

Phytochemical Profiling and Bioactivity Evaluation of Butanol *Spartium junceum* Flower Extract: GC-MS Characterization, Anticancer, Antioxidant, Antibiofilm, and Anti-Obesity Potential with Public Health Implications

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The phytochemical composition and biological activities of *Spartium junceum* flower extracts were studied after fractionation using solvents of increasing polarity. Among the tested fractions, the butanol extract exhibited the highest levels of total phenolics, flavonoids, flavonols, and tannins, suggesting enrichment in polar bioactive metabolites. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the major constituents were mainly fatty acids and unsaturated lipid derivatives, including n-hexadecanoic acid, linoelaidic acid, oleic acid, and linoleic acid methyl ester. The butanol fraction demonstrated significant cytotoxic activity against MCF-7 breast and PC-3 prostate cancer cell lines, with IC₅₀ values of 96 and 89.8 µg/mL, respectively, while showing lower toxicity toward normal WI-38 cells. Moderate antioxidant activity was confirmed using (2,2-diphenyl-1-picryl-hydrazyl-hydrate), (2,2 (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and total antioxidant capacity assays, with the strongest scavenging effect observed in the ABTS method. Additionally, the extract showed pancreatic lipase inhibitory potential (IC₅₀ = 79.6 µg/mL) and concentration-dependent antibiofilm activity against *Staphylococcus aureus* and *Escherichia coli*. These findings support *S. junceum* flowers as a promising source of multifunctional natural therapeutics.

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INTRODUCTION

Medicinal plants remain an essential source of bioactive secondary metabolites with wide therapeutic relevance. In recent years, plant-derived compounds, such as phenolics, flavonoids, terpenoids, and glycosides, have gained increasing attention as safer alternatives to synthetic drugs, particularly for chronic diseases including cancer, obesity,

and microbial infections. Natural products are widely recognized for their antioxidant, anticancer, antimicrobial, and enzyme inhibitory properties, making them valuable candidates for pharmaceutical and functional applications (Mohi-Ud-Din *et al.* 2022; Sharma *et al.* 2022).

Cancer remains a leading cause of mortality worldwide, prompting continuous exploration of polyphenol-rich plant extracts as potential anticancer agents. Recent reviews confirm that plant polyphenols exert cytotoxic effects through apoptosis induction, inhibition of tumor proliferation, and regulation of oxidative stress and inflammatory signaling (Campos-Pérez and Martínez-López 2015). Oxidative stress, caused by excessive reactive oxygen species (ROS), plays a central role in the development of cancer, cardiovascular disorders, and neurodegenerative diseases. Therefore, antioxidant-rich plant extracts are increasingly investigated as protective agents capable of scavenging free radicals and modulating redox pathways (Kotha *et al.* 2022). Obesity is another global health challenge closely associated with metabolic and cardiovascular complications. Pancreatic lipase inhibition is considered an effective strategy to reduce dietary fat absorption, and recent research emphasizes the promising role of natural plant metabolites as safer lipase inhibitors compared with synthetic drugs (Oliveira *et al.* 2022). In addition, bacterial biofilms represent a serious clinical problem due to their resistance to antibiotics. Plant-derived phenolics and flavonoids have recently been reported to interfere with quorum sensing, adhesion, and extracellular matrix formation, providing new antibiofilm approaches against pathogens (Kashi *et al.* 2024). *Spartium junceum* L. (Fabaceae), commonly known as Spanish broom, is a Mediterranean medicinal shrub traditionally used for inflammatory and infectious conditions. However, the phytochemical composition and multi-biological potential of its flowers remain underexplored (Zahrddin *et al.* 2022). Despite its traditional use, the floral parts of *Spartium junceum* remain insufficiently studied, particularly regarding solvent fractionation and comprehensive biological evaluation. Integrated investigations linking chemical profiling of specific fractions with multiple bioactivities are limited. Therefore, further characterization of the butanol fraction is warranted to clarify its therapeutic potential.

To the best of the authors' knowledge, this study represents the first comprehensive investigation of the butanol fraction derived from *Spartium junceum* flowers that integrates solvent-dependent extraction, GC–MS chemical profiling, and simultaneous evaluation of anticancer, antioxidant, pancreatic lipase inhibitory, and antibiofilm activities within a single experimental framework. Unlike previous reports that mainly have focused on essential oils or crude extracts, the present work provides a targeted assessment of a polar fraction and correlates its phytochemical composition with multiple biological effects, offering a broader understanding of its therapeutic potential.

EXPERIMENTAL

Plant Material

Flowers of *Spartium junceum* were supplied by the Agricultural Research Center. The collected floral material was air-dried and then mechanically pulverized using an electric grinder to obtain a homogeneous fine powder. The powder was subsequently sieved and preserved in clean glass containers until further use.

Quantitative Phytochemical Screening

Quantitative phytochemical analysis of *S. junceum* flower extract was conducted to evaluate the total contents of phenols, tannins, flavonoids, and flavonols using well-established spectrophotometric colorimetric methods (Khormi *et al.* 2025). Total phenolic content was determined by the Folin–Ciocalteu assay, where the reaction mixture was incubated in the dark and absorbance was measured at 760 nm; results were calculated from a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of extract (GAE/g). Total tannins were estimated using the Folin–Denis method following precipitation with (polyvinylpyrrolidone) PVPP to separate non-tannin phenolics. Absorbance was recorded at 725 nm, with values reported as mg tannic acid equivalents per gram (TAE/g). Total flavonoid content was quantified through the aluminum chloride complex formation assay, with absorbance measured spectrophotometrically and concentrations derived from a quercetin standard curve, expressed as mg quercetin equivalents per gram (QE/g). In addition, total flavonols were assessed using the $AlCl_3$ colorimetric technique in the presence of sodium acetate, with absorbance monitored at 440 nm and results presented as mg rutin equivalents per gram of extract. All experiments were performed in triplicate, and data were expressed as mean \pm standard deviation (SD).

