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FULL PAPER

# Total phenolic and total flavonoid contents and biological properties of Spartium junceum L. grown in Lebanon

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The proposed study experimentally intends to obtain the amount of phenolic and flavonoid contents in stems and legumes of *Spartium junceum L.* grown in Lebanon, to assess their anticancer activity on selected cell lines by XTT assay, and their antibacterial potential and finally, to evaluate their antioxidant activity using two different methods. The obtained results indicated that this plant exerted good antioxidant, antibacterial, and anti-proliferative potentials and are rich with active compounds that can be used to prevent and treat many diseases like cardiovascular diseases among others.

**KEYWORDS**

Anticancer activity; antioxidant activity; total phenolic; total flavonoid; Spartium junceum L.

## Introduction

Phenolics are essential plant elements present in fruits, vegetables, cereals, and legumes. They play a variety of activities in plants, including pigmentation, growth, reproduction, and resistance to pathogens or predators. Coumarins, flavonoids, phenolic acids, stilbenes, and tannins are the five primary classes [1,2]. Phenolics are secondary metabolites that are mostly generated in plants via the phenylpropanoid pathway from shikimic acid. They are also formed as byproducts of the monolignol pathway and as a result of the breakdown of lignin and cell wall polymers in vascular plants [1,2].

Phenolics are well-known medicinal chemicals for their usefulness in the treatment of an extensive variety of diseases, including diabetes, cardiovascular and neurological diseases, and cancer. Their anti-diabetic activity is mediated by changes in glucose metabolism [1]. Their anticancer action is mostly due to their antioxidant activity, as they are powerful radical scavengers [1,3]. Furthermore, their anti-carcinogenic activities are linked to their ability to inhibit cell proliferation, angiogenic, oncogenic signaling

cascades producing apoptosis, and limiting cellular migration and metastasis [1,4].

However, multiple studies have shown that phenolic acids and their derivatives have antibacterial capabilities due to their bactericidal properties. The fact that phenolic acids are weak organic acids with varied lipophilicity explains a lot of this. Compounds' solubility in microbial membranes and, as a result, antibacterial activity is regulated by their dissociation constant and lipophilicity [5]. Undissociated phenolic acids frequently infiltrate the cell membrane by passive diffusion, acidifying the cytoplasm by breaching the cell membrane, initiating the outflow of important intracellular constituents, and eventually leading to breakdown of microbial cells [5-7]. Furthermore, the antibacterial activity of p-coumaric acid was examined, and it was discovered that p-coumaric acid caused bacterial cell death by two primary mechanisms: disintegration of the microbial cell membrane and/or attaching to the microbial genomic material [8]. A subsequent investigation discovered that phenolic acids, including chlorogenic acid and the hydroxycinnamic acids, caffeic acid, p-coumaric acid, and ferulic acid, were antibacterial at pH 4.5 and bacterial-static at higher pH against particular types of bacteria [9]. However, more research into their methods of action is required to validate the possibility of bacteriostatic effects.

*Spartium junceum L.* (Spanish broom) is a flowering perennial medicinal shrub in the Fabaceae family. The blooms of this plant are rich in flavonoids, saponins, and cytisine-type alkaloids [10,11]. Previous study has found that the flowers have anti-ulcerogenic, anticancer, analgesic, anti-inflammatory, antiviral, and antioxidant properties [12-14].

In our study, we were concerned to establish “the total phenolic content (TPC)” and “the total flavonoid content (TFC)” of legumes and stems of *Spartium junceum* grown in Lebanon. On the other hand, we wanted to assess the antimicrobial, anti-proliferative and antioxidant activities of these parts of this plant.

## Materials and methods

### *Preparation of crude extracts*

The stems and the legumes of *S. junceum* were washed and kept to dry at room temperature for 4 days. After that, they were grinded to become powder using an electric grinder. Separately, 500 mL ethanol was added to 10 g powdered legumes and *S. junceum* stems. They were mixed for 5 hours at 25 °C before resting overnight. The extracts were filtered with 0.45 Millipore filter paper. The remaining ethanol was removed using a rotary evaporator set to 40 °C, and the extracts were kept at -20 °C.

