



## Cytotoxic Activity of *Citrullus colocynthis* (L.) Schrad Fruit Extract on Gastric Adenocarcinoma and Breast Cancer Cell Lines

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### ABSTRACT

Cancer is one of the most common diseases in developed and developing countries. Medicinal plants due to some benefit are used to treat cancer in all centuries. Therefore, the aim of this study was investigate the anti-proliferative and cytotoxic activity of the hydro-alcoholic extracts of *Citrullus colocynthis* (L.) Schrad on the AGS and MCF-7 cell lines. After preparation extracts by maceration procedure, different concentrations (control, 0.001, 0.01, 0.1 and 1 mg/ml) of *C. colocynthis* (L.) Schrad fruit extracts were added to cell lines and incubated for 24, 48 and 72 h. The viability of cells was evaluated by MTT Assay. Then optical density of each cell was readied by ELISA at 570 nm. Our results were showed that hydro alcoholic extracts of *C. colocynthis* (L.) Schrad had significant anti-proliferative effect on MCF7 and AGS cell lines. Data analysis was showed that there were significant differences in cell viability after 24, 48 and 72 h. These differences were showed in 72h a dose-dependent ( $p < 0.001$ ). In addition, this cytotoxicity effects is induced by apoptosis, probably. Finally, our findings suggest further research on *Citrullus colocynthis* (L.) Schrad, a potential chemotherapeutic agent against MCF-7 and AGS cell lines in gastric adenocarcinoma and breast cancer, respectively.

**Keywords:** *Citrullus colocynthis*, Breast cancer, Cytotoxicity, MTT, Medicinal plant.

### INTRODUCTION

Cancer is one of the mortal disease and important public health subjects in both developed and developing countries<sup>1, 2</sup>. Cancer belongs to a group of diseases which cells abnormally are proliferating and extending in the adjacent tissues. Uncontrolled division of cells can lead to create tumors<sup>3</sup>. Breast and gastric cancer are the five most common types of cancer which diagnosed in 2012<sup>4, 5</sup>. According to the World Health Organization comments, breast cancer is the most prevalent cancer among women worldwide and very rare in men. It is the second most common cancer in women after skin cancer in USA. Each year there are about 2,300 new cases of breast cancer in men and about 230,000 new cases in women. Stomach cancer known as gastric cancer is the fifth leading cause of cancer and the third leading cause of death from cancer making up 9% of deaths. It infected 950,000 people and caused 723,000 deaths, in 2012<sup>5</sup>.

Therefore, offering pivotal treatment options will play an important role in development society health<sup>6</sup>. Surgery, radiation therapy, chemotherapy, hormone therapy and targeted therapy are used for treatment of cancer, but due to the lack of selective toxicity have negative side effects<sup>7</sup>.

Nowadays, medicinal plants have been considered as a natural source of anti-cancer agents due to some

advantages for example, having antioxidant compounds and anti-mutagenic effects against chemicals and environmental factors, low side effect, low cost, and being easily accessible, may play a significant role in the treatment of the cancer<sup>1, 6</sup>. *Citrullus colocynthis* (L.) Schrad, a valuable cucurbit plant, belongs to the family of Cucurbitaceae and native to Asia (Figure 1).



**Figure 1:** *Citrullus colocynthis* L.

The fruit of *Citrullus colocynthis* (L.) Schrad is locally called bitter apple/colocynth in English and traditionally have been used for treatment diseases such as diabetes, jaundice, asthma, ulcer, inflammation, urinary, microbial diseases and cancer<sup>7-10</sup>. Phytochemical studies have shown that this plant contains flavonoids, alkaloids, carbohydrates, fatty acids, essential oils and especially colocynth fruit is a main source of cucurbitacins<sup>7, 11</sup>.

In the current study, we investigated the anti-proliferative and cytotoxic activity of the hydroalcoholic extracts of *Citrullus colocynthis* (L.) Schrad fruits on the AGS (Adenocarcinoma Gastric) and MCF-7 (Michigan Cancer Foundation-7) cell lines using MTT assay method.