GC-MS Analysis for the Best Solvent Butanol Flower Extract of *S. junceum*

Prior to GC–MS injection, the butanol extract obtained from *S. junceum* flowers was dehydrated using anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture. The dried sample was then filtered through a 0.45- μ m syringe membrane filter to ensure clarity and prevent particulate interference during analysis. The GC–MS profiling was performed using a Trace GC Ultra–ISQ system (Thermo Scientific, USA). The chromatographic separation was performed under a programmed oven temperature gradient, beginning at 70 °C, followed by an increase to 280 °C at a rate of 5 °C/min, where it was held for 2 min. The temperature was then further raised to 300 °C at a rate of 10 °C/min. Identification of the detected compounds was achieved by comparing their retention times and mass spectral fragmentation patterns with those available in the Wiley 09 mass spectral library database.

Anticancer Potential of Butanol Flower Extract of *S. junceum*

The *in vitro* cytotoxic effect of the butanol extract from *S. junceum* flowering aerial parts was evaluated against PC-3 prostate cancer cells, MCF7 breast cancer cells, and WI-38 normal lung fibroblasts (Vacsera, Egypt). Cells were seeded in 96-well plates (5×10^4 cells/well) and cultured in RPMI-1640 medium supplemented with 10% (Fetal bovine serum) FBS and antibiotics. After 24 h, cells were treated with different extract concentrations (31.25 to 1000 μ g/mL) and incubated for another 24 h at 37 °C in 5% CO_2 . Cell viability was determined using the MTT assay by adding MTT solution (5 mg/mL), followed by incubation for 4 h. Formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 490 nm. Untreated cells served as controls, and morphological changes were observed using a phase-contrast microscope (Soliman *et al.* 2025).

Assay for Biofilm Inhibition

The antibiofilm activity of the butanol extract from the aerial flowering parts of *S. junceum* was assessed against *E. coli* ATCC 25922 and *S. aureus* ATCC 35556 using the

microtiter plate (MTP) method with slight modifications from previously described protocols (Soliman *et al.* 2023; Soliman *et al.* 2024). Briefly, bacterial suspensions were prepared in Mueller–Hinton broth, diluted (1:100) in tryptic soy broth (TSB) supplemented with 1% glucose, and incubated with graded concentrations of the extract in 96-well plates at 37 °C for 24 h. After incubation, planktonic cells were removed and wells were gently washed with PBS (pH 7.4). The adhered biofilms were fixed with 95% methanol for 10 min, stained with 0.3% crystal violet for 15 min, and excess stain was rinsed off with distilled water. Bound dye was then solubilized using 30% acetic acid, and biofilm biomass was quantified by measuring absorbance at 540 nm using a microplate reader. Results were expressed relative to untreated control wells.

Anti-obesity Activity of Butanol Flower Extract of *S. junceum* via Lipase Inhibition

According to Kim *et al.* (2010), the inhibition of lipase activity by varying doses of butanol extract of the aerial flowering parts of *S. junceum* and the standard control (Orlistat) (7.8 to 1000 µg/mL dissolved in DMSO) was detected using the substrate 4-nitrophenyl butyrate. Potassium phosphate buffer (pH 7.0, 0.1 mM) was employed to prepare a stock solution of lipase (5 mg/mL). The reaction mixture consisted of 5 µL of 15 mM 4-nitrophenyl butyrate, 35 µL of enzyme, 220 µL of potassium phosphate buffer, and 5 µL of flowers plant extract, respectively. All these components were preserved for 10 min at 37 °C. The released quantity of 4-nitrophenol from the reaction was estimated at 405 nm. Lipase inhibition was measured from the subsequent Eq. 1:

$$\text{Lipase inhibition (\%)} = \frac{\text{Abs.control} - \text{Abs.sample}}{\text{Abs.control}} \times 100 \quad (1)$$

Antioxidant Activity

DPPH radical scavenging procedure

The butanol extract from the aerial flowering parts of *S. junceum* were evaluated using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) procedure for their ability to scavenge free radicals. To put it briefly, a 0.1 mM DPPH solution in ethanol was prepared. Next, 3 mL of various extracts were mixed with 1 mL of this solution at several concentrations (7.8 to 1000 µg/mL). After vigorous shaking, the combination took 30 min to recover at room temperature. Then, using a UV–visible Milton Roy spectrophotometer, absorbance was determined at 517 nm. Three replicates of the experiment were run, using ascorbic acid as the reference standard compound (Patel *et al.* 2010). The sample's IC₅₀ value, or using a log dosage inhibition curve, the concentration of the sample essential to inhibit 50% of the DPPH free radical was established. A reaction mixture with lower absorbance was taken as a sign of increased production of free radicals.

ABTS assay

The ABTS (2,2 (3-ethylbenzothiazoline-6-sulfonic acid) was acquired from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA) and served as a test for assessing antioxidant properties. The assessment of test samples' capacity to scavenge free radicals generated from the ABTS reagent was conducted, as outlined by Ilyasov *et al.* (2020). The identical quantities analyzed in the DPPH assay were evaluated in this test.