The aqueous extraction preparation was done using the same procedure used for ethanolic extraction with increasing of the extraction temperature till 60 °C [15].

### *Extraction of phenolic mixture*

The approach developed by Djeridane *et al.* was utilized to extract phenolic mixture (PM) [16]. Powdered stems and legumes (1 g) were incubated in 50 mL of EtOH (70% concentration) for 48 hours at 25 °C. Following the shaking stage, a 2-hour immersion in a water bath was performed. After that, the mixture was filtered, and the ethanol was extracted using a rotary evaporator set at 40 °C. Defatting the leftover aqueous solution with petroleum ether and n-hexane yielded the bottom phase. The extraction was then carried out in the presence of ammonium sulphate (20%) and 2% meta-phosphoric acid in ethyl acetate. The ethyl acetate fraction was then dried to remove the solvent. The precipitate was dissolved in MeOH before being kept at -20 °C [16].

*Total phenolic content*

100 µL of each plant extract concentration has been mixed with 0.5 mL of "Folin-Ciocalteau reagent" (1/10 dilution) and 1.5 mL of $Na_2CO_3$ 2% (w/v). This combination was then held in the dark at 25 °C for 15 minutes. The absorbance was determined at 765 nm [17]. The results were quantified in: "milligrams of gallic acid equivalent (GAE) per gram of dry plant powder".

*Total flavonoid content*

1 mL of 2% methanolic $AlCl_3$ was mixed with 1 mL of various extract strengths. These mixes were stored in the dark at 25 °C for 15 minutes before being measured at 430 nm. All the obtained results were expressed in: "mg per gram of rutin equivalent (RE)" [18].

*Hydrogen peroxide scavenging activity*

At a basic pH of 7.4, 40 mM $H_2O_2$ was added to PBS. After 10 minutes, aqueous extract quantities were added to the $H_2O_2$ solution, and the absorbance of $H_2O_2$ was measured at 230 nm against a blank solution containing phosphate-buffered saline (PBS) but no $H_2O_2$ [19]. Ascorbic acid was used as the reference standard.
The below equation was used to compute the percentage of scavenging activity of $H_2O_2$:

**"% *Scavenging* = [($A_{control} - A_{sample}$) / $A_{control}$] × 100"**

*Chelation effects on ferrous ions*

500 µL of various extract strengths were mixed to 0.5 mL of FeSO4 (0.12 mM) and 500 µL of Ferrozine (0.6 mM). The absorbance at 562 nm was measured after a 10-minute incubation period at R.T. [20]. The blank was ultra-pure water, and the reference standard was EDTA-Na2. Measurements were carried out in triplicate. To determine the sample's ability to collect $Fe^{2+}$ ions, the following equation was used:

"***Ferrous ion - chelating ability (%)*** **= [($A_{control} - A_{sample}$) / $A_{control}$] ×100"**

## Antibacterial activity assay

*Microbial strains and culture conditions*

The antimicrobial activity of aqueous and ethanolic extracts of *Spartium junceum* stems and legumes, as well as phenolic extracts of both plant parts, was tested against two classes of microbial strains: Gram-positive (Staphylococcus epidermidis CIP 444) and Gram-negative (Escherichia coli ATCC 35218) strains. The bacteria were cultured in Mueller-Hinton broth (MHB) for 24 hours at 37 °C.

*Minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration values of the aqueous and ethanolic extracts of *Spartium junceum* stems and legumes, as well as the phenolic extracts, were determined using the in broth micro dilution method, as indicated by the "Clinical Laboratory and Standard Institute" [21]. Each plant extract was produced at 800mg/mL. In a 96-well plate (200L/well) (Greiner Bio-One, Essen, Germany), serial 2-fold dilutions of the various extracts were performed. The wells were injected with 5 x $10^5$ bacteria/mL. After incubation the plates for 24 hours at 37 °C, the MIC was calculated.
MIC was calculated according to the following equation:

**"Weight of powder (mg) =**
**Volume of solvent (ml) X Concentration (µg/ml) / Potency of powder (µg /mg)"**

## Anticancer activity assay

*Cell culture preparation*

MCF7 and 293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS) and 2% penicillin-streptomycin solution at 37°C in a humidified atmosphere with 5% $CO_2$. A hematocytometer was used to count the cells. The trypan blue exclusion method was used to assess cell viability. MCF (10 cells) were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM F12 Ham), 10% (v/v) FBS, and 2% penicillin-streptomycin solution (control). The medium needed to be supplemented with 50 µL of hydrocortisone, 25 µL of insulin, and 5 µL of cholera toxin.

