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CYTOTOXIC EFFECT OF *POTENTILLA REPTANS* L. RHIZOME AND AERIAL PART EXTRACTS

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Abstract: *Potentilla reptans* L. belongs to the least studied of the plants from Rosaceae family, *Potentilla* genus. There are no data on cytotoxicity of *P. reptans* extracts, though traditionaly it was used as antiinflammatory and antiinfective. The aim of these studies was to investigate potential antitumor activity of aqueous extracts (rhizome and aerial parts) of *P. reptans* on 4T1 mouse breast cancer cell line. Aqueous extracts of rhizome and aerial parts of *P. reptans* were tested for cytotoxicity by the MTT colorimetric assay on 4T1 cancer cell line in concentration range 100-800 μg/mL. Aqueous extracts of *P. reptans* rhizome and aerial parts show concentration dependent cytotoxic effect in the range of tested concentrations. IC_{50} value of *P. reptans* rhizome extract was 280.51 ± 1.16 μg/mL. IC_{50} value of *P. reptans* aerial parts extract was 310.79 ± 1.22 μg/mL. The significant difference in cytotoxicity among tested concentrations was observed. Aqueous extracts of *P. reptans* rhizome and aerial parts demonstrated weak cytotoxic activity on 4T1 mouse breast cancer cell line, which is in correlation with current cytotoxicity data for aqueous herbal extracts. Rhizome extract of *P. reptans* has slightly higher antitumor activity than aerial parts extract. The results represent the first report on cytotoxicity for this plant and further research on human cell lines is indicated.

Keywords: *Potentilla reptans*, cancer cell line, cytotoxicity

Potentilla reptans L., perennial herbaceous plant with thick vertical rhizome belongs to Rosaceae family, *Potentilla* genus (1). Species from this genus are growing in Europe, Asia and North America (2). *P. reptans* is native for different areas in Serbia (3). Aerial parts and rhizome were part of traditional medicine (4). *P. reptans* is the least studied from *Potentilla* genus and its chemical composition have not yet been completed. Eight compounds are identified in aerial parts of *P. reptans* (flavonoids, tannins, phenol carboxylic acids). Underground parts have not been analyzed (2). Dry leaves of *P. reptans* were used traditionally in the treatment of ulcers, inflammations, cardiovasculary, and gastrointestinal problems. Recent research confirmed its traditional use (5 – 8).

Antioxidant properties of aerial parts of *P. reptans* (5), indicate possible antitumor activity, often tested on specific cancer cell cultures (9, 10). *In vitro* and *in vivo* cytotoxicity of some *Potentilla* species was conducted. *P. chinensis, P. multicaulis* and *P. erecta* showed antitumor activity on cell line *in vitro*, while *P. fulgens* was active against Dalton's lymphoma cells in Swiss albino mice (11–14). Currently, there are no data on cytotoxicity of any part of *P. reptans*.

The aim of this study was to investigate antitumor potential of aqueous extracts of aerial parts and rhizome of *P. reptans* on 4T1 mouse breast cancer cell line.

EXPERIMENTAL

Plant material

Aerial and underground parts of *P. reptans* were collected at three different locations in Serbia in May to August 2010. Plant material was dried under the shade. Voucher specimens were deposited in herbar-

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ium of botanical garden of Biology Faculty, University of Belgrade, Serbia, no. BEOU 16405.

Preparation of herbal extracts

Aqueous extracts of rhizome and aerial parts of *P. reptans* were prepared as infusion (15). Extraction of dry plant material was by boiling distilled water for 20 min with occasional stirring. Filtration of extracts and evaporation under reduced pressure at 40°C in rotating vacuum evaporator (RV05 basic IKA, Germany) followed.

Absence of cytotoxicity data for aqueous extracts (13) forced the authors to perform pilot screening for detecting effective cytotoxicity concentration. The experiments were started with extract concentration range from 5 to 160 µg/mL and positive result was detected at concentration of 160 µg/mL. Therefore, the experiments were further proceed using 100–800 µg/mL concentrations in estimation of cytotoxicity. Concentrations 100, 200, 400, 600 and 800 µg/mL were prepared by dilution with RPMI-1640 medium (Gibco, England).

