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Research Article

Investigation of *In Vitro* antiproliferative activity properties of *Spartium junceum* L. (Spanish broom) against MDA-MB-231 and HepG2 cancer cell lines

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Abstract: Cancer is among the top global public health burdens leading to millions of deaths each year. The study aims to investigate the antiproliferative effect of *Spartium junceum* L. flowers on different cancer cell lines. The ethanolic extract of the flowers was prepared in the present study. Phytochemical analysis of the plant extract revealed the presence of several phenolic compounds such as cinnamic acid and its derivatives (chlorogenic, *p*-coumaric, ferulic acids), protocatechuic acid, epicatechin and luteolin. This extract was tested against human breast (MDA-MB-231) and liver (HepG2) cancer cell lines to find out its antiproliferative activity. It was determined that the extract was effective against both cell lines with IC_{50} values of 2.37 ± 0.47 and 0.98 ± 0.01 µL/mL for MDA-MB-231 and HepG2, respectively. Particularly, the extract was found to be more effective in the liver cancer cell line than the breast cancer cell line. All these obtained findings led us to believe that this medicinal plant could be a promising antiproliferative agent candidate for the treatment of human liver and breast cancers.

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1. INTRODUCTION

Cancer is one of the significant global public health burdens leading to millions of deaths worldwide every year. This fatal disease is characterized by the transformation of normal body cells into abnormal ones that divide at an uncontrollable rate and can invade other parts of the body causing metastasis (Wu *et al*., 2019).

Carcinogenesis can affect any part of the body, and cancer is named after the part of the body in which it originated (Sahayarayan *et al*., 2021). Among the cancer types, hepatocellular carcinoma (HCC) is the second cause of cancer mortality and the rate of its incidence has continuously increased day by day (Llovet *et al*., 2022). Similarly, breast cancer is the most commonly diagnosed cancer in women and is responsible for nearly 900 thousand deaths per

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year (Allugunti, 2022). According to the World Health Organization's cancer report, there were nearly 10 million deaths in 2020 (World Health Organization, 2023).

The main components of cancer treatment include radiation therapy, surgery, and chemotherapy (van den Boogaard *et al*., 2022). Chemotherapy is an effective treatment option that increases the survival rate of people suffering from cancer. In this method, malignant cells that can harm healthy cells are killed by strong chemicals (Dennis *et al*., 2022). However, modern chemotherapeutics are associated with severe unpleasant side effects such as neurotoxicity, nephrotoxicity, cardiotoxicity, hepatotoxicity, and ototoxicity. Furthermore, the resistance of tumor cells to specific chemotherapeutics is one of the significant problems of chemotherapy (van den Boogaard *et al*., 2022). For all these reasons, there is an urgent need for more research to explore new and safe treatment strategies.

Increasing evidence has shown that some medicinal plants represent an excellent source for screening new and safe chemotherapeutics. The plant-based anti-cancer chemical compounds such as taxol, topotecan, irinotecan, vincristine, vinblastine, and etoposide are used clinically worldwide (Imran & Shahid, 2022).

Spartium junceum L. (Spanish broom) is a flowering perennial medicinal shrub belonging to the Fabaceae family. Flowers of this plant are rich in various secondary metabolites such as flavonoids, saponins, and cytisine-type alkaloids (Nadaf *et al*., 2012; Yeşilada *et al*., 2000a; Rammal *et al*., 2021). In previous studies, the flowers have been found to have anti-ulcerogenic, antitumor, analgesic, anti-inflammatory, antiviral, and antioxidant properties (Yeşilada *et al*., 2000b; Nanni *et al*., 2018; Menghini *et al*., 2006; Duman *et al*., 2019).

In the present work, the antiproliferative activity potential of the ethanolic extract prepared from the flowers of *Spartium junceum* L. was evaluated in different human cancer cell lines: breast adenocarcinoma (MDA-MB-231) and liver hepatocellular carcinoma (HepG2). The study material was chosen considering the presence of the plant's antiproliferative activity in different cell lines in previous reports (Abusamra *et al*., 2015; Cerchiara *et al*., 2012). As a result of the literature review, there is no study in the literature investigating the antiproliferative activity of the plant in the cell lines we selected for this study.

2. MATERIAL and METHODS

2.1. Plant Material and Extraction

Flowers of *Spartium junceum* L. (SJ) were collected from Eğirdir/Isparta on the date of May 19, 2021 (Figure 1). The herbarium sample was authenticated by Assoc. Prof. Gülsen Kendir and deposited in the Herbarium of the Faculty of Pharmacy of Ankara University under voucher number AEF 30711. To prepare the ethanolic extract, 50 g of dried flowers were subjected to maceration with 500 mL of 95% ethanol. The extract was filtered and the filtrate was evaporated to dryness at 36 °C using a rotary evaporator (Heidolph Hei-Vap Rotary Evaporator). At the end of the process, the crude extract remaining in the flask was weighed at 9 g and the yield of the extract was calculated as 18 % and transferred to a vial.