*XTT assay*

The XTT assay was used to assess the cytotoxicity of extracts [13,22]. In a 96-well plate, MCF7 and 293T cells were seeded (25,000 cells/well) and subsequently treated with various extract doses (0.1, 0.3, 0.5.0.7, and 1 mg/mL). In the absence of extracts, MCF7 and 293T cells were used as controls. Following treatment, the 96-well plate was incubated under the same conditions for 24 hours. The plate was next incubated for 2 hours with 25 µL of XTT reagent (0.6 mg/ml) containing 25 µM N-methyl dibenzopyrazine methyl sulfate (PMS) under the same experimental conditions. The absorbance was measured at 490 nm using an ELX800 Microplate Reader, and the cytotoxicity % was evaluated as follows:

**"% Cytotoxicity = [($A_{Control} - A_{Tested}$) / $A_{Control}$] ×100"**

## Results and discussion

*TPC and TFC*

Traditional extraction procedures are the most often utilized for separating these chemicals in a variety of samples due to their simplicity and low cost. The solvent used in the extraction procedure is crucial for the successful isolation and identification of physiologically active chemicals from plant material. Extraction using aqueous organic solvents was more successful than extraction with water alone [23-25]. It was shown that acetone-based mixtures lead to extract more phenolic mixture than methanol-based mixtures [26]. However, another study demonstrated that ethanol and ethanol/water solutions were best for phenolic extraction [27]. Results obtained revealed that the different parts of our plant contain various amounts of phenolics and flavonoids depending on the used solvent in the extraction (Table 1). The phenolic mixture represented the higher TPC and TFC in the two studied parts.

The total phenolic content (TPC) in ethanolic extract of legumes of *Spartium junceum* (32±0.05 mg GAE/ g extract) was found to be lower than that of the extract of the stem (38±0.03 mg GAE/ g extract). Similarly, the TPC of the water extract of the stem (23± 0.03 mg GAE/ g extract) is much higher than that of legumes extract in the same solvent (13± 0.03mg GAE /g extract). On the other hand, the total flavonoid content (TFC) in the water extract of the stem (13±0.07 mg/g RE) is higher than that of the legumes (6±0.05 mg/g RE). However, ethanolic extracts of both legumes and stem show very close TPC values, 23±0.03 and 24±0.03 mg/g RE, respectively.

**TABLE 1** TPC and TFC in legumes and stems of S. junceum

| | Legumes | | Stem | |
|---|---|---|---|---|
| | **Water** | **EtOH** | **Water** | **EtOH** |
| **TPC** (mg GAE/g dry weight) | 13±0.03 | 32±0.05 | 23±0.03 | 38±0.03 |
| **TFC** (mg/ g RE) | 6±0.05 | 23±0.07 | 13±0.07 | 24±0.03 |

*Antioxidant activity*

Various mechanisms are used to determine the antioxidant potential of plants without a single broad-spectrum system give a precise and quantitative prediction [28]. In our present study, two techniques are proposed to establish the antioxidant activity. Table 2 reveals the $EC_{50}$ values which correspond to the concentration effective in producing 50% of the maximal activity. There is a correlation between TPC, TFC and antioxidant activity of the plant [28]. This explains why the ethanolic extract of the stem has higher scavenging activity and iron-chelating activity than the other extract with lower $EC_{50}$ values of $2.6x10^2$ and $2.9x10^2$ µg/mL, respectively, while the other extracts either the aqueous or the ethanolic ones show moderate activity.

