Antibacterial, antifungal, antibiofilm, and cytotoxicity activity of *Astragalus baba-alliar* extract against main causes of dental root canal infections

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Abstract

The objective of endodontic treatment is paramount: to completely eradicate bacterial infection within the dental pulp and root canal system. This study aimed to evaluate the Antimicrobial, antibiofilm, and cytotoxicity activity of *Astragalus baba-alliar* (*A. baba-alliar*) extract against the main causes of dental root canal infections (*Enterococcus faecalis* and *Candida albicans*). After the preparation of the methanolic extract from *A. baba-alliar*, phytochemical analysis was conducted to determine the content of secondary metabolites, followed by the determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) against *Candida albicans (C. albicans) and Enterococcus faecalis (E. faecalis*). Subsequently, the ability of the methanolic extract to inhibit biofilm formation was investigated using the microtiter plate method. The cytotoxic effects of the methanolic extract on normal human gingival fibroblast cells (HGF1) and oral cancer cells (KB) were evaluated using the MTT reduction method. Based on the phytochemical results, the presence of flavonoids, terpenoids, saponins, and polysaccharides in this plant extract was confirmed. The total phenol and flavonoid content were determined to be 4.23 mg GEA/g DW and 2.61 mg QE/g DW, respectively. The methanol extract of the plant, both alone and in combination with nystatin, exhibited a significant anti-candidal effect against *C. albicans*, while alone and especially in combination with chlorhexidine, it demonstrated a significant antibacterial effect against *E. faecalis*. Moreover, the extract alone and in combination with nystatin induced biofilm formation in *C. albicans* with an MBIC50 of 4.6 µg/ml, 64 µg/ml, and 0.25 µg/ml, respectively. Similarly, the extract alone and combined with chlorhexidine inhibited biofilm formation in *E. faecalis* with a minimum biofilm inhibitory concentration (MBIC50) of 42.6 µg/ml and 1.16 µg/ml, respectively. The calculated Selectivity Index (SI) exceeding 2 (SI=2.72) indicates the extract's selective cytotoxicity towards cancer cells while maintaining negligible toxicity towards normal. Based on the antimicrobial properties uncovered in this research, the study is anticipated to lay the groundwork for clinical trials and subsequent investigations into the plant's effective compounds. Such endeavors hold potential for application across various industrial sectors including food, pharmaceuticals, and medicine.

Keywords: antimicrobial, Candida, viability, biofilm, methanolic extract

1. Introduction

The objective of endodontic treatment is paramount: to completely eradicate bacterial infection within the dental pulp and root canal system (1). However, achieving this goal proves challenging due to the persistence of microorganisms within dentinal tubules even after thorough chemical-mechanical preparation, highlighting the inadequacy of this approach as a standalone solution (1). Consequently, the necessity arises for the integration of sealers endowed with robust sealing capabilities and potent antimicrobial properties to effectively eliminate residual microorganisms (2).

Debates surrounding the antimicrobial efficacy of sealers against commonly isolated bacteria in infected teeth, alongside concerns regarding their varying degrees of cytotoxicity, compound the challenge faced by clinicians in selecting appropriate sealers for endodontic procedures (3, 4). Moreover, the alarming rise in antibiotic-resistant bacterial strains and the adverse effects associated with synthetic drugs have fueled a burgeoning interest in exploring herbal alternatives within the realm of dentistry (5, 6).

Despite the extensive utility of medicinal plants across various medical disciplines, their application in dentistry remains relatively underexplored (7, 8). Among the plethora of medicinal plants, the genus *Astragalus* emerges as a significant contributor to natural medicine. Comprising over 2,500 species distributed across 100 subgenera, *Astragalus* epitomizes diversity within the Fabaceae family (9). Its presence spans diverse geographical regions, with notable concentrations in Southwest Asia, the Chinese Himalayan region, Northwestern America, South America, and Europe (9).

