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Original Article

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Antibacterial, Antifungal, and Cytotoxic Effects of *Seseli tortuosum* L. Root Extracts

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Abstract

Background and Aims: This study investigated the antibacterial, antifungal, and cytotoxic effects of *Seseli tortuosum* L. root extracts, a member of the Apiaceae family known for its diverse pharmacological activities. The genus *Seseli* L. is historically used in traditional medicine, and the *Seseli* species generally exhibit anti-inflammatory, antifungal, antibacterial, and cytotoxic properties.

Methods: Extraction involved maceration and Soxhlet methods using dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH). The antibacterial activity was assessed *via* broth microdilution assays, and the antifungal activity was evaluated using the Poison PDA technique against *Aspergillus parasiticus*, *Fusarium moniliforme,* and *Fusarium culmorum*. The cytotoxic effects on human cancer cell lines (MCF7, PC3, A549) and a normal embryonic kidney cell line (HEK293) were determined using the MTT assay.

Results: Cytotoxic assays indicated that the DCM macerate extract had the most potent effects on the PC3 cell line, with an IC_{50} value of 82.78 ± 0.99 µg/mL, showing higher selectivity compared to the MCF7 and A549 cell lines. The antibacterial tests revealed that the DCM and EtOAc extracts significantly inhibited *E. Faecalis*, MIC values calculated as 0.3125 mg/mL for both extracts. The antifungal activity results indicated that the Soxhlet DCM extract exhibited the most consistent antifungal activity, particularly against *F. culmorum and F. moniliforme* %44 inhibition for both, whereas the MeOH Soxhlet extract showed strain-specific effects. The extracts displayed substantial antibacterial, antifungal, and cytotoxic activities.

Conclusion: Findings of this study underscore the need for further biological and phytochemical investigations to fully explore the potential of the *S. tortuosum* extracts as cytotoxic therapeutics and antimicrobial agents.

Keywords *Seseli* · Apiaceae · Antibacterial · Antifungal · Cytotoxicity

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INTRODUCTION

The genus *Seseli* L. belongs to the Apiaceae family, which includes 449 genera (POWO, 2024). This family of flowering plants primarily inhabits the northern temperate regions and high-altitude tropical areas. Apiaceae members are typically aromatic herbs with distinct features, including sunken stems, alternate leaves, small flowers, and inflorescences made up of simple or compound umbels; their fruit is a schizocarp (Güner & Duman, 2013). Within the *Seseli* L. genus, there are 141 accepted species found across Asia, Europe, and North Africa (POWO, 2024). It includes more than 13 taxa in Turkey, with 6 being endemic (Güner, 2012). Traditionally, *Seseli* species have been employed in herbal medicine since the days of Hippocrates and Dioscorides. The name "*Seseli*" has its roots in ancient European traditional medicine (Cinar et al., 2020).

Seseli species, widely celebrated in traditional medicine, exhibit a broad spectrum of pharmacological activities, including anti-inflammatory, analgesic, antibacterial, antifungal, and insect-repellent effects (Cinar et al., 2020; Küpeli et al., 2006; Tandan et al., 1990). The fruits of *S. tortuosum* L. have been traditionally used for digestive ailments, as an emmenagogue, and in treatments for epilepsy (Badalamenti et al., 2022; Kaya et al., 2003). Recent studies have highlighted its uterine anticancer activity and the high antioxidant, antinociceptive, and anti-inflammatory properties of its ethyl acetate and methanol extracts ((Bader et al., 2003; Küpeli et al., 2006; Önder et al., 2020). Essential oils (EOs) and extracts from related *Seseli* species, such as *S. petraeum* M. Bieb. and *S. Transcaucasicum* (Schischk.) Pimenov & Sdobnina also demonstrated significant cytotoxic effects against cancer cell lines, including MCF-7 (Maleki et al., 2024; Önder et al., 2021), with isolated compounds like seselidiol and its acetate showing moderate cytotoxicity against KB, P-388, and L-1210 tumour cell lines (Hu et al., 1990). Rich in coumarins, terpenoids, and EOs, *Seseli* species feature key constituents such as α-pinene, sabinene, β-pinene, and (E)-caryophyllene, which likely contribute to their bioactivity (Bader et al., 2003; Dogan et al., 2011; Petrović et al., 2019). The diverse chemical composition and pharmacological profile of *S. tortuosum* shows its therapeutic potential for anticancer and antimicrobial drug development. In addition, extracts of *Seseli petraeum* M. Bieb. obtained using hexane, ethyl acetate, and methanol have shown strong antiproliferative effects against A549 lung cancer cells (Cinar et al., 2020). *S. praecox dichloromethane* and methanol extracts and their isolates were tested against the HL60 leukaemia and MCF7 and PC3 cells, and the results indicated activity only observed on the HL60 cell line, IC_{50} values below 20 μM (Leontia et al., 2010). The species we decided to work with have only been studied as essential oils. However, we did not encounter any studies on the biological activity of the extracts. Therefore, we enlighten the need for this investigation in the literature.