**TABLE 2** EC50 of extracts from the two parts of S. junceum (µg/mL)

| | **Extracts** | | |
|---|---|---|---|
| | | **$H_2O_2$** | **Fe** |
| | **Ascorbic acid** | 10 | - |
| | **EDTA** | - | 5 |
| **Legumes** | **Aqueous** | $3x10^2$ | $3.6x10^2$ |
| | **EtOH** | $2.9x10^2$ | $4.4x10^2$ |
| **Stems** | **Aqueous** | $2.8x10^2$ | $3.2x10^2$ |
| | **EtOH** | $2.6x10^2$ | $2.9x10^2$ |

*Antibacterial activity*

Because of the surge in the number of people dying from infectious diseases, it has become crucial to look for novel medications and effective antimicrobial agents that may overcome microorganism resistance and prevent fatal opportunistic infections [29].

The findings of the antimicrobial tests revealed that the water extracts of *S. junceum's* stem and legumes had no antibacterial effectiveness against either Gram-positive bacteria (*S. epidermidis*) or Gram-negative bacteria (*E. coli*). Despite the fact that water extracts containing flavonoid components are responsible for antibacterial action [30-32]. Regardless, the antibacterial action of *S. junceum* appears to be unrelated to the flavonoids found in the plant. Nonetheless, the ethanolic extract of the stems hasn't

shown any antimicrobial effect against neither strains of the bacteria used, where that of the legumes has shown better activity against *S. epidermidis* (23.5 mg/mL) than against E. coli (47 mg/mL). Phenolic mixture of both stem and legume extracts has better antimicrobial efficacy against *S. epidermidis* (20.25 and 18.25 mg/mL, respectively) than against *E. coli* (40.5, 36.5 mg/mL respectively) (Table 3).

**TABLE 3** Antibacterial potential of the two parts of S. junceum

| | | S. epidermidis | E. coli |
|---|---|---|---|
| **Stems** | **EtOH** | No effect | No effect |
| | **Water** | No effect | No effect |
| | **PM** | 20.25 | 40.5 |
| **Legumes** | **EtOH** | 23.5 | 47 |
| | **Water** | No effect | No effect |
| | **PM** | 18.25 | 36.5 |

*Cancer cell proliferation*

It has been demonstrated that phenolic mixture has a low inhibition activity against 293T cancer cells [33]. As shown in Figure 1, the cytotoxicity percentage is concentration-dependent. As the concentration in the phenolic mixture increases in the stem and the legumes, the cytotoxicity increases to reach a value of 45 and 53%, respectively. The anticancer activity of PM is connected to its antioxidant activity. Plant phenolic compounds' antioxidant activity, however, is not restricted to direct radical scavenging. Polyphenols can also influence a cell's redox status by inhibiting metabolic pathways involved in the generation and neutralization of free radicals [34]. Aqueous extracts of *S. junceum* legumes inhibited 293T cell proliferation by 72% at 1mg/mL, while ethanolic extracts of legumes and stems inhibited cell proliferation by 54% in both cases (Figure 2).

