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Research Paper

Biological activities of *Artemisia changaica* Krasch grown in Mongolia

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ABSTRACT

Mongolia is rich in medicinal plants. In recent years, interest in plant-derived food additives has increased. Many *Artemisia* species have a characteristic scent or taste, which in many cases are the reason for their application in Mongolian traditional medicine. This study was conducted to evaluate the antioxidant and cytotoxic activities of aerial parts and ethanol extracts from *Artemisia changaica* Krasch grown in Mongolia. The antioxidant and cytotoxic activities of the ethanol crude extracts were determined using DPPH and MTT assays. The ethanol extracts showed higher antioxidant activity than essential oil. The results clearly showed that the ethanol extracts presented satisfactory cytotoxic activity against three human tumor cell lines A549 (human lung cancer cell line), A431 (human epithelial carcinoma cell line), and SK-BR-3 (human breast adenocarcinoma cell line) tested. The present study showed that the ethanol extracts and essential oil of *A. changaica* Krasch grown in Mongolia have potential as sources of new antioxidant and cytotoxic compounds, respectively.

Key words: *Artemisia changaica* Krasch; essential oil, ethanol extract, antioxidant, cytotoxic activity.

INTRODUCTION

A number of aromatic medicinal plants used for treating infectious diseases have been mentioned in different phytotherapy manuals due to their availability, fewer side effects, and reduced toxicity. The essential oils of these aromatic plants (Vandendool et al., 1963) are responsible for their fragrance as well as biological properties (Kalemba et al., 2003). Essential oils are complex mixtures of volatile secondary metabolites that are responsible for both the fragrant and biological effects of aromatic medicinal plants (Salzer, 1977; Angioni et al., 2003; Senatore et al., 2004). An important characteristic of essential oils and their constituents is their hydrophobicity, which enables them to partition in the lipids of bacterial cell membranes and mitochondria, thus disturbing the structures and rendering them more permeable (Sikkema et al., 1995).

Artemisia changaica Krasch belongs to the genus *Artemisia* of the family *Asteraceae (Compositae). Artemisia (Wormwood)* is a large, diverse genus of plants with between 200-500 species which are mainly found in Asia, Europe and North America (Bora et al., 2011). Among them, 105 species grow in Mongolian Forest-steppe and Desert-Gobi (Grubov, 1982). Many *Artemisia* species, which are known by such common names as mugwort, sagebrush, sagewort, and wormwood, have a vast range of biological activities, including antimalarial, cytotoxic, antifungal, antibacterial, antioxidant, and other useful effects (Bora et al., 2011).

Mongolia is rich in essential oil medicinal plants. Mongolian traditional medicine has long history of more than 2500 years. There are about 60 clans, 200 species, 300 types of essential oil plants and 600 kinds of herbal plants that have been registered; among of them, 150-200 types are commonly used (Shatar et al., 2000). Many essential oil plants are yet to be studied. It is important to investigate their chemical compositions and biological activities using traditional medicine (Shatar, 1989; Ligaa et al., 2005).

The aim of this study was to evaluate the antioxidant and cytotoxic effects of essential oil and ethanol extracts from *A.*

changaica Krach grown in Mongolia.

Antioxidant activities of the essential oil and the ethanol extracts were tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) colorimetric method was used for determining the cytotoxic activity of the samples.

To the best of our knowledge, there are no published reports on the biological activities of the essential oil and ethanol extracts of *A. changaica* Krach grown in Mongolia. Therefore, it is important to develop a better understanding of their mode of biological action for new application in human health.

MATERIALS AND METHODS

Plant material and study area

Samples were collected from mountain Buyant sum, Khovdaimag (Mongolia) in July 2010. Voucher specimens were deposited at the herbarium of the Institute of Botany of the Mongolian Academy of Science. Ulaanbaatar, Mongolia.

