Research Article

In vitro Anticancer Activity of *Caralluma acutangula* (Decne.) N.E.Br. Extract

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ABSTRACT

In the present investigation, the methanolic extract of *Caralluma acutangula* was screened (using the MTT colorimetric assay) for its *in vitro* inhibition capacity in two human cancer cell lines (hepatocellular carcinoma (HEPG2) and breast cancer (MCF-7) in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. The anticancer activity results indicated that the plant extract showed growth inhibition activity against the tested cancer cell lines but with varying intensities extents from moderate to strong growth inhibition in comparison to the anticancer drugs, 5-Flurouracil and Doxorubicin. Our data demonstrated that the methanolic extract of *Caralluma acutangula* has a potential cytotoxic and anticancer activity on the two cancer cell lines.

Keywords: *Caralluma acutangula*, MTT assay, cytotoxicity, anticancer activity, human cancer cell lines.

INTRODUCTION

Cancer is a life-threatening disease, accounted for 8.2 million deaths, in 2012.[1,2] According to World Health Organization, the annual cancer cases will rise from 14 million in 2012 to 22 million within next two decades[2] It is second most occurring disease after cardiovascular disease and causes a great burden to both single human lives and the society as a whole.[3] Although there has been good progress in the development of prevention and treatment of cancer, the successful treatment of cancer still remains a challenge. Cancer cells often adapt to develop resistance to commonly used chemotherapeutic agents. Therefore, it is important to develop novel chemotherapeutic agents, which are more potent tumor-selective cytotoxic agents and can circumvent drug-resistant cancer cells. Natural products have for long played an important role in drug discovery, especially in the area of cancer pharmacology. Many natural or natural based anti-tumor drugs such as bleomycin, doxorubicin, mitomycin, vinblastine, vincristine, etoposide (VP16), topotecan, irinotecan, paclitaxel, and combretastatins have been clinically used in recent years[4]. These drugs have not shown any improvement in survival, and severe adverse effects have been frequently observed in treated patients. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Therefore, the identification of new anti-cancer drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology[5].

Southwestern of Saudi Arabia is rich of wild plants, comprise about 70% of total flora of Saudi Arabia. One of the largest families in this part of the country is Apocynaceae. Many species of this family is used for the treatment of many diseases by the traditional medicine practitioners of Saudi Arabia[6].

The genus *Caralluma* belongs to the family Apocynaceae (sub family Asclepiadoideae), which comprises some 200 genera and 2500 species. Plants belonging to this genus are rich in esterified polyhydroxypregnane glycosides, some of which showed antitumor activity and others were postulated as precursors of cardenolides[7]. The genus is also characterized by the presence of flavone glycosides[8].

Certain species of *Caralluma* are edible and form part of the traditional medicine system of many countries[9]. These are commonly used in folk medicine as remedies to treat wide variety of diseases and health conditions[10].

In Saudi Arabia this genus represented by 14 species found in west and southwest regions[11]. *Caralluma* has significant anti-inflammatory and antitumor activity[7,8,12], anticancer, cytoprotective and antiulcer activity[13,14], antinociceptive[15], antioxidant, hypolipidemic[16], antihyperglycemic[17], antidiabetic[18], treating paralysis and joint pains, antipyretic[19].

In some reports, it was observed that *C. fimbriata*, can be used in weight reduction[20,21]. Some active ingredients of *Caralluma* have been shown to possess antitumor and anti-cancer activities.

In our preliminary results, Aly and Masrahi, (2012) demonstrated that *C. acutangula* has effective action on breast, hepatocellular carcinoma and prostate cancer[22].

The aim of the present study was to determine the *in vitro* anticancer activity of methanolic extract of *Caralluma acutangula* against two human cancer cell lines, hepatocellular carcinoma cell line HEPG2 and breast carcinoma MCF-7 cell line on cell viability and growth

inhibition. The cytotoxic potency of the plant extract was studied in comparison to two known anticancer drugs, 5-Flurouracil (5-FU) and Doxorubicin (DOX).