## MATERIALS AND METHODS

### Reagents

RPMI 1640 medium, Fetal bovine serum (FBS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Phosphate Buffer Saline (PBS), Trypsin, Ethylene Diamine Tetra Acetic Acid (EDTA), antibiotic, and dimethylsulfoxide (DMSO) were purchased from Sigma.

### Plant Material and extraction

The fruits of *Citrullus colocynthis* (L.) Schrad were purchased from a local herb market. In addition, the fruits were confirmed by Professor Dr Mohammad Azadbakht and representative voucher specimens were deposited at the herbarium of the Department of Pharmacognosy in Faculty of Pharmacy of the Mazandaran University of Medical Science, Sari in Iran. Then, dried fruits and their seeds were powdered using an electric grinder. Extraction was performed using 80% ethanol by maceration method. Briefly, 300 g fine powders of fruits and seeds were macerated in 3 L of solvent by shaking for 48 h and filtering through Whatman paper for several times. The mixture was first concentrated using a rotary evaporator to remove the solvent and dried using freeze dryer. The dried extracts were weighed and stored at -20°C for further analysis<sup>12,13</sup>.

### Cancer cell lines and culture conditions

The MCF-7 and AGS cell lines were purchased from north research center-Pasteur institute, Amol, in Iran. Cell lines were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% antibiotic penicillin/streptomycin in a humidified incubator at temperature of 37°C and containing 5% CO<sub>2</sub>. When cells reached to 80% confluency, their medium were removed, and washed by PBS and separated by 1% trypsin-EDTA from bottom flask and centrifuged at 1500 rpm for 7 min. Cell sediments were prepared in 1 ml of culture medium to suspension. Then, 10 µl of cell extracts transferred to Neubauer slide and counted by Inverted microscope. Figure 2 shows the AGS and MCF-7 cell microscopic images.<sup>1</sup>

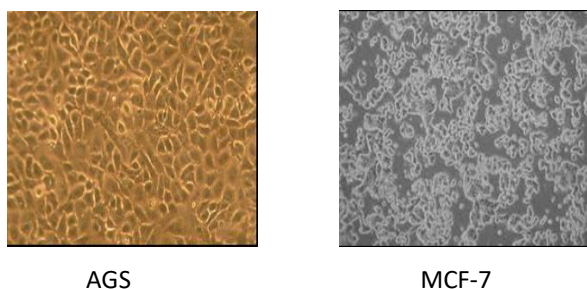


Figure 2: Cancer cell lines.

### Cytotoxicity assay

Anti-proliferative and cytotoxic activity of fruit extracts against AGS and MCF-7 cell lines were determined using MTT assay. Briefly, AGS and MCF-7 cells were implanted on a 96-wells microplate at a density of  $5 \times 10^3$  and  $10 \times 10^3$  with 100 µl medium, respectively. In addition, cells incubated at a temperature of 37°C in 5% CO<sub>2</sub>. After 24 h of incubation, medium of cell were removed and replaced by 200 µl of fresh medium with different concentration of extracts (0, 0.001, 0.01, 0.1, 1 mg/ml) and incubated for 24, 48 and 72 h. For each concentration of extracts repeated three well. After this period of time, the culture medium was discarded and washed by PBS. Then, MTT solution with concentration of 5 mg/ml was added to each wells and incubated for 3 h at 37 °C which their results were formed formazan crystals. Formazan crystals were dissolved using 100 µl of DMSO and incubated for 15 minute in the room temperature. Finally, optical density of each cell wells was readied by ELISA at 570 nm. Viability of the cell lines were compared with control group and IC<sub>50</sub> value of cells were calculated. In addition, Vincristine as a power cytotoxic drug (40µg/ml) was used as positive control. The experiment was performed in triplicate<sup>14,15</sup>.