Astragalus is revered for its multifaceted medicinal properties, including hepatoprotection, blood sugar regulation, anti-osteoporosis, anti-fatigue, anti-inflammatory, anti-cancer, antioxidant, and immunomodulatory effects (10, 11). Noteworthy compounds such as Formonontin and calycosin

derived from *Astragalus* membranous have exhibited promising antidiabetic properties, while *Astragalus* membranous polysaccharides (APS) represent typical active constituents with potential antidiabetic effects (12).

Given the imperative to revitalize traditional medicine and unveil the antimicrobial potential of plants possessing profound therapeutic attributes, this study endeavors to evaluate the Antimicrobial, antibiofilm, and cytotoxicity activity of *Astragalus baba-alliar* extract against main causes of dental root canal infections (*Enterococcus faecalis* and *Candida albicans*). Through this investigation, we seek to contribute to the evolving landscape of endodontic therapy by exploring natural alternatives with the potential to complement or even supersede conventional treatment modalities.

2. Materials and Methods

2.1. Plant collection

Aerial parts of the *A. baba-alliar* were collected in May 2023 from rural regions of Noorabad district, Lorestan province, Iran. The collected plant was then identified by a botanist (Dr. Javad Ghasemian Yadegari) and a voucher sample was deposited in Herbarium of Razi Herbal Medicines Research Center, Iran (No. 1402.245).

2.2. Preparation of *A. baba-alliar* methanolic extract

First, aerial parts of the plant were ground, and five grams of the sample were extracted in 50 ml of pure methanol (Merck, German) for 72 hours in a shaker. Then, the extracts were placed inside the hood and at room temperature by rotary at 50 °C and concentrated to evaporate excess methanol. Finally, the dried extracts were kept in the freezer and the dark until use.

2.3. Phytochemical analysis and secondary metabolites content

Phytochemical analysis of *A. baba-alliar* methanolic extract was done to confirm the presence of tannins, saponins, alkaloids, flavonoids, and glycosides (13).

2.4. Total phenolic compounds (TPC)

To determine the content of phenolic compounds in *A. baba-alliar* methanolic extract, it was measured by the colorimetric method of folin-siocaltio and according to gallic acid. In this method, the extract was added to Folin's reagent, and then the absorbance of the sample was read at 760 nm by a spectroscopic device. Finally, the total phenol content was expressed as gallic acid equivalents (mg of gallic acid/g of extract weight) (14).

2.5. Total flavonoid compounds (TFC)

The amount of total flavonoid was measured by aluminum chloride ($AlCl_3$) colorimetric method. The amount of light absorption at 510 nm reading and thus the amount of total flavonoid was obtained in terms of mg/g extract (14).

2.6. Antimicrobial activities of *A. baba-alliar* methanolic extract

2.6.1. Bacterial strain

The bacterial strain utilized in the study was *Enterococcus faecalis* ATCC 9854, which was obtained in lyophilized form from the Biotechnology Institute affiliated with the Iranian Research Organization for Sciences and Technology in Tehran. The bacteria were cultivated on tryptic soy agar (TSA) from Liofilchem in Teramo, Italy, and then incubated at 37°C overnight. The optical density of the bacterial suspension was standardized to the McFarland 0.5 turbidity standard (equivalent to 1.5×10^8 colony-forming units per milliliter) using spectrophotometry.