MATERIALS AND METHODS

Our target plants, *Caralluma acutangula* was collected from different locations from South region of Saudi Arabia and was identified by one of us (Dr. Yahya Masrahi, Department of Biology, Faculty of Science, Jazan University, Saudi Arabia).

Preparation for extracts

The plant was collected, washed and dried. Then it was ground in a grinding machine to fine powder and passed through a 24-mesh sieve and the extract is weighted and stored at room temperature.

Extraction of plant material

The powdered sample (20g) of *Caralluma acutangula* was successively extracted with 200ml of solvent (methanol) using magnetic stirrer and stirred for 3hrs. Then it was filtered using whatmann filter paper. Again the residue was dissolved with 200ml solvent and stirred for 2hrs. The solvent containing the extract is dried under reduced pressure. The aqueous extract was prepared with 10g of powder in 100ml of distilled water& stirred for 3 hrs. The supernatant was boiled up to minimum volume.

Determination of anticancer activities

Cell culture

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Human hepatocellular carcinoma HepG2 and breast cancer MCF-7 cells were obtained from National Cancer Institute, Cairo University. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 µg of streptomycin/ml in a humidified incubator with 5% CO_2 at 37°C.

In vitro cell proliferation and cell viability assay—Trypan blue exclusion assay

Trypan blue exclusion assay was performed to assess the effect of methanolic extract of *Caralluma acutangula* on viability of HEPG2 and MCF7 cells. Approximately 0.75×10^5 cells/ml was seeded in a six well tissue culture plate and different concentrations of the extract were added after 24 h.

For the determination of growth rate, smaller aliquots were collected in a 0.5 ml tubes, trypan blue (0.4%) was added to the cell suspension, and the number of cells (viable-unstained and non-viable-blue) was counted using a haemocytometer.

The media was not changed during the induction period. Each experiment was repeated a minimum of three times.

MTT assay

The methanolic extract of *Caralluma acutangula* was subjected to a screening system for evaluation of its anticancer activity against hepatocellular carcinoma HEPG2 cell line and breast carcinoma MCF-7 cell line in comparison to the known anticancer drugs: 5-FU and DOX.

3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay was used to evaluate the antiproliferative activities of the tested extracts against the cancer cell lines. The assay depends on the cleavage of the tetrazolium salt (MTT) into formazan blue by the mitochondrial enzyme succinate dehydrogenase. The conversion takes place only in living cells and the amount of formazan produced is proportional to the number of viable cells present. Thus, the MTT assay is potentially useful for assaying antiproliferative activities of materials[23,24]. Exponentially growing cells (HEPG2 and MCF-7) were plated in triplicate in 96-well sterilized plates at a density of 1×10^4 cells/well. After 24 h, cells were treated with escalating doses of the extract under investigation and incubated in 5% CO_2 atmosphere with high humidity.

After 48 and 72 h of the extract exposure, the cells were incubated with MTT (0.5 mg/ml) for another 4 h at 37°C. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of N,N dimethylformamide and 10% of sodium dodecyl sulphate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multiwell ELISA plate reader.

The mean absorbance of medium control was the blank and was subtracted. IC50 values (concentration of the extract causing 50% inhibition of cell growth) were estimated after 72 h exposure of the extract. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control.

The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and extract concentration is plotted to get the survival curve of both cancer cell lines with the specified extract.

A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of products.

RESULTS AND DISCUSSION

Cytotoxicity is the quality of being toxic to cells. Cells exposed to a cytotoxic compound can respond in a number of ways. Cytotoxicity assays are used widely in drug discovery research to predict which compounds might have safety concerns in humans before significant times and expense are incurred in their development[25,26].

Other researchers have studied the mechanisms of cytotoxicity as a way of better understanding of the normal and abnormal biological processes controlling cell growth, division, and death[27].

In this investigation, the methanolic extract of *Caralluma acutangula* were evaluated for their *in vitro* cytotoxic activity against human hepatocellular carcinoma (HEPG2) and breast carcinoma (MCF-7) cell lines using MTT assay. Doxorubicin and 5-Fluorouracil, which are two of the most effective anticancer agents, were used as a reference drugs.

