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Research article

ANTIPROLIFERATIVE EFFECT OF *SPILANTHES ACMELLA* EXTRACTS ON HUMAN CERVIX ADENOCARCINOMA AND HUMAN MYELOGENOUS LEUKEMIA CELLS

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Reactive oxygen and nitrogen species (RONS) play a crucial role in the pathogenesis of various chronic diseases, including cancer, by reacting with fundamental biomolecules. While conventional anticancer treatments such as ionizing radiation and chemotherapy have significant adverse effects, some medicinal plants exhibit free radical scavenging and anticancer activities. *Spilanthes acmella* L., commonly known as the toothache plant, is reported to have various bioactive compounds with antioxidant and anticancer properties. The aim of this study was to investigate and evaluate antiproliferative potential of *Spilanthes acmella* ethanolic extract on human cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562) cancer cell lines. The stock solution of *Spilanthes acmella* extract was prepared in ethanol at concentration of 1 mg/mL and diluted with complete nutrient medium RPMI-1640. The medium was supplemented with 3 mM l-glutamine, 100 μg/mL streptomycin, 100 IU/mL penicillin, 10% heat-inactivated fetal bovine serum (FBS), and 25 mM Hepes, adjusted to pH 7.2. Cell survival was determined by the MTT assay 72 hours post-treatment. The IC_{50} values were calculated using a dose-response growth curve. The *S. acmella* ethanolic extract demonstrated significant cytotoxic (antiproliferative) effects on both HeLa and K562 cancer cell lines. The extract exhibited higher cytotoxicity towards K562 cells, with an IC_{50} value of 29.1 μg/mL, compared to HeLa cells, which had an IC_{50} value of 48.8 μg/mL. *Spilanthes acmella* extract possesses considerable potential as an anticancer agent and warrants further *in vivo* investigations to confirm its efficacy.

Keywords: *Spilanthes acmella;* Antiproliferative effect; Cytotoxicity; Hela cells; K562 cells

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INTRODUCTION

The eukaryotic cells, containing electron transport systems, may produce reactive oxygen or nitrogen species [1]. Their free electrons are prone to react with lipids, proteins and nucleic acids causing a variety of chronic diseases, including cancers such as colorectal, prostate, breast, lung, and bladder cancers [2]. According to the World Health Organization (WHO), cancer is the second leading cause of death worldwide [3]. Based on data from the Cancer Registry of the Institute for Public Health of Serbia „Dr. Milan Jovanović Batut" in 2020, 1,087 newly diagnosed women were registered in Serbia, and 453 women died of cervical cancer. The estimates of the European Information System for the year 2020 indicates that Serbia ranks fifth in Europe, with an incidence rate of 26.3 per 100 000 women. Cervical cancer death rates are also high at 9.1 per 100 000 women [4].

WHO estimated that 80% of world's population relies on traditional medicines [5]. Traditional medicine is also widely used in Serbia. However, only 5–15% of the 250,000 higher plants have ever been tested for the presence of bioactive compounds [6].

Spilanthes acmella L. (fam. *Asteraceae*) commonly known as the toothache plant, is found all over the world and widely distributed throughout the tropics and subtropics [7]. Extracts of several of *Spilanthes* species were shown to exhibit a broad spectrum of activities such as antioxidant, gastroprotective, antiproliferative, immunomodulatory, diuretic, vasorelaxant, anti-inflammatory, enzyme inhibitory, antimicrobial, insecticidal, and larvicidal [8].

S. acmella extract is composed of a diverse group of active constituents, with spilanthol identified as a major active ingredient having strong antimicrobial, antifungal, and antioxidant properties [7,9]. For example, some authors suggested that the antioxidant properties of the *S. acmella* might be due to the presence of highly valuable bioactive compounds such as phenolics, coumarins, and triterpenoids [9]. Others showed that flavonoids exhibited antiproliferative activity on several cancer cell lines [10].

Therefore, our reasearch aimed to investigate and evaluate the antiproliferative potential of *S. acmella* extract against two selected human cancer cell lines: cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562).

MATERIAL AND METHODS

Cytotoxicity Analysis

The stock solution of the extract was prepared in ethanol at a concentration of 1 mg/mL and then diluted with complete nutrient medium (RPMI-1640) supplemented with 3 mM L-glutamine, 100 μg/ mL streptomycin, 100 IU/mL penicillin, 10% heat-inactivated fetal bovine serum (FBS) and 25 mM Hepes, and adjusted to pH 7.2 with a bicarbonate solution. Human cervix adenocarcinoma HeLa cells were cultured as

monolayers in nutrient medium, while human myelogenous leukemia K562 cells were maintained as a suspension culture. The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere.

HeLa cells (2,500 cells per well) were seeded into 96-well microtiter plates and 20 hours later, after cell adherence, five different concentrations of the extract were added to the wells. Final concentrations were in the range from 5 to 80μg/mL. Only nutrient medium was added to the cells in the control wells. The investigated extract was added to a suspension of leukemia K562 cells (5,000 cells per well) 2 h after cell seeding, in the final concentrations from 2.5 to 40 μg/mL. Nutrient medium with the corresponding concentrations of compounds, but devoid of cells was used as a blank.