Cell line

Mouse breast cancer 4T1-cell line (ATCC - American Type Culture Collection) was maintained in RPMI-1640 medium (2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, 1500 mg/L sodium bicarbonate, 20% FCS) in an incubator for 24 h (37°C, 5% CO_2). Cells were harvested using 0.25% trypsin (Hyclone) at 70–80% confluence in culture flasks (16).

Cytotoxicity experiment

Cytotoxicity was assessed by the MTT colorimetric assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA) to formazan (17, 18). Cells were seeded into 96-well microtiter plate at a density of 4000 cells per well and cultured in RPMI-1640 medium at 37°C with 5% CO_2. After overnight grow, cells were treated with increasing concentrations (100, 200, 400, 600 and 800 µg/mL) of rhizome and aerial parts extracts, respectively. The same tumor cell line, cultured in the RPMI medium with no treatment, served as the negative control. Content of each well was taken out and 100 µL of MTT solution was added to each well and incubated in humidified atmosphere of 5% CO_2 at 37°C for 4 h. At the end of incubation, MTT was poured off and 150 µL DMSO/well was added to dissolve formazan crystals. Reaction mixture was incubated for 30 min at room temperature with continuous stirring. The optical density (OD) was measured at 590 nm with a multiplate reader (Zenith 3100, Anthos Labtec Instruments GmbH, Austria) (17, 18). Each assay has been performed three times with seven replicate each.

Viability was calculated according to relevant equation:

% Viability = (mean absorbance of treated cells / mean absorbance of negative control) × 100

% Cytotoxicity = 100 - % Viability

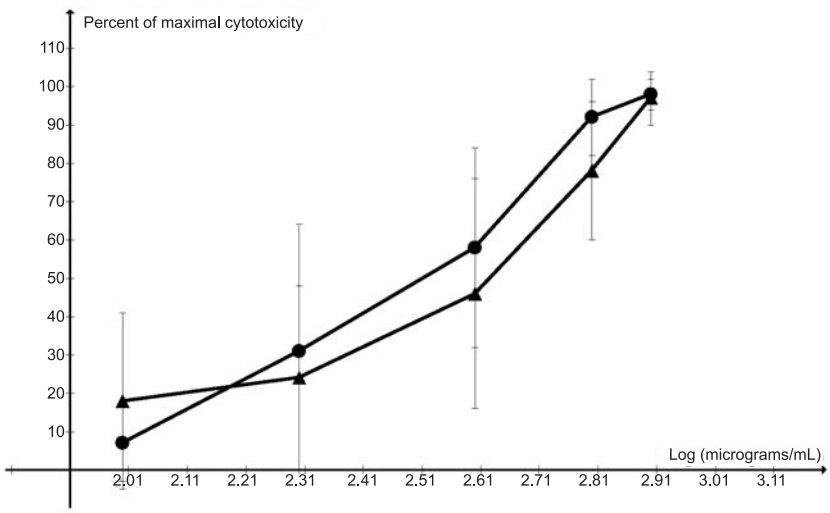

Figure 1. Concentration dependent cytotoxic effect for both *P. reptans* extracts, rhizome (•) and aerial parts (▲)

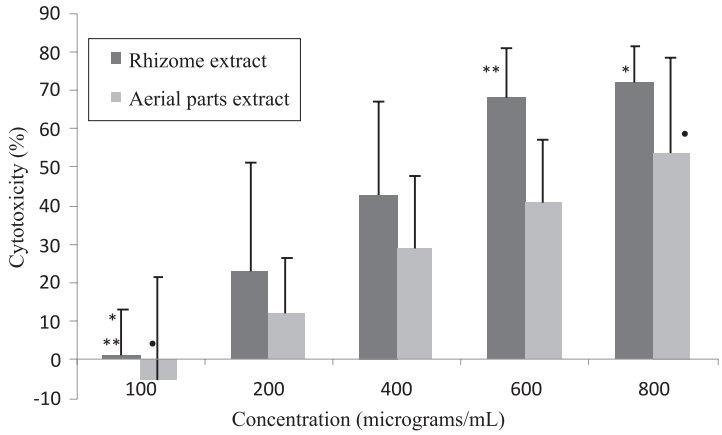

Figure 2. Cytotoxicity (%) of both *P. reptans* extracts (rhizome and aerial parts). * Significance in comparison of rhizome extract concentrations 100 µg/mL to 800 µg/mL ($p = 0.000$). ** Significance in comparison of rhizome extract concentrations 100 µg/mL to 600 µg/mL ($p = 0.000$). • Significance in comparison of aerial parts extract concentrations 100 µg/mL to 800 µg/mL ($p = 0.009$)