Our results showed that the methanolic extract of *Caralluma acutangula* exhibits a moderate to strong growth inhibition between 0-50 µg/ml concentrations in comparison to the reference anticancer drugs.

The relationship between surviving fraction and extract concentration was plotted to obtain the survival curve of each of the two cell lines. The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. The extract of *Caralluma acutangula* showed cytotoxicity to the breast carcinoma cell line with IC50 values of 7.06±0.86 and 6.16±0.80 µg/ml respectively versus 4.21±0.45µg/ml for doxorubicin.

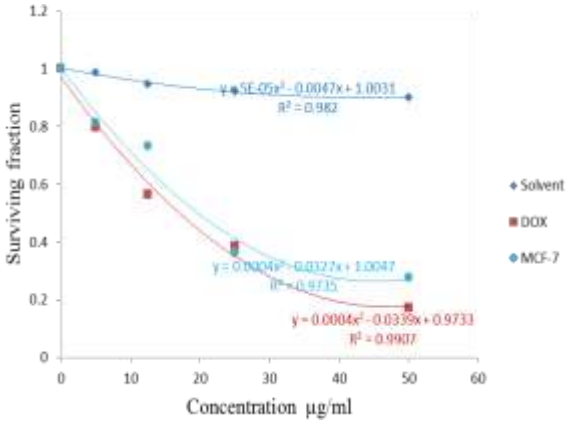

Figure 1: The cytotoxic activity of the methanolic extract of *Caralluma acutangula* against breast MCF7 cancer cell line.

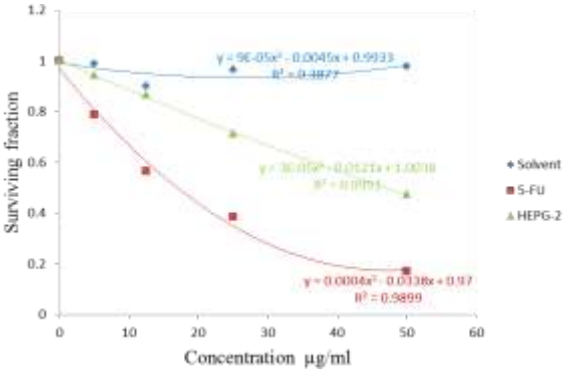

Figure 2: The cytotoxic activity of the methanolic extract of *Caralluma acutangula* against hepatocellular carcinoma HEPG-2 cancer cell line.

Figure 1 showed the cytotoxic activity of the methanolic extract of *Caralluma acutangula* against breast MCF7 cancer cell line. Whereas the extract showed cytotoxicity to the hepatocellular carcinoma cell line with IC50 values of 7.06±0.86 and 6.16±0.80 µg/ml respectively versus 4.21±0.45µg/ml for 5-fluorouracil. Figure 2 showed the activity against HEPG2 cell line in comparison to the reference drugs: 5-FU.

Our results indicate that the cytotoxic effect strengthens with increase in the concentration of extract. Due to the mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan produced is directly proportional to the number of viable cells[28]. Polyphenol compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells[29]. The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death[30]. The main objective of this assay is to check the cytotoxicity brought about by the extract and find the toxicity levels in terms of IC50 dose when live and dead cell percentages are equal, which is considered as the optimum dose for the various assays. It has been shown that the methanolic extract possesses antiproliferative activity at lower concentration.

The methanolic extract of *Caralluma acutangula* was cytotoxic to both cell lines, which was clearly observed when viewed under inverted microscope (Figure 3). MTT assay was used to evaluate cytotoxicity based on metabolic reduction of MTT. Thus, the methanolic extract of *Caralluma acutangula* is non-toxic to the normal cells and also has both anticancer and anti-proliferative activities against the cancerous cells.