Cell survival was determined by the MTT test according to the method of *Mosmann* [11] and modified by *Ohno and Abe* [12] 72 hours after the investigated extract was added. Briefly, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well. Samples were incubated for a further four hours at 37 °C under a humidified atmosphere with 5% CO_2. Then, 100 μL of 10% sodium dodecyl sulfate (SDS) was added to the wells. Absorbance was measured at 570 nm the next day. To determine cell survival (%), absorbance at 570 nm of a sample was divided by the absorbance of the control sample (the absorbance of cells grown only in nutrient medium), after subtraction of absorption of the blank. Concentrations of the extract which induced a 50% decrease in malignant and normal cell survival (IC_{50} values) were calculated from a dose-response growth curve using Microsoft® Excel® 2019 software.

RESULTS

In Vitro Cytotoxic Activity

The cytotoxicity of the *S. acmella* extract was tested on selected cancer cell lines: human cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562). Concentrations of the ethanol extract which induced IC_{50} are presented in Table 1.

Table 1. Concentrations of the ethanol extract which induced a 50% decrease (IC_{50}) in malignant cells survival

IC50 [μg/mL] Av ± SD*	
HeLa	**K562**
48.8 ± 1.5	± 0.9

*Average ± Standard deviation

The examined extract possess high to moderate cytotoxicity on the investigated tumor cells. The highest cytotoxicity was found in K562 cells (IC_{50} = 29.1 μg/mL), and somewhat weaker towards HeLa cells (IC_{50} = 48.8 μg/mL) (Figure 1).

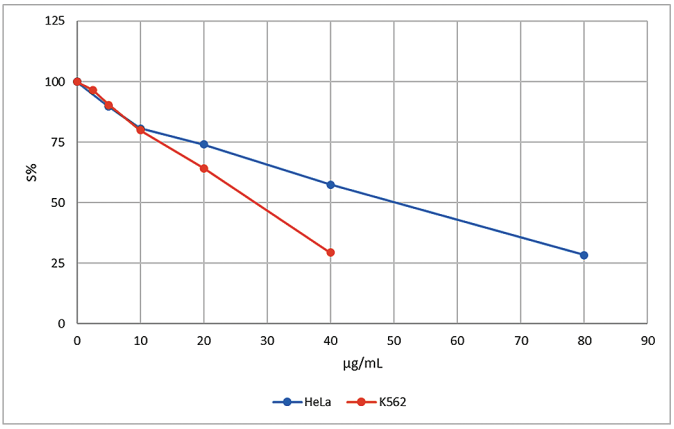

Figure 1. Survival of tumor cells as determined by MTT test, after 72 hours of continuous exposure to various concentrations of *S. acmella* extract.

DISCUSSION

Reacting with fundamental biomolecules, reactive oxygen and nitrogen species play a major role in the pathogenesis of a variety of chronic disases, including various cancers [13]. In addition to killing growing tumor cells, ionizing radiation and most anticancer drugs cause serious adverse effects [2,14]. Nevertheless, some medicinal plants, because of their free radical scavenging and anticancer activities, maintain the health and vitality of individuals and have the potential to improve various diseases, including cancer [3].

Recently, some authors documented that the strong antioxidant activity of *S. acmella* was due to the high concentration of flavonoids (72.14 QE mg/g) and phenols (84.52 GAE mg/g) in the leaf extract. These studies demonstrated that *S. acmella* could be an important source of natural antioxidants, which might help in preventing the progress of various oxidative stress-related diseases [15,16].

Shivsharan *et al.* [17], showed that *S. acmella* ethanolic extract is a source of bioactive compounds: proteins, phenolics, alkaloids, and flavonoids. Their anticancer properties on human liver (HEP-2) and colon (HT-29) cancer cell lines might be explained by the reduction of free radical formation and apoptosis induction, including growth regulators. Using MTT assay, Mishra *et al.* [18], observed that *Spilanthes paniculata* petroleum ether extract had the lowest effect on the antiproliferative activity on hepatic carcinoma cells (Huh-7 cells). However, the authors demonstrated that ethyl acetate and ethanol extracts possessed antiproliferative effects (20-90% inhibition and 4-90%

inhibition, respectively) due to their induction of caspase-3 enzymes and inhibition of phosphorylation of various tyrosine kinases [18].

Many studies showed that plant extracts had cell-selective activity, suggesting that plant extracts are prefentially active on specific cancer cells. Aqueous extract of *Acmella caulirhiza* caused a significant decrease in the proliferation of mouse glioma cells GL-261, melanoma cells B16-F1 and YUMM 1.7 after 24 hours, with respective IC_{50}s of 296.81, 487.94 and 149.42 μg/mL and after 48 hours with respective IC_{50}s of 232.29, 180.26, and 31.99 μg/ml [19]. Due to alkaloids, flavonoids, tannins and terpenoids, this extract exhibited the highest antiradical scavenging activities and was more cytotoxic on YUMM 1.7 cells compared to the other cancer cell lines. Results also showed that this extract induced cell death through the underexpression of inflammation, growth factors and anti-apoptotic protein genes.

