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Research Article

CYTOTOXIC ACTIVITY OF EXTRACTS AND PURE COMPOUNDS OF *BRYONIA ASPERA*

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ABSTRACT

Cytotoxic activity of the root extracts and isolated compounds from *Bryonia aspera* Stev. ex Ledeb was 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 cucurbitacins 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[1]. 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[2]. 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[3]. The ethnomedicinal value of plants provides evidence of their biological activity that can be further utilized for the drug discovery process.[4]

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.[5]

Ethnopharmacological information indicates that roots of *Bryonia aspera* Steven. 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[6,7].

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-Oglucosyl dihydrocucurbitacin D and 2-O-glucosyl dihydrocucurbitacin D. Isolation and structure elucidation data of compounds have been reported[8].

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 streptomycin. The cells were grown as monolayer in tissue culture flasks (BD Falcon) in humidified atmosphere under the conditions of 37°C / 5% of CO_2 in incubator.

MTT Assay

The MTT assay was used to measure the cytotoxicity[9]. Briefly, 1×10^4 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% CO_2 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]-2,3-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.[10] The plates were shacked 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 IC_{50} 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 ethnobotanical 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,

chloroform and methanol extracts were assayed against different tumor cells, including: MCF7 (human breast adenocarcinoma), HepG2 (hepatocellular carcinoma), WEHI (mouse fibrosarcoma) and one normal cell MDBK (bovine's kidney epithelial cells). The antiproliferative activity was measured using MTT assay and the results are shown in Table 1.

Table 1: The cytotoxicity (IC_{50} (µg/ml), mean, n=3) of the fractions obtained from *Bryonia aspera*

Cell lines	**MCF7**	**HepG2**	**WEHI164**	**MDBK**
Bryonia aspera				
Methanol extract	14.2	>100	>100	51.05
Chloroform extract	4.65	>100	>100	23.88
Petroleum ether extract	21.2	>100	>100	86.54
5-fluorouracil	5.06	N.D[a]	N.D[a]	36.17
tamoxifen	3.69	4.38	8.19	6.5

[a] N.D = not determined

Activity was attributed to the plant extracts expressing IC_{50} < 100 µg/ml. The conventional anticancer drugs in clinical use, 5-fluorouracil and tamoxifen were used as positive control. According to the data presented in table 1 none of the fractions showed important activity on cell lines HepG2 and WEHI, but all of them were cytotoxic on MCF7 cell line with IC_{50} <100 µg/ml. Although petroleum etheric and methanolic fractions demonstrated good activity on MCF7 cell line, antiproliferative activity was more pronounced at the chloroformic fraction in tumor cell line as well as normal cells. Moreover the IC_{50} obtained from chloroformic fraction is similar to antineoplastic drugs 5- fluorouracil and tamoxifen in MCF7 cell line. Therefore isolated cucurbitacins from chloroformic extract were selected for further cytotoxic evaluation. The cytotoxic properties of these compounds were further investigated on three cell lines (MCF7, HepG2 and MDBK) and the limit of activity was defined as IC_{50}< 50 µg/ml. The results are summarized in Table 2.

Table 2: Cytotoxic activity (IC_{50} (µg/ml); mean, n=3) of compounds isolated from chloroformic extract of *Bryonia aspera*

Cucurbitacins	**MCF7**	**HepG2**	**MDBK**
Dihydrocucurbitacin D	> 50	> 50	9.3
Iso dihydrocucurbitacin D	21.46	41.16	> 50
Dihydrocucurbitacin B	41.07	>50	>50
Epi-iso dihydrocucurbitacin B	23.86	> 50	> 50
Cucurbitacin L	12.2	> 50	> 50
Neocucurbitacin C	13.05	> 50	>50
7β-hydroxy dihydrocucurbitacin D	>50	> 50	> 50
25-O-glucosyl dihydrocucurbitacin D	> 50	>50	> 50
2-O-glucosyl dihydrocucurbitacin D	> 50	> 50	> 50

None of the compounds except dihydrocucurbitacin D and iso dihydrocucurbitacin D exhibited more than 50% inhibition at 50 µg/ml on HepG2 and MDBK cells. Most of the compounds demonstrated to be more active on MCF7 cells (IC_{50} = 12.2 – 41.07 µg/ml). Some of them showed strong inhibitory activity and especially high was the inhibition for cucurbitacin L and Neocucurbitacin C on MCF7 cells (IC_{50} = 12.2, 13.05 µg/ml) but glycosylated compounds and 7β-hydroxy dihydrocucurbitacin D had no cytotoxic effect on cell lines.

As drug development requires analogues that enhance the biological activity[11], naturally occurring analogous isolated from this plant provides an opportunity to evaluate the effect of structural changes on cytotoxic activity of compounds.

Lipophilicity is one of the major factors that influences the transport, absorption, and distribution of chemicals in biological systems[11]. Since the presence of glucose increases greatly both the polarity and the volume of the structure, glycosylation at C-25 and C-2 (25-O-glucosyl dihydrocucurbitacin D and 2-O-glucosyl dihydrocucurbitacin D) eliminate the cytotoxicity. Besides, existence of a hydroxyl group on C-7 in compound 7-β- hydroxy dihydrocucurbitacin D decreases toxicity against cell lines. These data confirm that compound's hydrophilicity decreases *in vitro* cytotoxicity.

An enhancement of cytotoxic activity was observed by unsaturation of C-1 (cucurbitacin L *vs.* dihydrocucurbitacin D). It has been also previously found that presence of double bond between C1 and C2 increases the basal toxicity of cucurbitacins[12]. The presence of carbonyl at C-2 and hydroxyl at C-3 (iso form) seems to be more favorable than that of a carbonyl at C-3 and hydroxyl at C-2 for the cytotoxicity (dihydrocucurbitacin D *vs.* iso dihydrocucurbitacin D) Study on the structure activity relationship of naturally occurred cucurbitacins in plant *Bryonia aspera* indicated that the lipophilicity of compounds, unsaturation of C-1 and position of carbonyl and hydroxyl groups in the first ring were important for the cytotoxicity. These findings provide a clue for further chemical modifications.

CONCLUSION

The root extract from *Bryonia aspera* Stev. ex Ledeb. (cucurbitaceae) was tested for cytotoxic activity using MTT assay against MCF7, HepG2, WEHI and MDBK cell lines. The chloroform extract of root strongly reduced growth of cancer cells. Therefore, pure compounds isolated from this fraction were likewise tested, and some of them showed cytotoxic activity. Since the compounds neocucurbitacin C and 7β-hydroxy dihydrocucurbitacin D have been isolated and identified for the first time from the root of *Bryonia aspera*, this is the first report of their cytotoxic activity. The potent activity exhibited by cucurbitacin L and neocucurbitacin C suggests that these compounds could be developed further as anticancer drugs.

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