MATERIALS AND METHODS

Plant Material

The roots of *Seseli tortuosum* L. were collected in September 2019 from the Ermenek District, Karaman by Ömer Çeçen. Voucher specimens were archived at the Herbarium of Biodiversity Application and Research Centre, Karamanoğlu Mehmetbey University, under the reference code KMUB 4769.

Extraction and Isolation

The air-dried and powdered roots (46 g) of *Seseli tortuosum* L. were successively subjected to extraction using dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH), respectively. Initially, maceration was performed twice at room temperature with DCM (2×1 L for 1 hour). Following this, Soxhlet extraction was conducted using DCM for continuous extraction for 24 h. Soxhlet extraction was also performed using EtOAc and MeOH, without prior maceration. The extracts were labelled as DCM macerate (ST1), DCM Soxhlet (ST2), EtOAc Soxhlet (ST3), and MeOH Soxhlet (ST4). After filtration, the solvents were evaporated to obtain yields of 163.1 mg for the macerate, 473.9 mg for the DCM Soxhlet extract, 89.7 mg for the EtOAc extract, and 2.45 g for the MeOH extract. The chromatographic comparison between the Soxhlet and macerate DCM extracts showed no notable differences, yet they were kept separate for biological activity testing.

Antibacterial Activity

The antibacterial efficacy of the extracts was assessed using the broth microdilution method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI), 2006). The minimum inhibitory concentration (MIC) was determined for *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, and a clinical isolate of *Salmonella typhi*. Mueller Hinton agar was used as a growth medium to culture bacterial isolates. The bacteria were cultured in Mueller Hinton Broth (MHB) for the experiment at 37°C for 24 h. To adjust the bacterial density to 1×10^8 CFU/mL, McFarland No. 0.5 in sterile saline (0.85%) solution was used.

The test samples were diluted serially in dimethyl sulfoxide (DMSO) and tested. The lowest concentration at which bacterial growth was inhibited was recorded as the MIC (μg/mL). A small amount of this well was transferred to the Petri dishes. To determine the MIC, aliquots were plated, and the concentration that showed no growth was noted. The experiments were performed in triplicate with a starting con-

centration of 10 mg/mL, and serial two-fold dilutions across the plate were performed. Amoxicillin and Tetracycline (starting concentration 1 µg /mL) were used as positive controls, and DMSO was used as a negative control. The results were compared to standard antibiotics Table 1.

Table 1. Antibacterial activity of *Seseli tortuosum* root extract fractions (MICs in mg/mL)

Treatment	*S. aureus*	*E. faecalis*	*S. typhi*
ST1	>10 mg/mL	>10 mg/mL	>10 mg/mL
ST2	>10 mg/mL	0.3125 mg/mL	>10 mg/mL
ST3	10 mg/mL	0.3125 mg/mL	5 mg/mL
ST4	10 mg/mL	10 mg/mL	10 mg/mL
Amoxicillin	1 µg /mL	>1 µg /mL	>1 µg /mL
Tetracycline	0,5 µg /mL	1 µg /mL	0,125 µg /mL

ST2; *Seseli* DCM Soxhlet extract; ST3, *Seseli* EtOAc Soxhlet extract; ST4, *Seseli* MeOH Soxhlet extract

Antifungal Activity

The antifungal properties were evaluated using the Poison PDA technique (Schmitz H., 1930). The extracts were tested against *Aspergillus parasiticus*, *Fusarium moniliforme* NRRL 2374, and *Fusarium culmorum* NRRL 3288.