In the experiment presented here, different concentrations of *S. acmella* ethanolic extract showed a cytotoxic (antiproliferative) effect, and IC_{50} was noticed at 29.1 μg/mL, conferring higher cytotoxicity upon K562 than HeLa cell lines (48.8 μg/mL). These results align with previous studies highlighting the broad spectrum of bioactive compounds in *S. acmella* and their potential to induce cytotoxic effects through mechanisms such as free radical scavenging and apoptosis induction. *S. acmella* menthol extract was most potent on lymphoma cells with an IC_{50} of 147.547 μg/mL, while it had no effect on lung carcinoma [20]. The antiproliferative analysis by MTT assay reveals that the methanolic extract of *Acmella ciliata* cell biomass inhibited or reduced the growth of melanoma SK-MEL 28 cell lines, and a lower concentration (24.144 μg/mL) was needed to inhibit the growth by 50% [21]. Also, this concentration is similar to the value (IC_{50}=24.84 μg/mL) for the standard anticancer drug cisplatin [22].

The exploration of traditional medical plants such as *S. acmella* offers a valuable source for the development of new anticancer therapies, potentially enhancing the treatment range for various malignancies. Given the promising *in vitro* results, further *in vivo* studies are warranted to validate the efficacy and safety of *S. acmella* extracts in animal models and clinical trials. These steps are crucial for translating the obtained results into practical therapeutic applications. Therefore, the findings of the present study show that *S. acmella* extracts possess potential for use as therapeutic agents and they could be recommended for further *in vivo* experiments.

CONCLUSION

The findings of our study demonstrate that the ethanolic extract of *Spilanthes acmella* exhibits significant antiproliferative effects on human cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562) cancer cell lines. The observed IC_{50} values indicate that the extract is more cytotoxic to K562 cells (29.1 μg/mL) compared to HeLa cells (48.8 μg/mL).

Authors' contributions

ZT proposed the key hypothesis, designed the study, and participated in writing the manuscript. IBŽ and ŽŽ carried out the planning and performance of the experimental setups and protocols and participated in writing. AJ and DO participated in writing, editing, and revising the manuscript. VB connected the obtained results with the hypotheses and the theoretical framework of the research. All authors have read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ANTIPROLIFERATIVNI EFEKAT EKSTRAKATA *SPILANTHES ACMELLA* NA ADENOKARCINOM GRLIĆA MATERICE I ĆELIJE MIJELOIDNE LEUKEMIJE KOD LJUDI

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Reaktivne vrste kiseonika i azota (RONS) igraju ključnu ulogu u patogenezi raznih hroničnih bolesti, uključujući i karcinom, reagovanjem sa osnovnim biomolekulima. Iako konvencionalni antikancer tretmani poput jonizujućeg zračenja i hemoterapije imaju značajne neželjene efekte, određene lekovite biljke pokazuju sposobnost uklanjanja slobodnih radikala i antikancerogene aktivnosti. *Spilanthes acmella L.*, poznata kao biljka za zubobolju, prema izveštajima sadrži različite bioaktivne supstance sa antioksidativnim i antikancerogenim svojstvima. Cilj ove studije bio je ispitati i proceniti antiproliferativni potencijal etanolnog ekstrakta biljke *Spilanthes acmella* na ljudske ćelijske linije adenokarcinoma grlića materice (HeLa) i ljudske mijeloidne leukemije (k562). Pripremljen je osnovni rastvor ekstrakta biljke *Spilanthes acmella* u etanolu u koncentraciji od 1 mg/mL i razblažen sa potpunim hranljivim medijumom RPMI-1640. Medijum je bio suplementiran sa 3 mM L-glutamina, 100 μg/mL streptomicina, 100 IU/mL penicilina, 10% toplotno inaktivisanog fetalnog goveđeg seruma (FBS), i 25 mM HEPES-a, podešenog na pH 7,2. Preživljavanje ćelija određeno je MTT testom 72 sata nakon tretmana. IC50 vrednosti su izračunate korišćenjem krive rasta zavisne od doze. Etanolni ekstrakt biljke *S. acmella* pokazao je značajne citotoksične (antiproliferativne) efekte na obe ćelijske linije, HeLa i k562. Ekstrakt je pokazao veću citotoksičnost prema ćelijama k562, sa IC50 vrednošću od 29,1 μg/mL, u poređenju sa HeLa ćelijama, kod kojih je IC50 vrednost bila 48,8 μg/mL. Ekstrakt biljke *Spilanthes acmella* ima značajan potencijal kao antikancerogeni agens i zahteva dalja in vivo ispitivanja kako bi se potvrdila njegova efikasnost.