Cytotoxic activity of the root extracts and isolated compounds from *Bryonia aspera* Stev. ex Ledeb were evaluated against three cancer cell lines (MCF7, HepG2 and WEHI) and one normal cell line (MDBK) using MTT assay. *Bryonia aspera* is used for the treatment of cancer and various health problems by the local healers throughout the Golestan province in the northern part of Iran. Different fractions of *Bryonia aspera* showed antiproliferative activity against human breast cancer cell line MCF7 and normal cells MDBK and the activity was much more pronounced at the chloroform fraction. Therefore, compounds isolated from chloroform extract have been selected for further cytotoxicity evaluation. Among the compounds, neocucurbitacin C and cucurbitacin L showed promising cytotoxic activity.

**Keywords:** *Bryonia aspera*, Cytotoxic, Cucurbitacin, MTT assay

**INTRODUCTION**

Plants have been a prime source of highly effective conventional drugs for the treatment of many diseases. There are different approaches for the selection of plants that may contain new biological agents. In the ethnomedical approach, credence is given to oral or written information on medicinal use of the plant and based on this information the plant is collected and evaluated. A retrospective analysis of the NCI program showed that the percentage of active leads based on ethnomedicine was substantially above that based on taxonomy, which itself was more than the active leads identified through random screening. The ethnomedical value of plants provides evidence of their biological activity that can be further utilized for the drug discovery process.

Cucurbit plants were early recognized in folk medicine to have biological values. They were used actively as traditional herbal remedies for various diseases and demonstrated anti-inflammatory, anti-tumor, hepatoprotective and immunomodulatory activities.

Ethnopharmacological information indicates that roots of *Bryonia aspera* Stev. ex Ledeb from the family cucurbitaceae, locally known as "andaz", are used in the Turkmen Sahra region, in the north of Iran for the treatment of cancer, liver problems and digestive disorders.

The present study was undertaken to evaluate the cytotoxic activity of the root extracts and some chemical constituents of *Bryonia aspera* against cancer and normal cells.

**Previously Isolated Compounds**

Previous phytochemical study on *Bryonia aspera* resulted in the isolation of some cucurbitane-type triterpenoids including: dihydrocucurbitacin D, iso dihydrocucurbitacin D, dihydrocucurbitacin B, epi-iso dihydrocucurbitacin B, cucurbitacin L, neocucurbitacin C, 7β-hydroxy dihydrocucurbitacin D, 25-O-glucosyl dihydrocucurbitacin D and 2-O-glucosyl dihydrocucurbitacin D. Isolation and structure elucidation data of compounds have been reported.

**MATERIAL AND METHOD**

**Plant material**

The roots of *Bryonia aspera* were collected from the Turkmen Sahra, Golestan province, Iran, in July 2006 and were identified by Mr. Ghorbani and Mr. Moazeni, Traditional Medicine & Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Voucher specimen of the plant (TMRC 252) has been deposited in the herbarium of the Traditional Medicine & Materia Medica Research Center.

**Preparation of Extracts**

The root of plant was air dried at room temperature, powdered and then successively extracted with solvents of decreasing lipophilicity (petroleum ether, chloroform and methanol) by maceration and with constant shaking for 24 h. The plant extracts were then filtered and the solvent was evaporated under vacuum by means of a rotary evaporator and stored at 4°C before evaluating biological activities.

**Cytotoxic Screening**

**Cell Culture**

Cell lines of human breast adenocarcinoma (MCF7), hepatocellular carcinoma (HepG2), mouse fibrosarcoma (WEHI) and Normal bovine's kidney epithelial cells (MDBK) were used for cytotoxic evaluation of plant extracts and compounds.

All cell lines were purchased from NCBI (National Cell Bank of Iran) and were cultured in DMEM and RPMI 1640 supplemented with 5% FBS (Gibco), 100 U/ml penicillin and 10 μg/ml streptomycyn. The cells were grown as monolayer in tissue culture flasks (BD Falcon) in humidified atmosphere under the conditions of 37°C / 5% of CO2 in incubator.

**MTT Assay**

The MTT assay was used to measure the cytotoxicity. Briefly, 1x10^4 cells were seeded into a 96-well plate and 24 h later cells were washed and maintained with different concentrations of extracts for 3 days, at 37°C under 5% CO2 atmosphere. The initial concentration of fractions and pure compounds were 100 μg/ml and 50 μg/ml in DMSO, which were serially diluted in complete culture medium to give six concentrations and added to cells in triplicate. After 72 h incubation, the medium in each well was replaced with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazodium bromide), and 4 h later DMSO was added to dissolve the formed violet formazan crystals within metabolically viable cells. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. The plates were soaked for 20 min and then the optical density was measured at 570 nm with a microplate reader. Non-treated cells were used as negative control and IC50 was calculated as the concentration of fractions and compounds causing a 50% inhibition of cell viability.

**RESULT AND DISCUSSION**

In this study the in vitro cytotoxic activity of extracts and isolated compounds from *Bryonia aspera* were evaluated. The plant was selected following an ethnomedical survey on plants of turkmen sahra region in Iran. In order to evaluate local uses of the root of *Bryonia aspera* as a treatment of cancer, petroleum ether,
Dihydrocucurbitacin D decreases data confirm that compound's hydrophilicity decreases in vitro cytotoxicity. Besides, glucosyl dihydrocucurbitacin D and 2-O-glucosyl dihydrocucurbitacin D) eliminate the cytotoxicity. Since the presence of glucose increases the basal toxicity of cucurbitacins greatly both the polarity and chemical modifications. The potent activity exhibited by cucurbitacin L and neocucurbitacin C suggests that these compounds could be developed further as anticancer drugs.

REFERENCES
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