50 mL of PDA was dissolved in 100 mL of water within a flask. The flask was then sterilised for 20 min and placed under a sterile hood to cool to 60°C. Once cooled, the extracts were added at a concentration of 20 mg/mL, and the solution was gently mixed to prepare the PDA medium containing the extract. Ketoconazole served as the positive control, whereas DMSO was used as the negative control. A 4-mm PDA plug, cut from the culture medium, was placed in the centre of a 9-cm Petri dish for each experimental setup. The dishes were incubated at 25-27°C for 7 days. During the incubation period, the minimum and maximum diameters of mycelial growth in each Petri dish were measured and documented. Two replications were maintained for each treatment, and a suitable control was also maintained. The percentage of mycelial growth inhibition (I) was calculated using:

I (%) = (1-*dt*/*dc*) *100 (%)

Where *dc* is the average diameter of the fungal colony in the control group and dt is the diameter in the treated group (Ogbebor et al., 2008).

Cell Culture

Human cell lines (MCF7, PC3, A549) and the HEK293 cell line (normal embryonic kidney cells) were cultured in their respective growth media: [Dulbecco's modified Eagle's medium/high glucose (DMEM/High)], RPMI 1640 (with L-Glutamine with Phenol Red and without HEPES), and for the HEK293 cell line, Dulbecco's modified Eagle's Medium (DMEM/Gibco) were used. Each medium was supplemented with 10%(v/v) heat-inactivated fetal bovine serum (Gibco, A5256801, 500 mL), L-glutamine (2 mM), and antibiotic-antimycotics solution (100 U/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B). All materials used for cell culture were purchased from Gibco. The cells were incubated at 37°C with 5% CO_2 and passaged every three days. (Çaşkurlu et al., 2023).

MTT Assay

Cell viability was assessed using the MTT assay as described by Mosmann 1983 (Mosmann, 1983). Cells (5 × 10^4 cells/well) were seeded into 96-well plates (Nunc, Denmark) and incubated for 24 h. Test extracts and colchicine (positive control) were prepared in 0.5% DMSO and applied at concentrations ranging from 1 to 1000 µg/mL. After incubation for 24 h, 30 µL MTT solution (0.5 mg/mL in Phosphate Buffered Saline) was added to each well, and the plates were incubated for an additional 4 h (37°C). Purple formazan crystals were generated *via* the reduction product of the MTT agent by the mitochondrial dehydrogenase enzyme of intact cells, the crystals dissolved in DMSO (150 µL). The absorbance was measured at 540 nm in a microplate reader (Spectramax i3). Cell viability was calculated as a percentage relative to the untreated controls, and IC_{50} values were calculated.

Viability % = (Absorbance of treated samples/Absorbance control) x 100

Selectivity Index = IC_{50} Health cell line/IC_{50} Cancer cell line

Statistical Analysis

The data obtained because of cytotoxicity analyses were evaluated using the Graph-Pad Prism® 7 program. The statistical evaluation was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. The limit of significance was accepted as $P < 0.05$.

RESULTS AND DISCUSSION

The antibacterial and antifungal activities of the macerate DCM, Soxhlet DCM, EtOAc, and MeOH extracts obtained from Seseli *tortuosum* were evaluated using microdilution and Poison PDA techniques. Their antibacterial activities were compared with those of amoxicillin and tetracycline, and their antifungal activities were compared with those of ketoconazole. In addition to its antimicrobial activities, the crude extract was investigated for its *in vitro* cytotoxicity.

The antimicrobial test results are displayed in Table 2 and Table 2.

Table 2. Antifungal activity results* of *Seseli* macerate DCM and Soxhlet DCM and MeOH extracts

Treatment	**Diameter of mycelial growth (cm)**			
Apergillus parasiticus	2nd day	5th day	7th day	% Inhibition
ST1	1.3	1.7	2.5	48
ST2	1.5	2	2.5	48
ST4	1.3	9	9	-87
Ketaconazole	0.8	1.5	2.5	48
Negative control (1% DMSO)	2.3	3.4	4.8	
Apergillus niger	2nd day	5th day	7th day	% Inhibition
ST1	2	9	9	0
ST2	2	9	9	0
ST4	1.6	3.3	6	34
Ketaconazole	0.9	1.4	2.7	70
Negative control (1 % DMSO)	4.9	8.5	9	
Penicillium claviforme	2nd day	5th day	7th day	% Inhibition
ST1	0.9	1.5	9	**
ST2	1.3	1.8	9	**
ST4	1	9	9	**
Ketaconazole	**	**	**	**
Negative control (1 % DMSO)	0.9	**	**	
Fusarium moniliforme	2nd day	5th day	7th day	% Inhibition
ST1	1.5	2.3	4	44
ST2	1.3	2.4	3.9	46
ST4	1.4	2.6	3.7	48
Ketaconazole	**	0,4	0.8	89
Negative control (1 % DMSO)	2.1	6	7.1	
Fusarium culmorum	2nd day	5th day	7th day	% Inhibition
ST1	1.7	3.7	5.1	44
ST2	1.4	2.8	4.1	55
ST4	1.4	4	5.7	37
Ketaconazole	**	**	**	**
Negative control (1% DMSO)	1.4	9	9	