Total antioxidant capacity assay

The total antioxidant capacity (TAC) of the butanol extract of *S. junceum* flowers was determined using the phosphomolybdenum method. Different extract concentrations were mixed with TAC reagent (sulfuric acid, sodium phosphate, and ammonium molybdate) and incubated at 95 °C for 90 min. After cooling, absorbance was measured at 695 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). All tests were performed in triplicate, with ascorbic acid as the reference standard, and IC₅₀ values were calculated from the dose–response curve (Silvestrini *et al.* 2023).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8.0 (USA). Data are expressed as mean ± SD (n = 3). One-way analysis of variance (ANOVA) followed by Tukey’s test was applied, with significance set at P < 0.05.

RESULTS AND DISCUSSION**Solvent-dependent Extraction Efficiency and Phytochemical Composition of *S. junceum* Flowers**

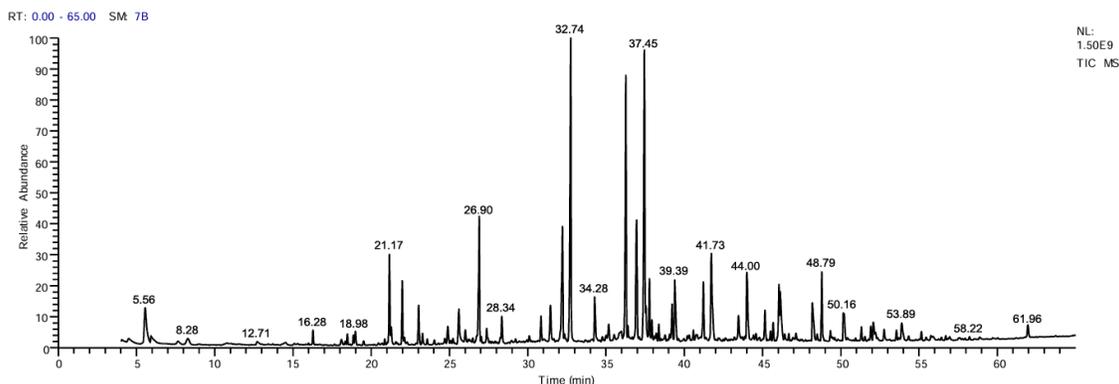
Quantitative analysis showed that total phenolic content varied significantly across solvents (butanol > methanol > water > ethyl acetate > petroleum ether). The butanol fraction exhibited the highest phenolic level (55.80 ± 0.82 mg GAE/g), while petroleum ether showed the lowest (16.07 ± 1.01 mg GAE/g) (Table 1). Flavonoid content followed a similar trend, with butanol and methanol extracts showing comparable high values (218.80 ± 1.04 and 201.20 ± 1.22 mg QE/g), whereas petroleum ether contained the least (77.67 ± 0.57 mg QE/g). Flavonols were also highest in butanol (108.02 ± 0.79 mg RTE/g), followed by methanol and water. Total tannins were significantly greater in the butanol fraction (42.81 ± 0.84 mg TAE/g), while petroleum ether remained the lowest (12.06 ± 1.01 mg TAE/g). Polyphenols and flavonoids are widely recognized for their antioxidant and protective roles against chronic diseases, including cancer, cardiovascular disorders, and neurodegeneration (Han *et al.* 2007; Kumar and Pandey 2013; Greenwell and Rahman 2015). Thus, the high phenolic and flavonoid richness of *S. junceum* flower extracts likely contribute to the antibacterial and antioxidant activities observed in this study, supporting its traditional medicinal use.

Table 1. Total Flavonoids, Flavonols, Phenols and Tannins of *S. junceum* Flower Extracts

| Extracts | Flavonoids (mg QE/g) | Flavonols (mg RTE/g) | Phenols (mg GAE/g) | Tannins (mg TAE/g) |
|-----------------|----------------------|----------------------|--------------------|--------------------|
| Petroleum ether | 77.67 ± 0.57 | 30.66 ± 0.59 | 16.07 ± 1.01 | 12.06 ± 1.01 |
| Ethyl acetate | 101.54 ± 0.54 | 38.99 ± 0.44 | 30.63 ± 0.65 | 14.10 ± 0.58 |
| Butanol | 218.80 ± 1.04 | 108.02 ± 0.79 | 55.80 ± 0.82 | 42.81 ± 0.84 |
| Methanol | 201.20 ± 1.22 | 87.92 ± 0.52 | 39.85 ± 0.6 | 26.83 ± 0.61 |
| Water | 127.62 ± 3.4 | 62.77 ± 0.91 | 34.57 ± 0.44 | 21.39 ± 0.43 |

Table 2. Top Phytochemical Constituents Detected in the Butanol Fraction of *S. junceum* Flower Extract Using GC–MS

| Rank | Rt (min) | Area % | M.W. | M.F. | Identified Compounds |
|------|----------|--------|------|-----------|--|
| 1 | 29.92 | 15.21 | 256 | C16H32O2 | n-Hexadecanoic acid |
| 2 | 29.08 | 8.36 | 270 | C17H34O2 | Hexadecanoic acid, methyl ester |
| 3 | 33.22 | 6.72 | 280 | C18H32O2 | 17-Octadecynoic acid |
| 4 | 33.13 | 6.61 | 280 | C18H32O2 | Linoelaidic acid |
| 5 | 7.73 | 6.36 | 548 | C29H40O10 | Proceroiside |
| 6 | 4.57 | 5.35 | 132 | C6H12O3 | Acetic acid, ethoxy-, ethyl ester |
| 7 | 32.30 | 5.17 | 294 | C19H34O2 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester |
| 8 | 39.99 | 4.27 | 390 | C24H38O4 | Diisooctyl phthalate (possible contaminant) |
| 9 | 32.42 | 2.27 | 400 | C28H48O | Cholestan-3-ol, 2-methylene- |
| 10 | 32.93 | 2.22 | 298 | C19H38O2 | Heptadecanoic acid, 16-methyl-, methyl ester |
| 11 | 8.80 | 2.14 | 128 | C6H8O3 | Furaneol |
| 12 | 33.67 | 2.08 | 282 | C18H34O2 | Oleic Acid |
| 13 | 19.52 | 1.89 | 162 | C6H12O6 | α -D-Glucopyranose |
| 14 | 30.43 | 1.75 | 284 | C18H36O2 | Hexadecanoic acid, ethyl ester |