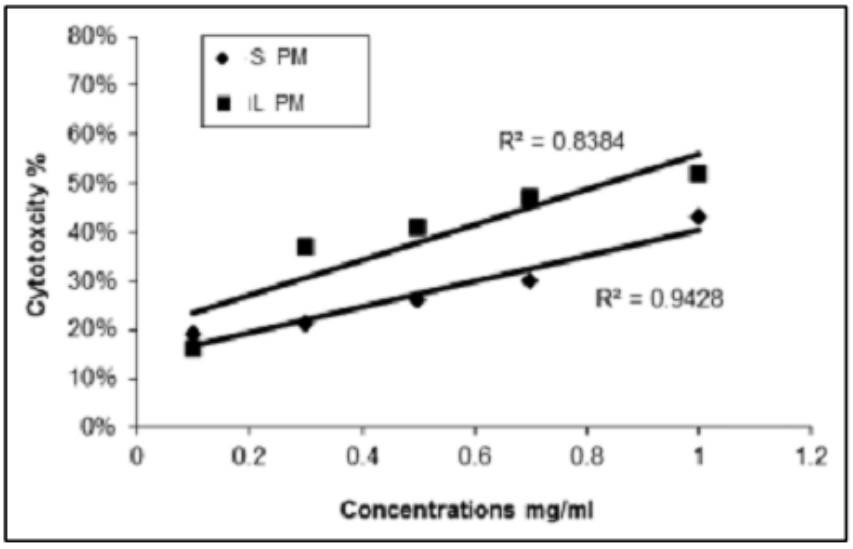


**FIGURE 1** Inhibition of the proliferation of 293T cell after the treatment with PM from legumes and stems

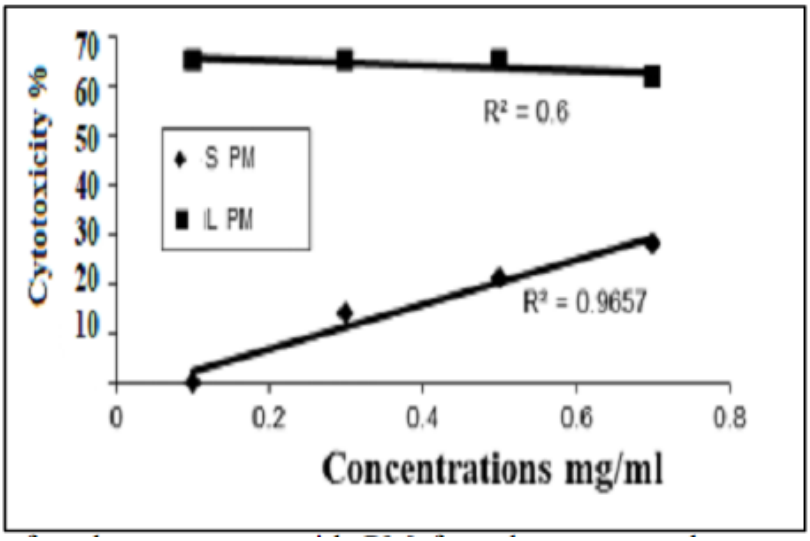


**FIGURE 2** Inhibition of the proliferation of MCF7 cell following the treatment with PM from legumes

The effect of *S. junceum* extracts was also performed on MCF7 cell line. PM revealed a noticeable growth inhibitory effect on this cell line in a concentration-dependent manner. As demonstrated in Figure 3, low concentrations of phenolic mixture extracted from stems and legumes starting from 0.1 to 0.7 mg/mL showed a maximum inhibition of 30 and 62% respectively at 0.7mg/mL.

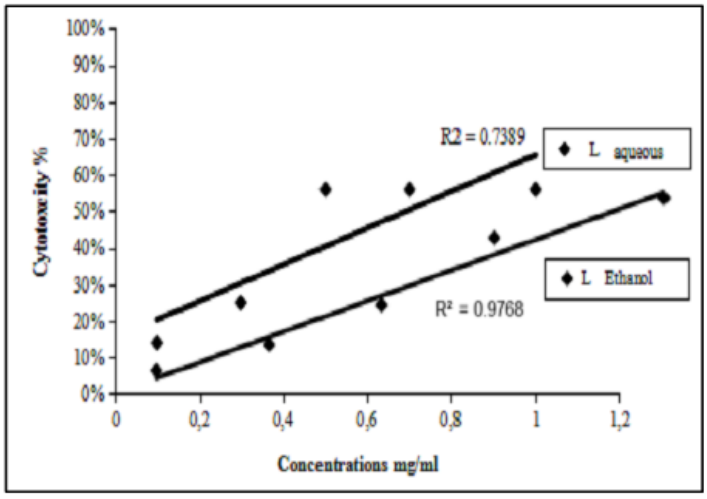


**FIGURE 3** Inhibition of the proliferation of 293T cell following the treatment with water and ethanolic extracts from legumes

Moreover, we observed a better inhibitory activity of the aqueous and ethanolic extracts of the legumes reaching at concentration of 1mg/mL comparable values of 66 and 47% respectively (Figure 4). On the other hand, both extracts of the stem reached a maximum value of approximately 65% (Figure 5).