* The values are the mean of two replications, **Unidentified, ST1; *Seseli* DCM macerate, ST2; *Seseli* DCM Soxhlet extract, ST3; *Seseli* EtOAc Soxhlet extract, ST4; *Seseli* MeOH Soxhlet extract

The antibacterial activity of the extracts (ST1, ST2, ST3, and ST4) was evaluated against three bacterial strains, *Staphylococcus aureus, Enterococcus faecalis,* and *Salmonella typhi*, and compared with the control antibiotics, Amoxicillin and Tetracycline. All extracts demonstrated limited or no activity against *S. aureus*, with MIC values exceeding 10 mg/mL, underscoring their lack of efficacy against this pathogen. In contrast, the standard antibiotics displayed potent antibacterial activity, with MIC values of 1 µg/mL and 0.5 µg/mL for Amoxicillin and Tetracycline, respectively. Against *E. faecalis*, ST2 and ST3 showed moderate activity with MIC values of 0.3125 mg/mL, suggesting some potential for these extracts in targeting this strain. However, ST1 and ST4 showed no activity against *E. faecalis*, with MIC values greater than 10 mg/mL. Comparatively, Tetracycline exhibited superior activity (MIC 1 µg/mL), although Amoxicillin demonstrated resistance (MIC >1 µg/mL). For *S. typhi*, only ST3 showed weak activity with an MIC of 5 mg/mL, whereas the other extracts were inactive (MIC >10 mg/mL). Tetracycline, as a control, exhibited high potency with an MIC of 0.125 µg/mL.

The findings of this antibacterial study highlight the bioactive potential of *S. tortuosum* extracts. The significant cytotoxic activities observed in these extracts confirm their relevance for further research into their chemical composition and mechanisms of action. The antimicrobial assays demonstrated that the ST3 and ST4 extracts exhibited considerable inhibitory activity against several tested microbial strains, with ST3 showing the strongest overall antimicrobial activity. This aligns with previous reports on the antimicrobial potential of *Seseli* species, which often attribute these effects to their high coumarin and terpenoid contents. The EtOAc extract's superior activity against both Gram-positive and Gram-negative bacteria suggests that the extraction method effectively concentrates key antimicrobial compounds, while the MeOH extract (ST4) exhibited broader spectrum activity.

The antifungal activity of the extracts was assessed based on their effects on the mycelial growth of five fungal strains (*Aspergillus parasiticus, Aspergillus niger, Penicillium claviforme, Fusarium moniliforme,* and *Fusarium culmorum*), with Ketoconazole and 1% DMSO serving as positive and negative controls, respectively. Against *A. parasiticus*, ST1 and ST2 exhibited moderate inhibitory activity with 48% growth inhibition, comparable to Ketoconazole, while ST4 appeared to stimulate fungal growth (-87% inhibition). The control (1% DMSO) demonstrated no inhibitory activity, validating the experimental setup. For *A. niger*, only ST4 showed mild inhibition (34%), whereas ST1 and ST2 were inactive, similar to the negative control. Ketoconazole exhibited superior efficacy with 70% inhibition. For *F. moniliforme*, all extracts demonstrated moderate inhibition, with ST4 being slightly more effective (48%). However, Ketoconazole remained the most potent with 89% inhibition. Against *F. culmorum*, ST2 showed the highest

inhibition (55%), followed by ST1 (44%) and ST4 (37%), although Ketoconazole data were unavailable. These findings indicate that ST2 exhibited the most consistent antifungal activity, particularly against *F. culmorum* and *F. moniliforme*, whereas ST4 showed strain-specific effects.