**Fig. 1.** GC–MS chromatographic profile of the butanol fraction derived from *S. junceum* flower extract

GC–MS Analysis of the Butanol Extract of *S. junceum* Flowers

The GC–MS analysis of the *S. junceum* flower butanol extract revealed that the major identified constituents were mainly fatty acids and unsaturated lipid derivatives, such as n-hexadecanoic acid, oleic acid, and linoleic acid methyl ester (Fig. 1) (Table 2). These compounds are widely recognized for their significant antioxidant and anticancer potential. Although unsaturated fatty acids are often highlighted for their antioxidant activity, saturated fatty acids such as palmitic acid have also been reported to exhibit important biological activities, including anti-inflammatory and protective effects. Although unsaturated fatty acids has been reported to their antioxidant activity, saturated fatty acids such as palmitic acid have also been reported to exhibit important biological activities, including anti-inflammatory and protective effects (Bermúdez *et al.* 2022). Moreover, exhibit anti-inflammatory and protective biological effects, supporting its role in reducing oxidative stress–related damage (Purushothaman *et al.* 2025). Likewise, oleic acid is

known to modulate immune and inflammatory pathways and has been linked to anticancer activity through apoptosis induction and inhibition of tumor progression (Santa-María *et al.* 2023). In addition, linoleic acid derivatives, such as 9,12-octadecadienoic acid methyl ester, have been extensively studied for their role in cancer prevention and antioxidant defense mechanisms (Chen *et al.* 2025). Moreover, the detection of glycosidic constituents, such as procero-side, suggests the presence of polar metabolites that may further enhance the therapeutic value of the butanol fraction. These findings are consistent with the phytochemical screening and quantitative analysis of *S. junceum* flower extracts, which demonstrated that the butanol fraction contained the highest levels of phenolics and flavonoids. Because polyphenols and lipid-derived bioactives often act synergistically, the richness of this fraction in such metabolites may explain the strong antioxidant and anticancer activities observed in the present study. Overall, the identified compounds support the traditional medicinal use of *S. junceum* flowers and highlight their potential as a promising source of natural antioxidant and anticancer agents.

***In vitro* Anticancer Activity of the Butanol Extract of *S. junceum* Flowers**

The anticancer activity of the butanol extract of *S. junceum* flowers was evaluated against MCF-7 breast cancer, PC-3 prostate cancer, and the normal WI-38 fibroblast cell line. The extract exhibited a clear dose-dependent reduction in cell viability in both cancer cell lines. At high concentrations (500 to 1000 µg/mL), viability decreased to less than 10% in MCF-7 and nearly 3% in PC-3 cells (Fig. 2).

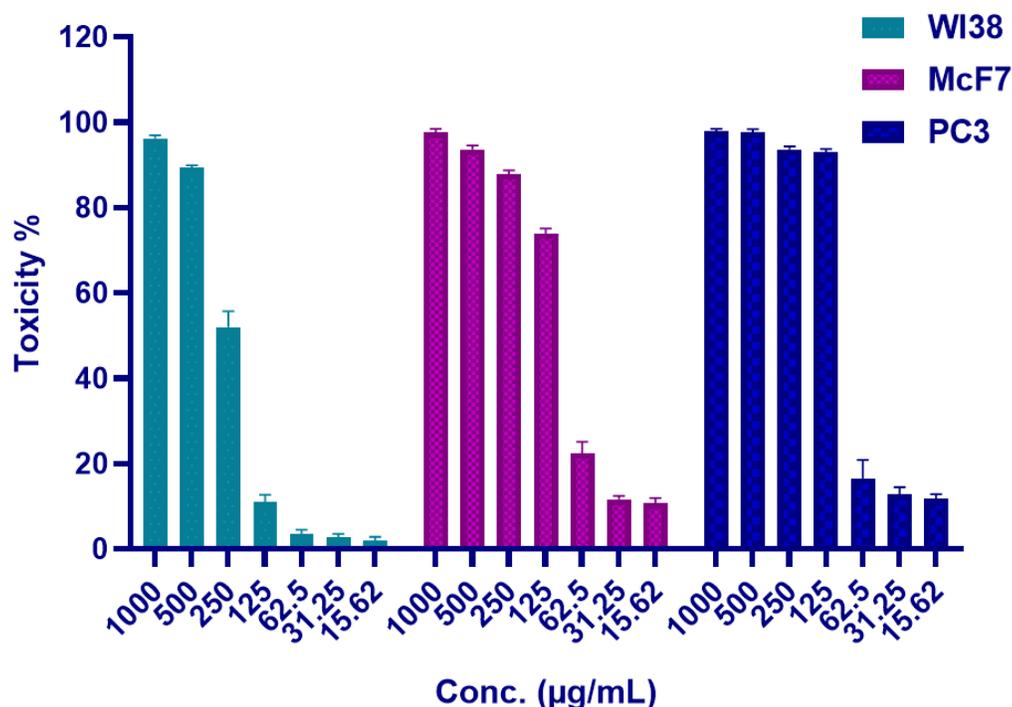


Fig. 2. Dose-dependent cytotoxicity of the butanol extract of *S. junceum* flowers on MCF7, Pc3, and Wi38 cell lines