The morphological changes of the cell lines treated cells with various concentrations of the methanolic extract of *Caralluma acutangula* were incubated for 24 h and compared with the untreated cells shown in Figure 3. Compared to control cells after the incubation period, morphology of the extract treated cancer cells significantly changed. The extract treated cells appeared less uniform with the loss of membrane integrity, although still intact at lower concentrations. Whereas at higher concentrations the extract treated cells showed remarkable difference with the control group. The significant changes such as loss of intact membrane, karyopyknosis, cell detachment from the plate, and

change of morphological features were evident when compared to untreated cells.

The most identifiable morphological features of apoptosis were observed by inverted light microscopy in the extract treated cells.

The treated cells appeared like cells undergoing apoptosis with prominent features such as detaching from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighbouring cells[31].

However the untreated cells appeared normal and were confluent.

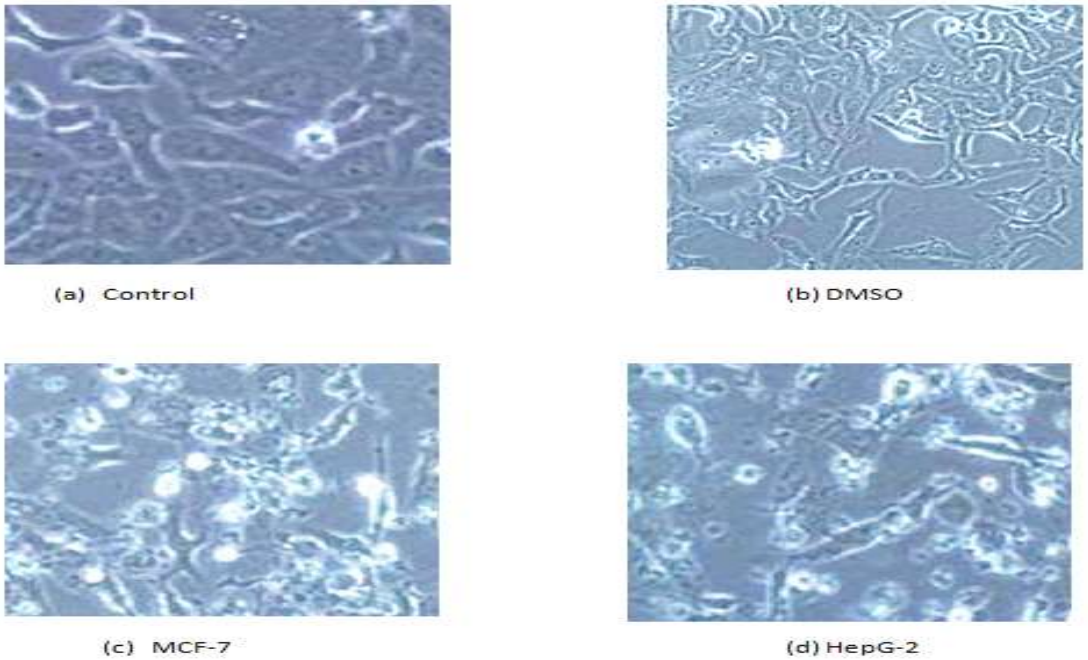

Figure 3: Morphological features of MCF-7 and HepG-2 cells after 24 h treatment with the methanolic extract of *Caralluma acutangula*. There were no significant visible differences in both control and 0.5% DMSO treated cells (a and b). The extract treated cells (c, and d) after 24 h showed loss of intact membrane, loss of contact with neighbouring cells, condensed and detached from the culture plate.

CONCLUSION

Chemoprevention and dietary modification studies are underway to identify promising candidates for reduced cancer risk. It is concluded that the methanolic extract of *Caralluma acutangula* possess anticancer properties against hepatocellular carcinoma HEPG2 cell line and breast carcinoma MCF-7 cell line. Further experimental analysis on this plant would definitely reveal the important chemical constituents responsible for cancer cell death because the probable inhibitions with active principles/chemical constituents would be higher than the extracts (due to the presence of mixture of varied constituents).

Thus, the current work on identification and evaluation of anticancer activity of *Caralluma acutangula* may prove its importance in improving human health. The elucidations of their mechanisms as cancer therapeutics are warranted.

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