2.6.2. Fungal strain

The standard strain *C. albicans* (PTCC5027) used in this study was provided from Scientific Research Center of Iran and was cultured on Sabouraud dextrose agar medium (Merck, Germany)

at 35°C. Standardized inoculum for *Candida* spp, ranging from 2.5 to 5 × 10^3 CFU/mL, were prepared utilizing turbidimetric methods. The stock inocula were generated on the second day of culturing Candida species, which were cultivated on Sabouraud Dextrose Agar (SDA) at a temperature of 30 ˚C. A sterile normal saline solution (0.9%, 3 mL) was introduced to the agar slant, and the cultures were gently swabbed to facilitate the dislodgment of blastoconidia from the *Candida* sp.. Subsequently, the blastoconidia suspensions were transferred to sterile tubes, and the volume of these suspensions was adjusted to 4 mL using sterile saline solution. The resulting suspensions were allowed to settle for a duration of 5 minutes at 28 ˚C. The optical density of the suspensions was measured at 530 nm and adjusted to achieve 95% transmittance. The suspensions were then diluted to a ratio of 1:2000 in RPMI-1640 medium, which was supplemented with l-glutamine and devoid of sodium bicarbonate. To achieve an inoculum size of 2.5 to 5 × 10^3 CFU/mL, the suspensions were buffered to a pH of 7.0 using a 0.165 mol/L solution of morpholine-propanesulfonic acid (15).

2.6.3. Antimicrobial tests

Determining the minimum inhibitory concentration (MIC) for the plant extract along with the standard drug nystatin (control for *C. albicans*) and chlorhexidine (control for *E. faecalis*), according to the instructions of Clinical and Laboratory Standards Institute (CLSI), 2017 (16) by micro method Broth dilution was done in sterile house 96 plate. After preparing serial dilutions of the methanolic extract and the drug, 100 µL of the extract and the drug were added to the fungal/bacterial suspension and incubated for 48 hours at 35 °C and the MIC was determined. According to the guidelines, the MIC is the lowest drug concentration at which the fungus/bacteria did not grow appreciably after 48 hours of incubation at that drug concentration. The turbidity was

read at the wavelength of 630 nm by an ELISA reader (AWARENESS, TechnilogyINC, Atat fax 2100) (17). The minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) were determined by subculturing 100 µL of the solution from the wells without turbidity on PDA and Mueller Hinton agar medium at 28°C. MFC and MBC were defined as the lowest concentration resulting in no growth on the subculture after two days (18).

2.7. Anti-biofilm activities of *A. baba-alliar* methanolic extract

The ability to inhibit biofilm formation in the treatment with methanolic extract was investigated using the microtiter plate method. 100 µL of each concentration was added to test wells of a microtiter plate (96 wells) under aseptic conditions and then 100 µL of *E. faecalis* / *C. albicans* suspension was added to each of these wells. In this test, the positive control well, negative control, and control well of the extract were considered the same as the MIC determination test. The microplate was incubated for 24 hours at a temperature of 37°C without movement. To check the inhibitory effect of the extract after incubation, the crystal violet staining method was used. The optical absorbance of each well was determined at a wavelength of 630 nm with an ELISA reader) AWARENESS, TechnilogyINC, Atat fax 2100) (19).

2.8. Cytotoxicity effect of on normal and cancerous oral cells.

2.8.1. Cell culture

The normal human gingival fibroblast cells (HGF1) and oral cancer cells (KB) were sourced from the American Type Culture Collection (ATCC). These cells were cultured in DMEM, which was supplemented with 10% FBS and antibiotics (penicillin/streptomycin at a concentration of 100 U/ml).

2.8.2. Cell viability assay

The cytotoxic effect of methanolic extract on normal human gingival fibroblast cells (HGF1) and oral cancer cells (KB) was evaluated by MTT (Methylthialazole Tetrazolium) reduction assay (20). The cell lines were cultured separately in a 25 cm^2 flask and after several passages were transferred to a 75 cm^2 flask and incubated in CO_2 5% incubator at 37°C. After cell counting, 100 µL of cells (1×10^5) were added in 96-well plates and incubated for 48 hours, under CO2 and at 37 °C, for the cells to adhere and grow to the bottom of the plate. Then, the supernatant culture medium of the cells was removed and the medium containing concentrations of methanolic extract, medicine, and free culture medium was added to the cells as a control. After 48 hours, 10 µL of MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, Germany) was added to each of the wells, and after 4 hours of incubation, 50 µL of DMSO was added to each of the wells, after 30 minutes, the cells were absorbed were read by the ELISA reader in 540 nm. Finally, the 50% cytotoxic concentration (CC50) of the drug was calculated using Probit test software (16).