The calculated IC_{50} values were 96.0 µg/mL for MCF-7 and 89.8 µg/mL for PC-3, indicating strong cytotoxic activity. In contrast, the extract showed lower toxicity toward normal WI-38 cells, with an IC_{50} value of 244.4 µg/mL, suggesting selective anticancer

potential of the butanol fraction. The strong cytotoxic activity of the *S. junceum* flower butanol extract against MCF-7 and PC-3 cell lines may be attributed to its richness in phenolic compounds, flavonoids, and lipid-derived metabolites. Recent studies have confirmed that polyphenol-rich plant extracts exert anticancer effects through apoptosis induction, inhibition of tumor cell proliferation, and modulation of oxidative stress pathways (Sharma *et al.* 2022). Moreover, unsaturated fatty acids and their derivatives, such as those identified in the GC-MS profile of the butanol fraction, have been reported to suppress cancer progression by disrupting membrane integrity and regulating inflammatory signaling (Akbar *et al.* 2024). The higher selectivity observed toward cancer cells compared with normal WI-38 fibroblasts suggests that the extract contains bioactive compounds with preferential toxicity against malignant cells, a key requirement for developing safe natural anticancer agents. Similar selective cytotoxic effects have been documented for butanol fractions of other medicinal plants enriched in flavonoids and fatty acids (Mohi-Ud-Din *et al.* 2022).

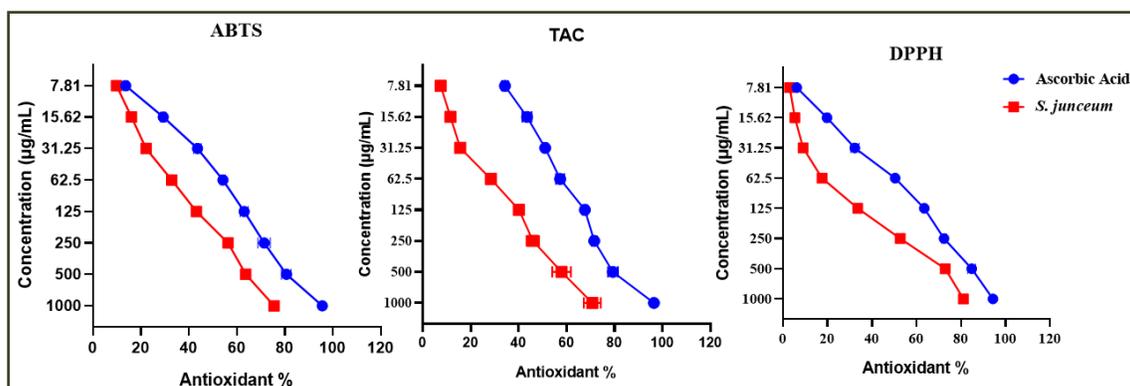


Fig. 3. Antioxidant activity of *S. junceum* flower butanol extract compared with ascorbic acid using ABTS, TAC, and DPPH assays

Radical Scavenging Activity of *S. junceum* Butanol Extract

The antioxidant activity of the butanol extract of *S. junceum* flowers was evaluated using TAC, DPPH, and ABTS assays across different concentrations (7.81 to 1000 µg/mL) (Fig. 3). The extract exhibited a clear concentration-dependent increase in antioxidant capacity in all tested methods. The calculated IC₅₀ values confirmed the moderate antioxidant potential of the butanol fraction, recording 340 µg/mL in the TAC assay, 233 µg/mL in the DPPH radical scavenging assay, and 190 µg/mL in the ABTS assay. Among the three methods, the lowest IC₅₀ value was obtained in the ABTS assay, indicating that the butanol extract showed stronger scavenging activity against ABTS radicals compared with DPPH and TAC. In comparison, ascorbic acid exhibited significantly lower IC₅₀ values (29.0 µg/mL for TAC, 60.5 µg/mL for DPPH, and 50.2 µg/mL for ABTS), confirming its higher antioxidant efficiency relative to the plant extract. The moderate antioxidant activity observed for the butanol extract of *S. junceum* flowers may be attributed to its richness in phenolic and flavonoid compounds, which are well recognized as major contributors to radical scavenging capacity. Recent studies have highlighted that polyphenols exert antioxidant effects through hydrogen atom donation, metal chelation, and modulation of oxidative stress pathways, thereby protecting biological systems from reactive oxygen species (ROS) damage (Rana *et al.* 2022). The lower IC₅₀ value obtained in the ABTS assay compared with DPPH suggests that the butanol extract may contain

both hydrophilic and lipophilic antioxidants, as ABTS is known to be more sensitive in detecting a wider range of radical scavengers (Kotha *et al.* 2022). Furthermore, the antioxidant potential of plant-derived butanol fractions has been widely associated with their high polarity and enhanced extraction of bioactive polyphenols, which often act synergistically to provide protective effects against chronic diseases, including cancer and cardiovascular disorders (Wang *et al.* 2025).