Figure 1. Flower of SJ plant (photograph taken by Fatma Tuğçe Gürağaç Dereli).

2.2. Reagent and Materials

Human liver hepatocellular carcinoma cell line (HepG2) (ATCC® HB-8065TM) and human breast adenocarcinoma cell line (MDA-MB-231) (ATCC® HTB-26TM) were purchased from American Type Culture Collection (ATCC, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco's modified eagle's medium high glucose (DMEM), glutamax and fetal bovine serum (FBS) were purchased from Sigma (USA), Gibco (Life Technologies, USA) or HyClone (USA).

2.3. In Vitro Antiproliferative Activity Studies

MDA-MB-231 and HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% glutamax under a humidified incubator of 5% CO_2 at 37 ºC. When the cells reached the level to be passaged (90% occupancy), the medium in the flask was removed. Cells were washed twice with phosphate buffer saline (PBS). The cells in flasks were passaged using trypsin-EDTA. The cells were seeded into 96-well plates at 5 x 10^3 cells/well density. A stock solution was prepared by dissolving 5 mg of extract in 1 mL of DMEM. After 24 h, the medium was replaced and the cells were exposed to the prepared extract dissolved in DMEM at different concentrations (0.1562, 0.3125, 0.625, 1.25, 2.5, and 5 µL/mL) for 48 h. After this period was completed, the medium in the wells was carefully removed. 5 mg/mL of MTT stock solution was added to each well, and plates were incubated for 2 h. After this period was completed, the medium was removed and 200 µL of dimethylsulphoxide (DMSO) was added to dissolve the formed formazone. It was stirred for half an hour in the dark and at room temperature. The absorbance values were measured with Promega reader device at 560 nm. GraphPad Prism 5 program was used for calculating IC_{50} values.

2.4. Phytochemical Screening

The phenolic profile of the ethanolic flower extract was defined by High-Performance Liquid Chromatography (HPLC) technique. HPLC conditions are presented in Table 1.

Table 1. Chromatographic conditions.

Chromatographic conditions	Time (min.)	A (%)	B (%)
Stok concentration injected to the HPLC system: 0.04 µg/mL Detector: SPD-M 10A vp DAD dedektör (λmax=278nm) Autosampler:SIL–10AD vp System controller: SCL-10A vp Pump: LC-10AD vp Degasser: DGU-14a Column heater: CTO-10 A vp Column: Agilent Eclipse XDB C-18 (250 mm × 4.6 mm), 5 µm Column temperature: 30 °C Mobile phases: A: acetic– water (3:97 v/v), B: methanol Flow rate: 0.8 mL / min. Injection volume: 20 µL	0	93	7
	20	72	28
	28	75	25
	35	70	30
	50	70	30
	60	67	33
	62	58	42
	70	50	50
	73	30	70
	75	20	80
	80	0	100
	81	93	7

3. RESULT and DISCUSSION

3.1. *In Vitro* Antiproliferative Activity Studies

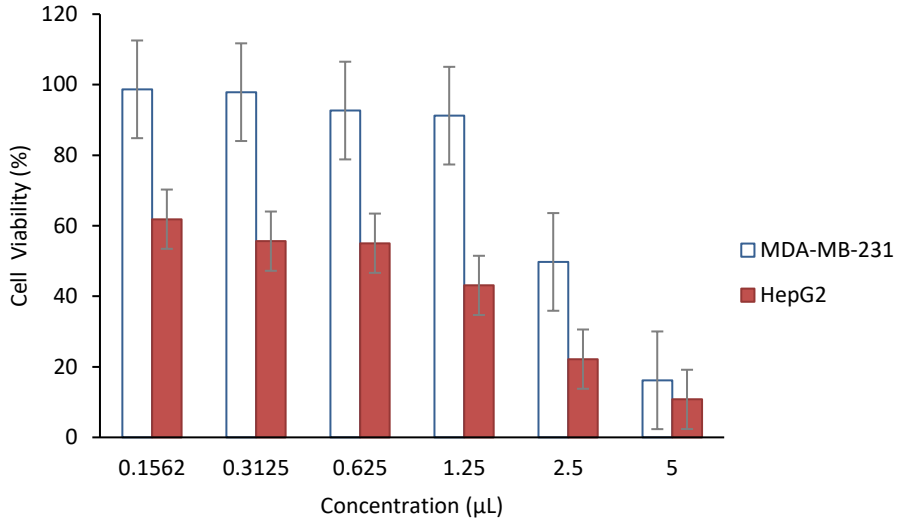
The antiproliferative activities of extract were evaluated against MDA-MB-231 and HepG2 cell lines for 48 h. The experiments were repeated twice. The results and calculated standard deviation values are given in the Table 2.