2.8.3. Selectivity index (SI)

The SI is employed to evaluate the comparative toxicity of the methanolic extract of A. baba-alliar on normal cells. This index is determined by calculating the ratio of the CC50 value for normal cells to the CC50 value for cancer cell line. An SI value exceeding 2 suggests a favorable safety profile for normal cells.

2.9. Statistical Analysis

All experiments were conducted in triplicate. Statistical analyses will be carried out utilizing SPSS version 25 software, with a significance threshold set at $p<0.05$.

3. Results

3.1. Phytochemical analysis and secondary metabolites content

Following the extraction process, a total of 18.7 g of methanolic extract, constituting 7.48% (w/v), was successfully obtained, showcasing the efficacy of the extraction method employed. Phytochemical analyses conducted on the extract unveiled a rich profile of bioactive compounds, including flavonoids, terpenoids, saponins, and polysaccharides, as delineated in Table 1. The presence of these compounds underscores the diverse chemical composition and pharmacological potential of the plant under study. Of particular interest are the secondary metabolites identified within the extract, shedding light on its therapeutic properties. The quantification of total phenolic and flavonoid contents yielded significant values, with 4.23 mg GEA/g DW and 2.61 mg QE/g DW, respectively. These findings underscore the extract's remarkable phenolic and flavonoid richness, indicative of its potential health-promoting attributes and antioxidant capacity. Such robust secondary metabolite profiles not only highlight the plant's pharmacological value but also provide insight into its possible mechanisms of action and therapeutic applications.

3.2. Antifungal and antibiofilm effects on *C. albicans*

The findings pertaining to the minimum Inhibitory Concentration (MIC) and minimum Fungicidal Concentration (MFC) of the alcoholic extract derived from the plant, both independently and in conjunction with nystatin, against *C. albicans*, are detailed in Table 1. Notably, the results underscore a significant anti-*C. albicans* effect elicited by the methanol extract of the plant, particularly when combined with nystatin. Intriguingly, the combination of the extract and nystatin exhibited the most pronounced anti-*C .albicans* effect ($P<0.001$), as evidenced by the lowest MIC and MFC values recorded. Furthermore, concerning the inhibition of biofilm production, our observations reveal a dose-dependent inhibition of biofilm formation by the extract alone and in synergy with nystatin against *C. albicans*. The results delineate a compelling dose-response relationship, with the MBIC50 values calculated at 4.6 µg/ml, 64 µg/ml, and 0.25 µg/ml for the

extract alone, the combination of extract and nystatin, and nystatin alone, respectively. This dose-dependent inhibition underscores the potent anti-biofilm properties of the methanol extract, particularly in combination with nystatin, thereby highlighting its potential as an adjunct therapeutic agent in combating *C. albicans* infections.

Table 1. Antifungal and antibiofilm effect of the methanolic extract alone and in combination with nystatin. Data are presented as Mean±SD. (n=3)

Antifungal compounds	Antifungal effects		Antibiofilm effects
	MIC (µg/ml)	MFC (µg/ml)	$MBIC_{50}$(µg/mL)
Extract	1.7.6±3.37	128± 0.0 *	64.0± 0.0*
Nystatin	2.66±0.94	3.33±0.47	1.66±0.23*
Nystatin + Extract	0.66±0.1*	0.83±0.11*	0.25±0.0*