Pancreatic Lipase Inhibitory Activity of *S. junceum* Flower Butanol Extract

The anti-obesity potential of the butanol extract of *S. junceum* flowers was evaluated through its inhibitory activity against pancreatic lipase, using orlistat as a standard reference drug. The extract demonstrated a concentration-dependent inhibition of lipase activity over the tested range (7.81 to 1000 µg/mL) (Fig. 4A). The calculated IC₅₀ value of the *S. junceum* butanol fraction was 79.6 µg/mL, indicating a moderate inhibitory effect compared with orlistat, which exhibited a significantly stronger activity with an IC₅₀ of 14.8 µg/mL. These results suggest that the butanol extract possesses promising lipase inhibitory properties, although its potency remains lower than the pharmaceutical control. The observed lipase inhibition may be attributed to the high phenolic and flavonoid content previously reported in the butanol fraction of *S. junceum* flowers. Recent studies have confirmed that plant-derived polyphenols can reduce lipid absorption by inhibiting digestive enzymes, such as pancreatic lipase, thereby contributing to anti-obesity effects through suppression of triglyceride hydrolysis (Hou *et al.* 2022). Furthermore, flavonoids and related secondary metabolites are known to interact with the catalytic site of lipase, leading to decreased fat digestion and improved metabolic regulation (Oliveira *et al.* 2022).

Antibiofilm Activity of the Butanol Extract of *S. junceum* Flowers

The antibiofilm potential of the butanol extract of *S. junceum* flowers was evaluated against two clinically relevant bacterial strains, *Staphylococcus aureus* ATCC 35556 and *Escherichia coli* ATCC 25922. The extract exhibited a clear concentration-dependent inhibition of biofilm formation across the tested range (7.81 to 1000 µg/mL), as shown in Fig. 4B. At the highest concentration (1000 µg/mL), the butanol extract inhibited biofilm development 52.8% against *S. aureus* and 58.3% against *E. coli*. A gradual reduction in antibiofilm activity was observed with decreasing concentrations, reaching minimum inhibition values of approximately 13.5% for *S. aureus* and 10.0% for *E. coli* at 7.81 µg/mL. Overall, the extract showed slightly stronger antibiofilm activity against *E. coli* compared with *S. aureus*, particularly at higher concentrations. The observed antibiofilm effect may be attributed to the richness of the butanol fraction in phenolic compounds and flavonoids, which are known to interfere with bacterial adhesion, quorum sensing, and extracellular polymeric substance (EPS) production. Recent studies have highlighted that plant-derived polyphenols can effectively inhibit biofilm formation by disrupting microbial communication systems and reducing bacterial virulence, making them promising alternatives to conventional antibiofilm agents (Kashi *et al.* 2024). Additionally, flavonoids have been reported to weaken biofilm matrix integrity and enhance bacterial susceptibility to antimicrobial stress (Rodríguez *et al.* 2023). Therefore, the moderate antibiofilm activity demonstrated by *S. junceum* flower butanol extract supports its potential application as a natural source of antibiofilm compounds, complementing its antioxidant and cytotoxic properties observed in this study. A comparative overview of selected studies investigating n-butanol fractions from medicinal plants is presented in Table 3. As shown, polar fractions

enriched with phenolic and flavonoid constituents consistently exhibit antioxidant and cytotoxic activities, supporting the relevance of solvent-dependent fractionation in identifying multifunctional bioactive metabolites.

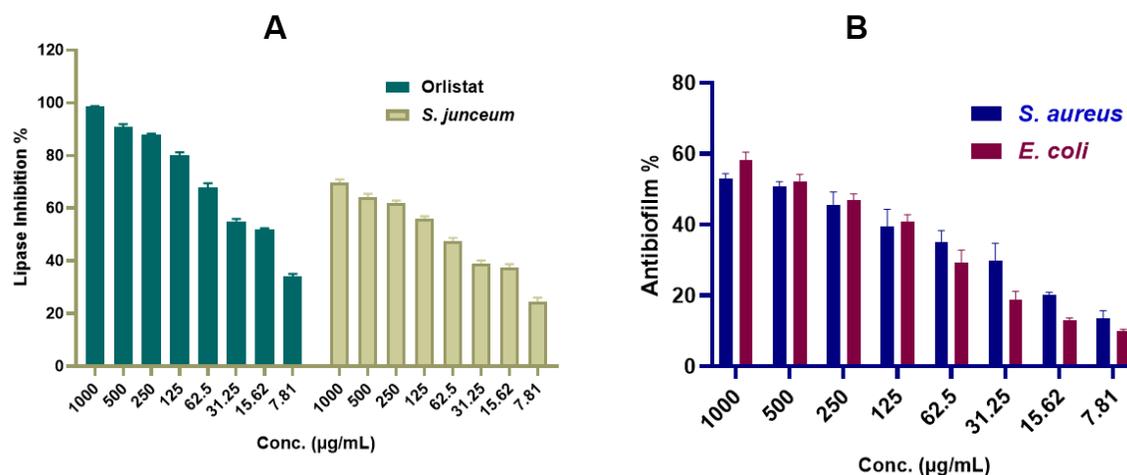


Fig. 4. Anti-obesity (A) and antibiofilm (B) potential of *S. junceum* flower butanol extract

Table 3. Comparison of Biological Activities of n-Butanol Plant Extracts

| Plant | Main fraction | Major reported activities | Reference |
|---|---------------|--|--------------------------------|
| <i>Spartium junceum</i> (present study) | Butanol | Antioxidant, Cytotoxic, Lipase inhibitory, Antibiofilm | Present work |
| <i>Ziziphus jujuba</i> fruit | n-Butanol | Antioxidant, Antimicrobial, Antifungal | (Perović <i>et al.</i> 2025) |
| <i>Tamarix nilotica</i> flowers | n-Butanol | Cytotoxic | (Fayed <i>et al.</i> 2023) |
| <i>Leptadenia pyrotechnica</i> aerial parts | n-Butanol | Antioxidant, Anti-Lipoxygenase, Cytotoxicity | (Khasawneh <i>et al.</i> 2011) |
| <i>Lepidium sativum</i> seeds | n-Butanol | Antioxidant, Antibacterial | (Ait-Yahia <i>et al.</i> 2018) |

CONCLUSIONS

1. The butanol fraction of *Spartium junceum* flowers showed the highest enrichment in phenolics, flavonoids, flavonols, and tannins among the tested extracts.
2. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the extract was dominated by fatty acids and unsaturated lipid derivatives.
3. The extract demonstrated selective cytotoxic activity against cancer cell lines compared with normal cells.
4. Antioxidant activity was confirmed using multiple *in vitro* assays, with strong radical-scavenging capacity.