Table 2. IC_{50} results for SJ in human cell lines.

Compound	IC_{50} (µL/mL)	
	MDA-MB-231	HepG2
SJ	2.37 ± 0.47	0.98 ± 0.01

The effect of the extract in proliferation varies partly depending on the studied concentrations (Figure 2). At 5 µL/mL of the extract, the viability ratio was obtained as 16.18% and 10.77% for MDA-MB-231 and HepG2, respectively. When the amount of extract used was halved (for 2.5 µL/mL), the cell viability rates increased to 49.75% and 22.18% for breast and liver cancer cell lines, respectively. It was observed that the extract used in amounts smaller than 1.25 µL/mL had a similar effect on cell proliferation, and there were no major differences. For the MDA-MB-231 cell line, the cell viability ratio was obtained as 91.22%, 92.67%, 97.88%, 98.69% at 1.25 µL/mL, 0.625 µL/mL, 0.3125 µL/mL, and 0.1562 µL/mL of the prepared extract, respectively. For the HepG2 cell line, the cell viability ratio was calculated as 43.09%, 55.05%, 55.63%, and 61.86% at 1.25 µL/mL, 0.625 µL/mL, 0.3125 µL/mL, and 0.1562 µL/mL of extract, respectively.

Figure 2. Antiproliferative activity of ethanolic extract of *Spartium junceum* L. Flowers.



3.2. Results of HPLC Analysis

HPLC analysis of the ethanolic extract of SJ flowers shows the presence of chlorogenic, cinnamic, ferulic, *p*-coumaric, and protocatechuic acids, epicatechin, and luteolin. Ferulic acid had the highest concentration (2583.3 µg/mL) followed by chlorogenic acid with a concentration of 571.2 µg/mL, then *p*-coumaric acid with a concentration of 545.6 µg/mL, with the presence of epicatechin, luteolin, cinnamic acid, and protocatechuic acid with concentrations of 280.1, 159.4, 34.7 and 12.9 µg/mL, respectively (Table 3). The chemical structures of determined compounds in the ethanolic extract are shown in Figure 3. The open structures of these molecules were drawn with the ChemDraw Professional software program.

Figure 3. Chemical structures of determined compounds in the ethanolic extract of SJ flowers.

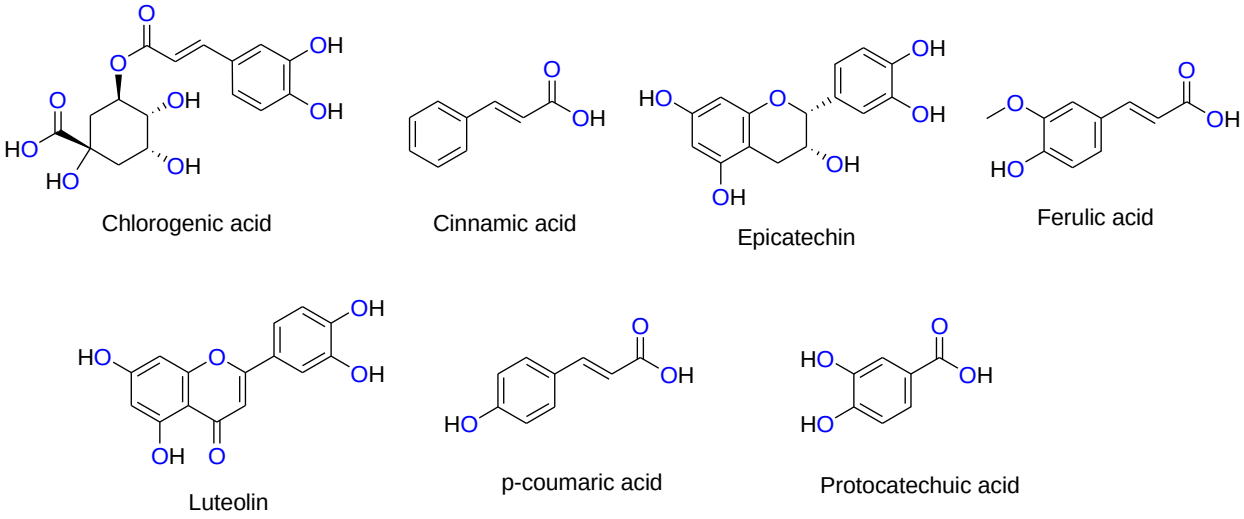


Table 3. Concentrations of the main phenolic compounds identified in the ethanolic extract of SJ flowers.