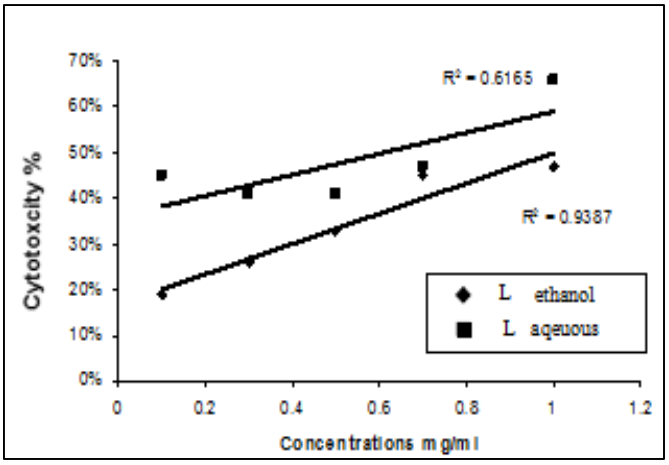


**FIGURE 4** Inhibition of the proliferation of MCF7 cell following the treatment with water and EtOH extracts from legumes

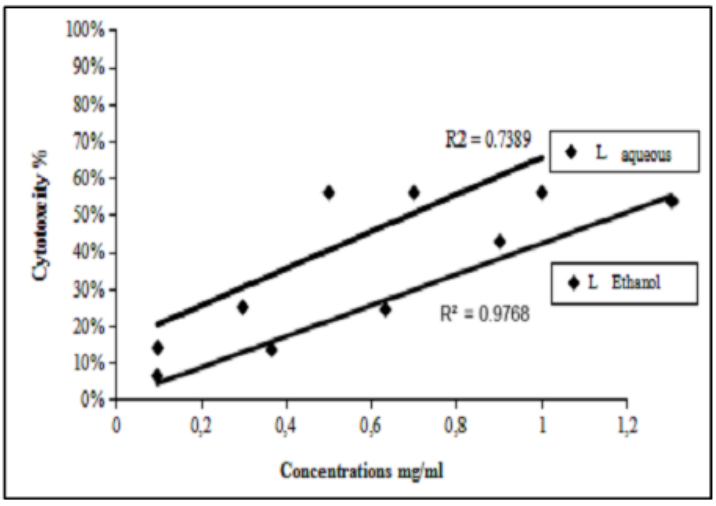


**FIGURE 5** Inhibition of the proliferation of 293T cell following the treatment with water and EtOH extracts from stems

In all the tested cases, the extracts from the stems and the legumes of *S. junceum* has shown inhibitory activity against the studied cancer cells similar to those from the flowers [35,36]. As mentioned before, the anticancer activity of *S. junceum* is related to the antioxidant activity of the medicinal components present in the plant extracts [37].

Some extracts with phytochemical bioactive molecules have the power to inhibit cancer cells. Extracts such as tannins have been used in treatment of tissue inflammation and with an important potential in cancer prevention. Consequently, plants containing such extracts might be considered as good source of bioactive compounds used in the prevention or treatment of cancer. Many studies were performed on different phenolic compounds extracted from Chinese herbs. Phenolic acids, flavonoids, tannins, coumarins, showed anticancer capacity, and thus would be used as natural antioxidants and would be beneficial chemo-preventive agents [38].

## Conclusion

As a conclusion, it was shown that the stems and the legumes of *Spartium junceum* have

antioxidant and antimicrobial activities; also, they have exhibited an anticancer activity against 293T and MCF7 tumor cells in a concentration-dependent manner. The overall findings suggested that *Spartium junceum* may be an important provider of active chemicals that can be used to prevent and treat a variety of disorders associated with oxidative stress. Extraction and purification of some of these active compounds should be done in the future to evaluate their biological activities to have a better sight on their relationship with the treatment of some diseases.

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**Authors' Contributions**

**Hawraa Zahrddin:** Hawraa Zahrddin led the fieldwork including all the extraction methods and the antioxidant, antimicrobial and anticancer activities, and she wrote the article. **Akram Hijazi:** Akram Hijazi was the lab supervisor, he checked all the experimental work and he helped in the analysis by providing important background information. **Mahmoud Khalil**: Mahmoud Khalil checked all the work and followed up with the biological part work and corrected the article and added notes. In other words, he did the editing and the finalization of the manuscript.

**Conflict of Interest:**

The authors declare no conflict of interest.

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