5. The extract exhibited pancreatic lipase inhibitory and concentration-dependent antibiofilm activities.

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REFERENCES CITED

- Ait-Yahia, O., Perreau, F., Bouzroua, S.-A., Benmalek, Y., Dob, T., and Belkebir, A. (2018). "Chemical composition and biological activities of n-butanol extract of *Lepidium sativum* L (Brassicaceae) seed," *Tropical Journal of Pharmaceutical Research* 17, 891-896. <https://doi.org/10.4314/tjpr.v17i5.20>
- Akbar, S., Rahman, A., Ahmad, N., Imran, M., and Hafeez, Z. (2024). "Understanding the role of polyunsaturated fatty acids in the development and prevention of cancer," *Nutrition and Dietary Interventions in Cancer* 191, 57-93. https://doi.org/10.1007/978-3-031-55622-7_3
- Bermúdez, M. A., Pereira, L., Fraile, C., Valerio, L., Balboa, M. A., and Balsinde, J. (2022). "Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, in inflammation, metabolic diseases and cancer," *Cells* 11, article 2146. <https://doi.org/10.3390/cells11142146>
- Campos-Pérez, W., and Martínez-López, E. (2015). "Role of polyunsaturated fatty acids in cancer prevention," *Revista Mexicana de Endocrinología, Metabolismo y Nutrición* 2(4), 194-203.
- Chen, Y., Xiao, J., Zhu, X., Fan, X., Peng, M., Mu, Y., Wang, C., Xia, L., and Zhou, M. (2025). "Exploiting conjugated linoleic acid for health: A recent update," *Food & Function* 16(1), 147-167. <https://doi.org/10.1039/D4FO04911J>
- Fayed, M. A. A., Bakr, R. O., Yosri, N., Khalifa, S. A. M., El-Seedi, H. R., Hamdan, D. I., and Refaey, M. S. (2023). "Chemical profiling and cytotoxic potential of the n-butanol fraction of *Tamarix nilotica* flowers," *BMC Complementary Medicine and Therapies* 23, article 169. <https://doi.org/10.1186/s12906-023-03989-8>
- Greenwell, M., and Rahman, P. (2015). "Medicinal plants: Their use in anticancer treatment," *International Journal of Pharmaceutical Sciences and Research*. 6(10), 4103-4112. [https://doi.org/10.13040/IJPSR.0975-8232.6\(10\).4103-12](https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12)
- Han, X., Shen, T., and Lou, H., (2007). "Dietary polyphenols and their biological significance," *International Journal of Molecular Sciences* 8(9), 950-988. <https://doi.org/10.3390/i8090950>
- Hou, X.-D., Qin, X.-Y., Hou, J., Tang, H., and Ge, G.-B. (2022). "The potential of natural sources for pancreatic lipase inhibitors: A solution of the obesity crisis?," *Expert Opinion on Drug Discovery* 17(12), 1295-1298. <https://doi.org/10.1080/17460441.2023.2156499>

- Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., and Terekhov, R. P. (2020). “ABTS/PP decolorization assay of antioxidant capacity reaction pathways,” *International Journal of Molecular Sciences* 21(3), article 1131. <https://doi.org/10.3390/ijms21031131>
- Kashi, M., Noei, M., Chegini, Z., and Shariati, A. (2024). “Natural compounds in the fight against *Staphylococcus aureus* biofilms: A review of antibiofilm strategies,” *Frontiers in Pharmacology* 15, Article Number 1491363. <https://doi.org/10.3389/fphar.2024.1491363>
- Khasawneh, M. A., Elwy, H. M., Hamza, A. A., Fawzi, N. M., and Hassan, A. H. (2011). “Antioxidant, anti-lipoxygenase and cytotoxic activity of *Leptadenia pyrotechnica* (Forssk.) Decne polyphenolic constituents,” *Molecules* 16, 7510-7521. <https://doi.org/10.3390/molecules16097510>
- Khormi, M. A., Abdelglil, M. I., Alharbi, H. M., Alwutayd, K. M., Albaqami, J. J., Zarah, R. K., Hamdi, H., Alfattah, M. A., and Soliman, M. K. Y. (2025). “Unwrapping the phytofabrication of bimetallic silver–selenium nanoparticles: Antibacterial, anti-virulence (targeting magA and toxA genes), anti-diabetic, antioxidant, anti-ovarian, and anti-prostate cancer activities,” *Green Processing and Synthesis* 14(1), article 20250087. <https://doi.org/10.1515/gps-2025-0087>
- Kim, Y. S., Lee, Y. M., Kim, H., Kim, J., Jang, D. S., Kim, J. H., and Kim, J. S. (2010). “Anti-obesity effect of *Morus bombycis* root extract: Anti-lipase activity and lipolytic effect,” *Journal of Ethnopharmacology* 130(3), 621-624. <https://doi.org/10.1016/j.jep.2010.05.053>
- Kotha, R. R., Tareq, F. S., Yildiz, E., and Luthria, D. L. (2022). “Oxidative stress and antioxidants—A critical review on *in vitro* antioxidant assays,” *Antioxidants* 11(12), article 2388. <https://doi.org/10.3390/antiox11122388>
- Kumar, S., and Pandey, A. K. (2013). “Chemistry and biological activities of flavonoids: An overview,” *The Scientific World Journal* 2013(1), article 162750. <https://doi.org/10.1155/2013/162750>
- Mohi-Ud-Din, R., Mir, R. H., Sabreen, S., Jan, R., Pottoo, F. H., and Singh, I. P. (2022). “Recent insights into therapeutic potential of plant-derived flavonoids against cancer,” *Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents* 22(20), 3343-3369. <https://doi.org/10.2174/1871520622666220421094055>
- Oliveira, A. K. d. S., de Oliveira e Silva, A. M., Pereira, R. O., Santos, A. S., Barbosa, E. Junior, V., Bezerra, M. T., Barreto, R. S., Quintans-Junior, L. J., and Quintans, J. S. (2022). “Anti-obesity properties and mechanism of action of flavonoids: A review,” *Critical Reviews in Food Science and Nutrition* 62(28), 7827-7848. <https://doi.org/10.1080/10408398.2021.1919051>
- Patel, A., Patel, A., and Patel, N. (2010). “Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* Linn leaves (Leguminosae),” *Pharmacognosy Research* 2(3), 152-158. <https://doi.org/10.4103/0974-8490.65509>
- Perović, T., Lazović, B., Adakalić, M., Džamić, A., Žarković, L., Gašić, U., Kostić, M., Petrović, J., Stojković, D., and Ćirić, A. (2025). “Insights into bioactivity guided chemical profiling of *Ziziphus jujuba* Mill. fruits wild-growing in Montenegro,” *Heliyon* 11, article e41361. <https://doi.org/10.1016/j.heliyon.2024.e41361>
- Purushothaman, R., Vishnuram, G., and Ramanathan, T. (2025). “Antiinflammatory efficacy of n-hexadecanoic acid from a mangrove plant *Excoecaria agallocha* L.