Phytochemicals	Concentrations (µg/mL)
Chlorogenic acid	571.2
Cinnamic acid	34.7
Epicatechin	280.1
Ferulic acid	2583.3
Luteolin	159.4
p-coumaric acid	545.6
Protocatechuic acid	12.9

4. DISCUSSION

SJ, also known as "Spanish broom", is a perennial erect shrub widespread in the Mediterranean. There are many studies proving that the flowers of this plant have anti-ulcerogenic, antitumor, analgesic, anti-inflammatory, antiviral, and antioxidant properties (Yeşilada *et al*., 2000b; Nanni *et al*., 2018; Menghini *et al*., 2006; Duman *et al*., 2019). However, when the literature is reviewed, it is seen that only a few studies show the anticancer activity of flowers (Abusamra *et al*., 2015; Cerchiara *et al*., 2012). In the previous studies, the anticancer activity of flowers was investigated on different cell lines. Abusamra *et al*. tested the cytotoxic effect of crude hydromethanolic extract prepared from SJ flowers towards the glioblastoma tumor cell line (U-373) (Abusamra *et al*., 2015). They found the IC_{50} value as 1602 µg/mL. So, the hydromethanolic extract of SJ flowers appeared to have weak cytotoxic activity. Cerchiara and coworkers screened the antitumor effect of SJ aromatic water against melanoma (RPMI 7932), leukemia (K562), breast (MCF7-Bart and MCF7-ICLC), and colon adenocarcinoma (SW480) cell lines (Cerchiara *et al*., 2012). They found that the SJ aromatic water had an antitumor effect on these cancer cell lines. Furthermore, they also investigated the toxic effect of SJ aromatic water on the healthy human cell line (NCTC 2544). They found that the aromatic water of SJ has selectivity in normal cell lines compared to cancer cell lines. In the present study, the prepared extract of SJ was screened towards MDA-MB-231 and HepG2 cell lines to find out its antiproliferative activity *in vitro*. It was determined that the extract, which was used in ranging from 0.1562 µL/mL to 5 µL/mL, was effective against both cell lines. Particularly, the extract was found to be more effective in the liver cancer cell line than the breast cancer cell line. In other words, in the study conducted by Cerchiara et al., as in our study, it was observed that the extract had a high cytotoxic effect on cancer cell lines (MDA-MB-231, HepG2) (Cerchiara *et al*., 2012).

Phytochemical analysis of plant extract revealed the presence of several phenolic compounds such as cinnamic acid and its derivatives (chlorogenic, *p*-coumaric, and ferulic acids), protocatechuic acid (3,4-dihydroxybenzoic acid), epicatechin and flavone luteolin. In the literature, there are many studies in which the antiproliferative effects of various plant extracts are attributed to the phenolic compounds in the phytochemical composition of the plants. For example, a study conducted by Vale et al. proved the antiproliferative and antimetastatic potential of cinnamic acid derivatives on melanoma (Vale *et al*., 2022). The antiproliferative effects of luteolin, *p*-coumaric acid, and protocatechuic acid against MCF-7 human breast cancer cell lines have been reported (Zheng *et al*., 2017). Some studies in the literature have shown that epicatechin-rich extracts have *in vitro* antiproliferative effects at high doses (Horie *et al*., 2005; Philips *et al*., 2009; Singh *et al*., 2011). *Ficus carica* L. latex was found to have antiproliferative activity toward numerous cell lines and Ultra Performance Liquid Chromatography coupled with mass spectrometry (UPLC-MS) analysis revealed that various

phenolic secondary metabolites could be responsible for this activity (Yahiaoui *et al*., 2022). A study conducted to analyze the change in the antiproliferative effect of Rhodiola after *in vitro* digestion revealed that gastrointestinal digestion significantly reduced the levels of total phenol and flavonoid content and antiproliferative activity potential of the extract (Zhang *et al*., 2022). Here, the prepared extract was tested in breast and liver cancer cell lines. The results show that the SJ extract had high antiproliferative activity on screened cell lines for 48 h incubation times.

5. CONCLUSION

In conclusion, this study indicated that SJ flower extract could inhibit proliferation in the selected cell lines, possibly due to its rich phenolic phytochemical profile, and the obtained findings from the current study led us to believe that SJ could be a promising antiproliferative agent candidate.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Fatma Tuğçe Gürağaç Dereli: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Senem Akkoç**: Investigation, Methodology, Validation, and Writing.

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