Plant extracts

The air-dried and powdered whole plant (170 g) was extracted with 70% ethanol (2 L × 3) using sonicator under room temperature. The resultant extracts were combined and evaporated in a rotary vacuum evaporator (Buchi R-205, Switzerland) at 40°C to afford crude extracts. The ethanol crude extract (28 g) were suspended in water and then fractionated successively with n-hexane, chloroform, ethyl acetate and butyl alcohol using the separation funnel, respectively.

Scavenging of DPPH radicals

The assay was carried out according to the method of Brand-William et al. (1995) to investigate the free radical scavenging activity of the samples. Briefly, the samples were dissolved in ethanol at the concentration of 100 mg/ml and then serially diluted with ethanol. On each well of a 96-well plate, 100 µl of samples of different concentration were mixed together with 100 µl of 60 µM DPPH prepared in ethanol. After incubation of 20-30 min for reaction, the absorbance of supernatants was measured at 517 nm using Multi-detection Reader (Bio Tek Co.). Ethanol was used as negative control and α-tocopherol as positive control. The scavenging capacity (SC) of the sample was calculated using the following formula:

$$SC\ (\%) = [1-A_S/A_C]\ 100$$

Where, A_S=is the net absorbance of the sample, A_C=is the net absorbance of negative control. The IC_{50} value of a sample is the concentration of sample at which 50% activity of DPPH (absorbance) is inhibited. It was calculated by linear regression.

Determination cytotoxic activity

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium-bromide (MTT) colorimetric method was used for determining cytotoxic activity of samples against A549, A431, SK-BR-3 cell lines as described by Mosman (1983).

The cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% (w/v) glutamine, sodium pyruvate 5%, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2.

Briefly, each sample was dissolved in DMSO at concentration of 100 mg/ml and serially diluted into different concentration of 1-100 µg/ml with culture media. On a 96-well plate, 200 µL of cell suspension at density of $2x10^4$ (in ml) were seeded. After 24 h incubation at 37°C, 5% CO_2 to allow cell attachment, the culture media were removed and replaced by 200 µl of cultured media containing different concentration of samples and incubated for 24 and 48 h under the same condition. In the control wells, the media were added without any samples. Finally, after 4 h of MTT reagent addition (final concentration of 0.5 mg/ml), the formazan crystals formed was resolved in DMSO (200 µl/well) and absorbance were measured at 570 and 630 nm using Multi-detection Reader (Bio Tek Co.). The cell growth inhibition (GI) of the sample was calculated using the formula:

$$GI\ (\%) = [1-A_S/A_C] \times 100$$

Where $A_{S=}$ is the absorbance in sample wells and $A_{C=}$is the absorbance in the control wells.

Chemical reagents

Dimethyl sulfoxide (DMSO), MTT, and DPPH were purchased from Sigma Chemical Company. RPMI 1640 medium, fetal bovine serum, penicillin, and streptomycin were purchased from GIBCO Co. (GIBCO BRL, Grand Island, NY, USA). The human alveolar basal epithelial cell line (A549), human epithelial carcinoma cell line (A431), human breast adenocarcinoma cell (SK-BR-3) lines were purchased from Korean cell line bank (KCLB, Korea). All other chemicals were of analytical grade and purchased from Sigma-Aldrich (USA) and DUKSAN Co. (Korea).

RESULTS

Antioxidant activity

DPPH is a free radical compound that has been widely used

Table1: Radical scavenging activity of ethanol extracts from branches, flowers and essential oil from *Artemisia changaica* Krasch grown in Mongolia.

Concentration (µg/ml)	*Ethanol extracts*[a]	
	Branches	**Flowers**
12.5	5.96±1.3	21.07±0.9
25	15.71±0.8	37.57±0.6
50	29.03±1.8	52.49±1.1
100	73.16±0.9	73.36±1.6
IC_{50}	71.89	49.4

Concentration (µg/ml)	***Essential oil***
10	4.04±1.5
50	5.38±1.9

[a]Result is average 3 replicates.