Statistics

Statistical analysis was performed using SPSS software (13.0; SPSS Inc., USA). Concentrations which inhibited growth by 50% (IC_{50}) were determined for both extracts by linear regression analysis ($p < 0.05$). Cytotoxicity, appearing as a function of extract concentration, was represented graphically. The significant difference, among tested concentrations of both extracts, was calculated by one-way and repeated measures by ANOVA ($p < 0.01$).

RESULTS

Aqueous rhizome extract of *P. reptans* showed concentration-dependent cytotoxic effect ($F = 27.16915$; $r = 0.87$; $p < 0.05$). IC_{50} value for aqueous rhizome extract was 280.51 ± 1.16 µg/mL.

Aqueous aerial parts extract of *P. reptans* showed also concentration-dependent cytotoxic effect ($F = 17.79516$; $r = 0.79$; $p < 0.05$), while IC_{50} value was 310.79 ± 1.22 µg/mL.

Concentration-dependent cytotoxic effect for both extracts is presented in Figure 1 (where the cytotoxicity was expressed as a percentage of maximal cytotoxic effect which extracts showed in the experiment). The significant differences in exerted cytotoxicity among tested concentrations, for both extracts, are presented in Figure 2.

DISCUSSION AND CONCLUSION

Plants used in traditional medicine are often researched for new anticancer compounds (19). Potential anticancer activity of a plant is screened by cancer cell culture specific assays (20). However, choice of solvent used in plant extraction seems to influence results obtained by such assays. Value of IC_{50} for cytotoxicity of aqueous plant extracts and organic solvent plant extracts (ethanolic, methanolic, etc.) may differ significantly. Though, concentrations of aqueous plant extracts exerting cytotoxicity are usually higher than concentrations of extracts obtained by other solvents, aqueous extraction seems to be unavoidable due to the preparation methods used in traditional medicine (21, 13).

Antitumor activity for most of species of *Potentilla* genus has been little explored, with exception of *P. erecta*; its ethanol extract of rhizomes showing cytotoxicity (13). *P. reptans* has not been tested on cytotoxicity up to date.

Motivation for evaluation of potential antitumor activity of this plant was based on its use in folk herbal medicine against inflammation and infection, which often disbalance homeostasis in cancer or pre-cancer states (4, 6). Therefore, the authors decided to perform investigations with aqueous extracts of different parts of the plant, being at the same time in concordance with method of plant preparation in its ethnopharmacological use (6). Both rhizome and aerial parts extracts of *P. reptans* expressed cytotoxic effect on 4T1 mouse breast cancer cell line. Effect was detected at 100 and 200 µg/mL concentrations, respectively. IC_{50} for rhizome extract was 280.51 ± 1.16 µg/mL and IC_{50} for aerial parts extract was 310.79 ±1.22 µg/mL. The results correlate well with general observation that

aqueous extracts achieve cytotoxic effect by concentrations starting from 250 μg/mL, demonstrating weak cytotoxic effect (21). Therefore, these results may be considered preliminary, representing the first information on antitumor properties of *P. reptans* and indicating possibility of further antitumor research of this plant.

The cytotoxic effect of aqueous extract of *P. reptans* (rhizome and aerial parts) was investigated in the present study. The concentration-dependent cytotoxic response was observed on 4T1 mouse breast cancer cell line for both extracts (rhizome and aerial parts). It could be considered that *P. reptans* aqueous extracts demonstrate weak cytotoxic activity on the investigated cancer cell line. Rhizome extract showed slightly higher antitumor activity than aerial parts extract. These results represent the first report on cytotoxicity of this plant, therefore it is indicated to continue investigation of cytotoxicity on other cancer cell lines (human).

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Conflict of interest

The authors have declared that there is no conflict of interest.

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