- through *in silico*, *in vitro* and *in vivo*,” *Pharmacological Research-Natural Products* 7, article 100203. <https://doi.org/10.1016/j.prenap.2025.100203>
- Rana, A., Samtiya, M., Dhewa, T., Mishra, V., and Aluko, R. E. (2022). “Health benefits of polyphenols: A concise review,” *Journal of Food Biochemistry* 46(10), article e14264. <https://doi.org/10.1111/jfbc.14264>
- Rodríguez, B., Pacheco, L., Bernal, I., and Piña, M. (2023). “Mechanisms of action of flavonoids: Antioxidant, antibacterial and antifungal properties,” *Ciencia, Ambiente y Clima* 6(2), 33-66. <https://doi.org/10.22206/cac.2023.v6i2.3021>
- Santa-María, C., López-Enríquez, S., Montserrat-de la Paz, S., Geniz, I., Reyes-Quiroz, M. E., Moreno, M., Palomares, F., Sobrino, F., and Alba, G. (2023). “Update on anti-inflammatory molecular mechanisms induced by oleic acid,” *Nutrients* 15(1), article 224. <https://doi.org/10.3390/nu15010224>
- Sharma, E., Attri, D. C., Sati, P., Dhyani, P., Szopa, A., Sharifi-Rad, J., Hano, C., Calina, D., and Cho, W. C. (2022). “Recent updates on anticancer mechanisms of polyphenols,” *Frontiers in Cell and Developmental Biology* 10, article 1005910. <https://doi.org/10.3389/fcell.2022.1005910>
- Silvestrini, A., Meucci, E., Ricerca, B. M., and Mancini, A. (2023). “Total antioxidant capacity: Biochemical aspects and clinical significance,” *International Journal of Molecular Sciences* 24(13), article 10978. <https://doi.org/10.3390/ijms241310978>
- Soliman, M. K., Talib, A. H., Mahmoud, R., Ali, Z. A., Al-Haideri, H. H., Abalkhail, A., Binsahya, A. S., Salem, M. H., Al-Otibi, F. O., and Yassin, M. T. (2025). “Ecofriendly magnesium oxide nanoparticles: Anticancer, antimicrobial, and antidiabetic potentials *in vitro*,” *AMB Express* 15(1), 1-20. <https://doi.org/10.1186/s13568-025-01950-1>
- Soliman, M. K., Abu-Elghait, M., Salem, S. S., and Azab, M. S. (2024). “Multifunctional properties of silver and gold nanoparticles synthesis by *Fusarium pseudonygamai*,” *Biomass Conversion and Biorefinery* 14(22), 28253-28270. <https://doi.org/10.1007/s13399-022-03507-9>
- Soliman, M. K., Salem, S. S., Abu-Elghait, M. and Azab, M. S. (2023). “Biosynthesis of silver and gold nanoparticles and their efficacy towards antibacterial, antibiofilm, cytotoxicity, and antioxidant activities,” *Applied Biochemistry and Biotechnology* 195(2), 1158-1183. <https://doi.org/10.1007/s12010-022-04199-7>
- Wang, L., Li, T., Wu, C., Fan, G., Zhou, D., and Li, X. (2025). “Unlocking the potential of plant polyphenols: Advances in extraction, antibacterial mechanisms, and future applications,” *Food Science and Biotechnology* 34(6), 1235-1259. <https://doi.org/10.1007/s10068-024-01727-5>
- Zahrddin, H., Khalil, M., and Hijazi, A. (2022). “Antibacterial, antioxidant, and repellency potential of the essential oil from *Spartium junceum* l. grown in Lebanon,” *BAU Journal-Science and Technology* 4(1), article 6. <https://doi.org/10.54729/UYHZ3197>

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