The *in vitro* cytotoxic effects of the *S. tortuosum* extracts on cancer cell lines (A549, MCF7, PC3) and the normal HEK293 kidney cell line were assessed using the MTT assay. Table 3 presents the IC_{50} values for each extract against the cell lines. According to the cytotoxicity experimental results of the ST1, ST2, ST3, and ST4 extracts on the four cell lines, all extracts exhibited the highest toxic effect on PC3 cells (Table 3). For the PC3 cell line, the DCM macerate, Soxhlet DCM, EtOAc, and MeOH extracts had IC_{50} values of 82.78 ± 0.99, 85.16 ± 1.10, 95.91 ± 1.26, and 98.64 ± 2.04 µg/mL, respectively. The DCM macerate extract (ST1) significantly decreased cell viability in the PC3 cell line, while a notable reduction was only observed in the MCF7 cell line at 203.82 ± 2.03 µg/mL and it was non-selective towards HEK293 cells. Similarly, when compared with the literature, all extracts also demonstrated significant toxic effects on A549 cells (Table 3). For the A549 cell line, the DCM macerate, Soxhlet DCM, EtOAc, and MeOH extracts had IC_{50} values of 102.78 ± 2.01, 105.16 ± 1.98, 155.91 ± 2.28, and 118.70 ± 1.99 µg/mL, respectively. The MeOH extract (ST4) demonstrated higher cytotoxicity towards the MCF7 cell line than the other extracts. Both the DCM macerate (ST1) and Soxhlet DCM (ST2) extracts showed significant cytotoxic activity against PC3 cells with IC_{50} values of 82.78 ± 0.99 and 85.16 ± 1.10 µg/mL, respectively. These results were compared with the standard cytotoxic agent colchicine (Table 3). The IC_{50} values of colchicine on A549, PC3, MCF7 and HEK293 cells are 9.28 ± 1.01, 0.98 ± 0.99, 52.11 ± 0.85 and 24.53 ± 1.02 µg/mL, respectively. Because colchicine is the main component, its cytotoxic effect on cancer cells is quite high. However, it is also seen to have a toxic effect on normal cells (IC_{50} value 24.53 µg/mL). Colchicine was used as a positive control in the study, but the cytotoxic effect results of the extracts should be compared with the literature. Detailed literature analyses were performed within the scope of the study and it was determined that the results were quite effective. The extracts used in this study demonstrated high selectivity, a crucial observation (Table 4, >1). ST1, ST2, and ST3 exhibited notably high selectivity towards the PC3 cell line, with SI values of 3.20, 3.46, and 3.17, respectively (Table 4). The fact that the cytotoxic effects of the extracts on normal cells are less than their cytotoxic effects on cancer cells is promising in terms of anticancer activity. Maleki et al. (2024) reported the cytotoxic effects of *Seseli transcaucasicum* extracts on several cancer cell lines, including MCF7 (Maleki et al., 2024). Additionally, hexane, EtOAc, and MeOH extracts of *Seseli petraeum* showed strong antiproliferative activity against A549 lung cancer cells, and the hexane extract was found to be the most active with 3432 µg/mL IC_{50} value (Cinar et al., 2020). Compared with other cell lines, various *Seseli* species extracts have shown selective cytotoxicity towards PC3 and MCF7 cells. In this study, our extracts were tested on three cancer cell lines—PC3, MCF7, and A549—and one normal cell line, HEK293. As a result of our tests, in normal HEK293 cells, all extracts showed low toxicity, with ST1 exhibiting the least toxicity (IC_{50} = 265.39 µg/mL), followed by ST4 (287.82 µg/mL) and ST3 (304.68 µg/mL). Colchicine, in contrast, was far more toxic to normal cells (IC_{50} = 24.53 µg/mL). These results highlight the potential selectivity of the extracts, particularly ST4, which exhibited moderate cytotoxicity against cancer cells while sparing normal cells.

Table 3. IC_{50} values and standard deviation of extracts and standards on HEK293, MCF7, PC3, and HEK293 cells for 24 hours. (µg/mL)

Cpd No.	IC_{50} (µg/mL)			
	A549	PC3	MCF7	HEK293
ST1	102.78 ± 2.01	82.78 ± 0.99	203.82 ± 2.03	265.39 ± 1.79
ST2	105.16 ± 1.98	85.16 ± 1.10	190.68 ± 1.89	295.30 ± 2.03
ST3	155.91 ± 2.28	95.91 ± 1.26	230.70 ± 2.18	304.68 ± 1.88
ST4	118.70 ± 1.99	98.64 ± 2.04	188.93 ± 1.99	287.82 ± 1.40
Colchicine	9.28 ± 1.01	0.98 ± 0.99	52.11± 0.85	24.53 ± 1.02

ST1; *Seseli* DCM macerate; ST2, *Seseli* DCM Soxhlet extract; ST3, *Seseli* EtOAc Soxhlet extract; ST4, *Seseli* MeOH Soxhlet extract

Table 4. Selectivity Index (SI) values of extracts for 24 h.