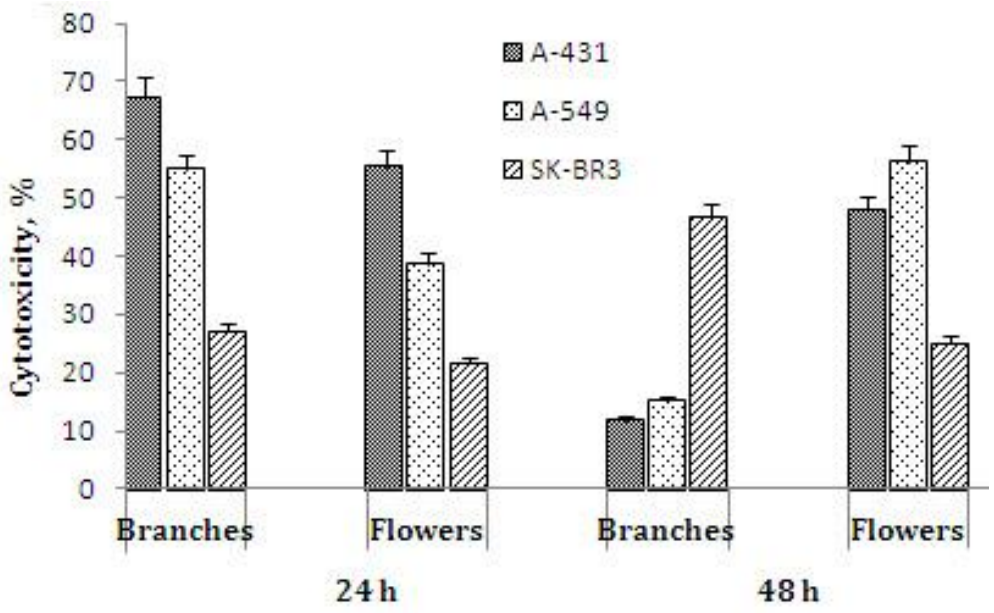

Figure 1: Cytotoxicity (%) of ethanol extracts from *Artemisia changaica* Krasch growing in Mongolia against A549, A431 and SK-BR-3 cell lines. Cell was treated with the fractions for 24 and 48 h at a concentration of 50 µg/ml.

to determine free radical scavenging activity (Brand-Williams et al., 1995). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form of DPPH (non radical) with the loss of this violet color (Molyneux, 2004: 26, 211-219).

The DPPH scavenging abilities of essential oil from *A. changaica* Krasch were 4.04 and 5.38% at concentrations of 10 and 50 µg/ml. The IC_{50} values were 71.89 and 49.4 µg/ml for ethanol extracts of branches and flowers, respectively. In this study, the ethanol extracts of *A. changaica* Krasch exhibited remarkable antioxidant activity than essential oil (Table 1).

Cytotoxic activity

To investigate the cytotoxic activity of ethanol extracts from branches and flowers of *A. changaica*Krasch, we evaluated its effect on a selection of human epithelial carcinoma cell line (A431), human alveolar basal epithelial cell line (A549) and human breast adenocarcinoma cell (SK-BR-3) lines using MTT assay (Mosman, 1983). These cell lines were submitted to growing concentrations of *A. changaica*Krasch ethanol extracts for 24 and 48 h. As shown in Figure 1, the

extracts of plants were significantly active against the chosen human cancer cell lines tested.

Conclusion

In recent years, interest in plant-derived food additives has grown. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically (Martinez-Tome et al., 2001). To the best of our knowledge, the present study is the first to evaluate the biological activities of *A. changaica*Krasch grown in Mongolia. The antioxidant activity of the ethanol extracts was moderate as compared with essential oil. The results clearly showed that the ethanol extracts presented satisfactory cytotoxic activity against three human cancer cell lines tested. The results of this study also demonstrate the potential of *A. changaica*Krasch ethanol extracts as a new antioxidant and cytotoxic agents for human health.

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