*:P<0.001

3.3. Antibacterial and antibiofilm activity of methanolic extract

The outcomes regarding the MIC and MBC of the methanolic extract, both as an independent agent and in conjunction with chlorhexidine, against *E. faecalis* are delineated in Table 2. Notably, our findings underscore a significant anti-*E. faecalis* effect exerted by the methanolic extract, particularly when combined with chlorhexidine. Remarkably, the combination of the extract and chlorhexidine demonstrated the most potent antibacterial effect (P<0.001), as evidenced by the attainment of the lowest MIC and MFC values recorded in our study. The outcomes regarding the MIC and MBC of the methanolic extract, both as an independent agent and in conjunction with chlorhexidine, against *E. faecalis* are delineated in Table 2. Notably, our findings underscore a significant anti-*E. faecalis* effect exerted by the methanolic extract, particularly when combined with chlorhexidine. Remarkably, the combination of the extract and chlorhexidine demonstrated

the most potent antibacterial effect (P<0.001), as evidenced by the attainment of the lowest MIC and MFC values recorded in our study.

Table 2. Antibacterial and antibiofilm effect of the methanolic extract alone and in combination with nystatin. Mean±SD. (n=3)

Compounds	***E. faecalis***		
	MIC (µg/ml)	MBC (µg/ml)	$MBIC_{50}$ (µg/ml)
Extract	85.3±36.4	106.6±3.6	42.6±1.84
Chlorhexidine	7.3±1.15	9.3±0.94	3.33±0.94
Extract + Chlorhexidine	2.3±1.52*	2.6±1.15*	1.16±0.76*

*:P<0.001 compared to chlorhexidine

3.4. Cytotoxicity effect on normal and cancerous oral cells

The cytotoxicity of the methanolic extract was assessed against KB cancer cells and normal HGF1-RT1 cells, with CC50 values determined to be 105.3 µg/ml and 286.6 µg/ml, respectively. Intriguingly, the calculated SI exceeding 2 (SI=2.72) indicates the extract's selective cytotoxicity towards cancer cells while maintaining negligible toxicity towards normal cells, as illustrated in Figure 1. This selective cytotoxicity profile holds significant promise for the extract's potential application as an anticancer agent, as it demonstrates the ability to target cancerous cells while sparing healthy cells, thereby minimizing adverse effects commonly associated with traditional chemotherapeutic agents. Such selective cytotoxicity highlights the extract's favorable therapeutic index and underscores its potential as a targeted therapeutic intervention in cancer treatment.