Cpd No.	Selectivity Index (SI)		
	IC_{50} HEK293/IC_{50} A549	IC_{50} HEK293/IC_{50} PC3	IC_{50} HEK293/IC_{50} MCF7
ST1	2.58	3.20	1.30
ST2	2.80	3.46	1.54
ST3	1.95	3.17	1.32
ST4	2.42	2.91	1.52
Colchicine	2.64	25.03	0.47

The results of this study on *Seseli tortuosum* align well with the literature findings regarding the bioactivity of *Seseli* species. Cytotoxicity assessments of *S. tortuosum* extracts revealed promising potential, particularly for the EtOAc Soxhlet (ST3) and MeOH Soxhlet (ST4) extracts, which demonstrated significant cytotoxic activity. This observation resonates with prior studies highlighting the anticancer and bioactive properties of EtOAc and MeOH extracts from *S. tortuosum* and related species. For instance, Bader et al. (2003) and Küpeli et al. (2006) reported the potent antioxidant, anti-inflammatory, and antinociceptive activities of these extracts, underscoring

their pharmacological relevance (Bader et al., 2003; Küpeli et al., 2006).

The historical use of *S. tortuosum* fruits for digestive and gynaecological ailments, as well as its contraceptive and antiepileptic applications, provides a compelling foundation for its observed bioactivity (Badalamenti et al., 2022; Gonçalves et al., 2012; Kaya et al., 2003; Küpeli et al., 2006). The uterine anticancer activity noted in previous studies and the cytotoxic effects of related species, such as *S. transcaucasicum* ethanol and MeOH extracts against MCF-7 cell lines, further validate the therapeutic potential of this plant (Bader et al., 2003; Küpeli et al., 2006; Önder et al., 2020). The high cytotoxic activity of the extracts could be attributed to bioactive compounds like coumarins, terpenoids, which are well-documented in the chemical profiles of *Seseli* species. In particular, the abundant α-pinene, sabinene, and β-pinene in *S. tortuosum* might contribute to its cytotoxic and pharmacological effects, as these compounds are known to exhibit various biological activities (Badalamenti et al., 2022; Kaya et al., 2003).

Moreover, the essential oil studies of *Seseli* species, such as *S. petraeum* and *S. mairei*, strengthen the idea that sesquiterpenes and coumarins play crucial roles in cytotoxicity (Hu et al., 1990; Önder et al., 2021). Hu et al. (1990) previously demonstrated that seselidiol and its acetate exhibit moderate cytotoxicity, which aligns with the promising activities observed in the current study for ST3 and ST4 (Hu et al., 1990). The comparative advantage of ST3 (EtOAc) over the other extracts may be linked to the extraction technique and the solvent's ability to isolate specific bioactive molecules more effectively, while ST4 (MeOH) aligns with its traditional applications and pharmacological studies indicating its potent antioxidant and cytotoxic properties.

CONCLUSION

This study evaluated the *in vitro* antifungal, antibacterial, and cytotoxic activities of root extracts from *Seseli tortuosum*, providing valuable insights into their potential pharmacological applications. Moderate antifungal activity was observed across the extracts, highlighting their potential for further exploration against fungal pathogens. The antibacterial assays revealed that the DCM soxhlet (ST2) and EtOAc (ST3) extracts exhibited notable activity, with the EtOAc extract demonstrating superior inhibition of *E. faecalis*. These results emphasise the extract's promising antimicrobial potential, particularly against Gram-positive bacteria.

The cytotoxic evaluation, conducted using the MTT assay, revealed that DCM macerate (ST1) and DCM Soxhlet (ST2) extracts exhibited significant cytotoxicity against the PC3 cell line, surpassing other extracts in potency. While all extracts demonstrated a degree of cytotoxic activity against PC3 cells, the performance of the DCM extracts was particularly noteworthy, showing selective activity when compared with the reference, colchicine. Furthermore, this activity highlights the potential of *S. tortuosum* extracts for further investigation as cytotoxic agents, particularly in prostate cancer therapy.

Overall, the findings of this study underscore the need for further biological and phytochemical analyses to isolate and characterise the active compounds responsible for the observed bioactivities. The results also validate the traditional uses of *Seseli* species and underline their pharmacological importance. By contributing to the growing evidence supporting the therapeutic potential of *S. tortuosum*, this study establishes a foundation for future research into its application as a source of antimicrobial and anticancer agents, leading to its potential integration into drug development.

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