitate formation indicates presence of alkaloid compounds. The sample which showed positive alkaloid was then subjected to further quantitative evaluation using Hager's test. Yellow colored precipitate formation indicate the presence of alkaloids [16].

Quantitative Estimation of total alkaloid

Bromocresol green (BCG) (Thomas Baker, India) was used to estimate total alkaloid using spectrophotometry method. The BCG solution was prepared by mixing 69.8mg of BCG with 3ml of 2N NaOH and 5ml DW then dissolved by heating. The volume was adjusted to 1000ml.

BCG assay: After the total alkaloids extraction (mentioned above), the pH was adjusted to 7 using 0.1 N NaOH. About 5ml of BCG solution and 5ml of phosphate buffer were added and mixed well. The complex further was extracted with 1, 2, 3 and 4ml chloroform with shaking. The absorbance of the solution was measured at 470nm. The total alkaloids were calculated according to of Quinine standard curve. The standard curve was constructed using (0.4, 0.6, 0.8, 1 and 1.2ml) of Quinine standard solution (0.1mg/1ml). [17]

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

Breast cancer cell line (MCF-7), and normal epithelial cell (WRL-68) were cultured on RPMI 1640 in CO₂ 5% at 37°C for 3 days then the medium was descended then cells were washed with PBS. Cells were detached using a trypsin /EDTA solution for 2min then resuspended culture medium supplemented with 10% fetal bovine serum. Cells were collected then centrifugation at 2000rpm for 10min. The cell pellet was resuspended in 1ml of media. The cells were seeded (10⁴ cells/well) in 96-well microtiter plates with 200µl of media in each well (in triplicates). The culture was incubated at 37°C and 5% CO₂ for 24 h. Alkaloid extract in different concentrations (50, 100, 200 and 400 µg/ml) were prepared and added to the cells were added in triplicates in addition to control (untreated cultures) then incubated at 37°C in 5% CO₂ for 4h. The medium then removed from the wells and

cells were incubated for 48h. About 10 μ l of MTT dye (Sigma-Aldrich, USA) (5mg/ml in PBS) were added to each well and were incubated again at 37°C for 4h. The plates were read at 570nm and the viability of cells was calculated [18].

$$\text{Cells viability} = \frac{\text{OD for treatment}}{\text{OD for control}} \times 100$$

RESULTS AND DISSECTION

Table (1) show alkaloids detection using Dragendorff's reagent and Hager's test, the result was positive in both tests. Table (2) show the total content of alkaloids were expressed as mg equivalent of Quinine per 100g plant powder. The concentration of total alkaloids in *Opuntia polyacantha* L. areal parts was (82.87 \pm 2.8) mg/100g powder.

The cytotoxicity assay tested against MCF-7 and normal cell line by MTT assay show high cytotoxic activity for cancer cell (57.20 \pm 6.20% viability) compared to normal cells (91.89 \pm 4.50% viability) at 400 μ g/ml of alkaloid extract with IC₅₀ 168.6 μ g/ml (Table 3).

Table 1: Qualitative detection of alkaloids in *Opuntia polyacantha* plant extract using different reagent

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

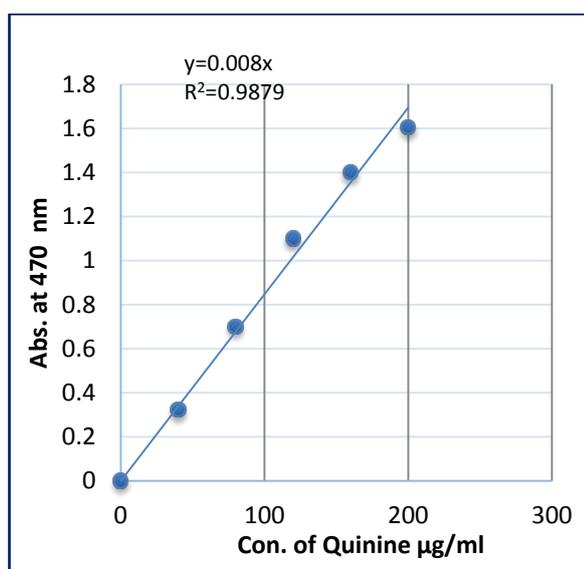


Figure 1: Calibration curve of the Quinine using BCG methods at 470nm

Table 2: The total alkaloid contents of *Opuntia polyacantha*

Plant	Part used	Amount in mg/ 100 g of dry plant \pm SD
<i>Opuntia polyacantha</i>	Aerial	82.87 \pm 2.8

Table 3: Cytotoxic activity of the total alkaloids of *Opuntia polyacantha* against the MCF-7 and WRL-68 cell lines.

Alkaloid extract conc. μ g/ml	% Viability of WRL-68 \pm SD	% Viability of MC-7 \pm SD	IC ₅₀ of MC-7 μ g/ml
400	91.89 \pm 4.50	57.20 \pm 6.20	168.6
200	90.5 \pm 3.30	70.8 \pm 3.27	
100	94 \pm 1.20	85.40 \pm 7.09	
50	94.60 \pm 3.13	93.63 \pm 2.50	

The result agree with [19, 20] where the Alkaloids proven to have antitumor activity against breast, colorectal, ovarian and other

tumor types. Canals *et al* [21] found that some plant alkaloids inhibit topoisomerase II in MCF-7 cancer cells. However, Neurotoxicity, hepatotoxicity, immunotoxicity, embryonic toxicity and reproductive toxicity were reported for some alkaloids [22, 23].

CONCLUSION

Opuntia polyacantha alkaloid extract show high cytotoxic activity against MCF-7 cells compared with its minor cytotoxic effect on normal cell line, which may open a new research in cancer therapy as either an alternative drug or immunoadjuvant agent especially its safety and plant availability.

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