### Statistical analysis

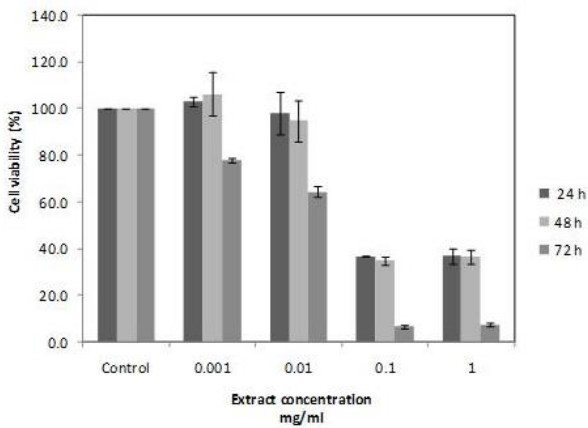
All data was analyzed statistically and their significance was evaluated by the Turkey's tests with critical value of  $P \leq 0.05$  using SPSS 21.0 software. The results are represented as mean  $\pm$  standard error of mean (SEM).

## RESULTS & DISCUSSIN

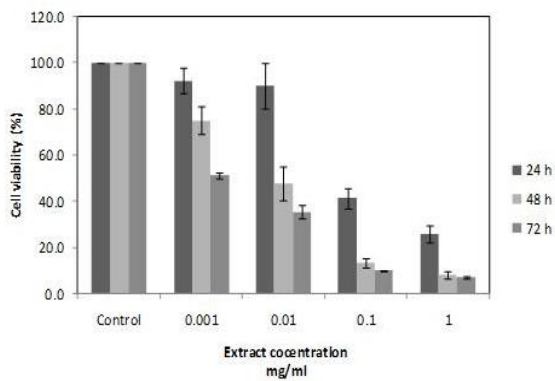
Cytotoxic effect of different concentration of the hydroalcoholic extracts *Citrullus colocynthis* (L.) Schrad fruits on the AGS and MCF-7 cell lines are demonstrated in figure 3 and 4.

AGS cell growth was analyzed after 24, 48 and 72 h. Data analysis showed that there were significant differences in cell viability after 24, 48 and 72 h. This differences in 72h a dose-dependent was showed ( $p < 0.001$ ). Statistical analysis showed that there was no significant difference in viability of AGS cell line between 0.001 mg/ml and 0.01 mg/ml for 24 and 48 h, but there was significant difference between 0.1 mg/ml to 1 mg/ml concentration for 24 and 48 h ( $p < 0.001$ ).

Cytotoxic effect of *Citrullus colocynthis* (L.) Schrad fruits against MCF-7 after 24, 48 and 72 h were determined and extract of *C. colocynthis* (L.) Schrad fruits fruit showed inhibitory effect on MCF-7 cell line. Data analysis showed that there was no significant difference in cell viability between 0.001 mg/ml and 0.01 mg/ml concentration in 24 h but there was significant difference between 0.1 mg/ml and 1 mg/ml concentration after 24h and also between 0.001 mg/ml to 1 mg/ml in 48h ( $p < 0.001$ ). In addition, viability of MCF-7 cells after 72 h a dose-dependent cytotoxic activity was showed ( $p < 0.001$ ).

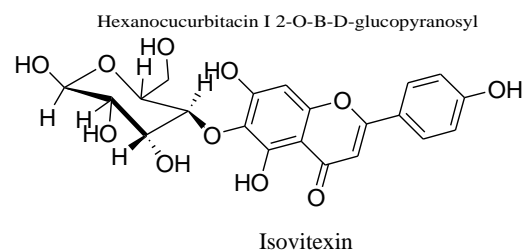
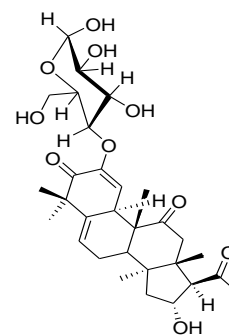
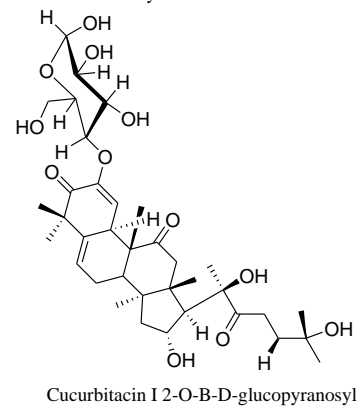
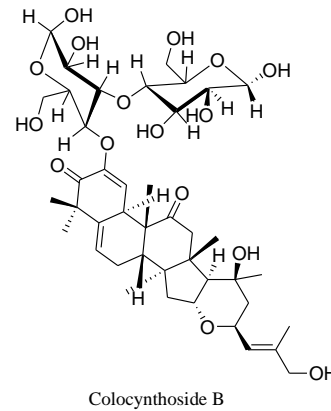
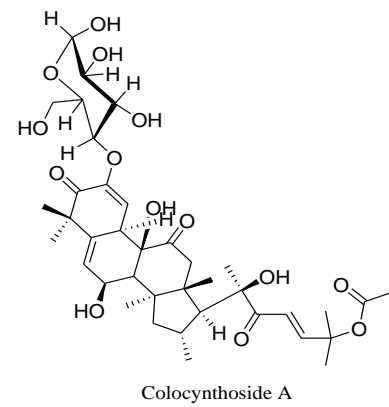
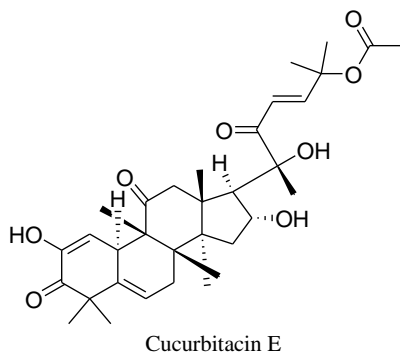


**Figure 3:** Cytotoxic effect of hydro alcoholic extracts of *Citrullus colocynthis* (L.) Schrad fruits against AGS cell line.



**Figure 4:** Cytotoxic effect of hydro alcoholic extracts of *Citrullus colocynthis* (L.) Schrad against MCF-7 cell line.

In recent years, medicinal plants have been used for cancer therapy research<sup>16-19</sup>. These plants due to their phytochemicals and low side effect are as suitable alternative and will prevent spreading of cancer<sup>20</sup>. *Citrullus colocynthis* (L.) Schrad belongs to Cucurbitaceae family is used in traditional medicine to treatment various disease<sup>7, 10</sup>. Therefore, *C. colocynthis* (L.) Schrad, as medicinal plant, is a potential cytotoxic for cancer cell lines. The results were showed that hydroalcoholic extracts of *C. colocynthis* (L.) Schrad fruits caused significant changes on MCF-7 and AGS cell lines in density 0.1 mg/ml and 1mg/ml. It seems, presence of different compounds in parts of the shell, core and pulp of the fruit, prevents growth and induces death in the cell lines.



**Figure 5:** Main phytochemicals in *C. colocynthis* (L.) Schrad.

*C. colocynthis* (L.) Schrad has phytochemicals including cucurbitacin E, colocynthoside A and B, cucurbitacin glucosides, flavonoids, tannins and sterols with antioxidant, antibacterial, and anticancer effects (Figure 5)<sup>11, 21</sup>.

It was reported an association between some flavonoids and breast cancer risk<sup>4</sup>. According to the type of cancer, different parts of the plant may be efficient for the treatment. Also, it was demonstrated that the fruit of *C. colocynthis* (L.) Schrad can decrease different cancer cell line proliferation<sup>22-24</sup>. These results could be an early step in examining and identifying of human gastric carcinoma and human breast anticancer effect of *C. colocynthis* (L.) Schrad. However, results obtained from our research were showed that the plant could be considered as a part of prevention and treatment of cancer protocols. According to the variety and large extent of plants, researchers have a long way for the investigation and disquisition in this regard.

### CONCLUSION

According to the results, *C. colocynthis* phytochemicals are cytotoxic to AGS and MCF-7 cell lines. We conclude that this plant and its compounds may act as potential chemotherapeutic drugs for the treatment of breast and gastric adenocarcinoma cancer.

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