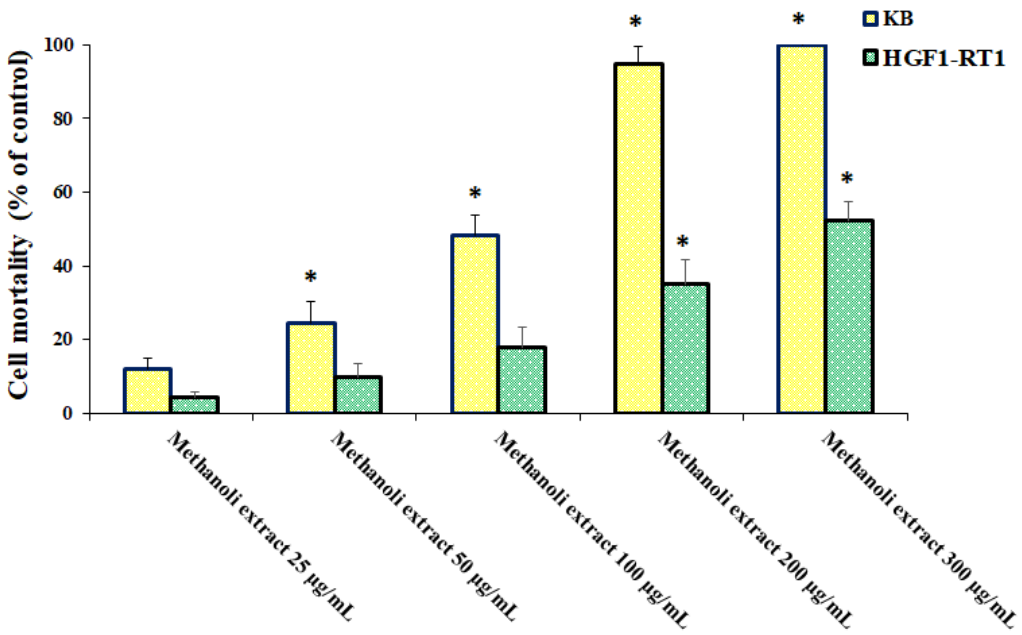

Figure 1. The effect of different concentrations of the methanolic extract of the plant on the viability of normal human gingival fibroblast cells (HGF1-RT1) and oral cancer cells (KB mean ± SD). *p<0.05 compared to the control group (normal saline) (n=3).

4. Discussion

The utilization of essential oils and plant extracts renowned for their antimicrobial properties holds significant promise in disease management (21). Recent years have witnessed a surge in studies across various countries aimed at substantiating the efficacy of essential oils and extracts in combating microbial infections. The findings of our investigation underscore the remarkable efficacy of the *A. baba-alliar* Methanolic Extract against both *E. faecalis* and *C. albicans*, thus contributing to the growing body of evidence supporting the therapeutic potential of natural remedies in microbial infections. The observed optimal antimicrobial effect of the extract against these pathogenic microorganisms underscores its potential as a viable alternative or adjunctive therapy in the management of endodontic infections and other microbial-related diseases. These findings underscore the importance of further exploration and utilization of natural compounds

derived from medicinal plants in combating microbial infections, highlighting their potential as valuable additions to the arsenal of antimicrobial agents available for clinical use.

Based on the results, the presence of flavonoids, terpenoids, saponins, and polysaccharides in the extract of this plant was confirmed and the total phenol and flavonoid content was 4.23 (mg GEA/g DW), and 2.61 (mg QE/g DW), respectively. The methanol extract of the plant alone and especially in combination with nystatin, and chlorhexidine showed a significant anti- *C. albicans,* and *E. faecalis*. The extract alone and together with nystatin also inhibited the produced biofilm in *C.albicans* with MBIC50 of 4.6, 64, and 0.25 μg/ml, respectively, and the extract alone and together with chlorhexidine produced biofilm in *E. faecalis* with MBIC50 of 42.6 and 1.16 μg/ml, respectively. The CC50 for KB and normal HGF1-RT1 cancer cells were reported as 105.3 and 286.6 μg/ml, respectively. The findings indicate that the methanolic extract of *A. baba-alliar* exhibits selective cytotoxicity towards cancer cells while demonstrating no toxicity to normal cells. In a study by Nayeem et al., the total phenolic and flavonoid content of *A. spinosus* methanolic extract was reported as 420 μg and 68 μg, respectively (22). Similarly, Asgarpanah et al. investigated the total phenolic constituents and flavonoid content of *A. squarrosus* Bunge, revealing values of 23.3 mg/g and 26.0 mg/g, respectively (23). These variations in the total phenol content observed across different species of *Astragalus* plants can be attributed to several factors, including inherent differences between species, extraction methodologies employed, the geographical source, harvesting season, and the specific plant part utilized for extraction. Phenolic compounds, ubiquitous in numerous plant species, play crucial roles in defense mechanisms against microbial pathogens (24-26). Flavonoids, characterized by their phenolic structure, possess notable antimicrobial properties, which may be attributed to their ability to disrupt cell membranes, form complexes with cell wall components, and induce alterations in extracellular proteins. The

variability in phenolic and flavonoid content among different *Astragalus* species underscores the importance of considering these factors when evaluating the therapeutic potential and antimicrobial efficacy of plant extracts. Additionally, further exploration into the specific mechanisms underlying the antimicrobial activity of phenolic compounds and flavonoids could provide valuable insights into their potential applications in combating microbial infections and enhancing human health.

Jaradat et al. reported an examination of the phytochemical compounds in four extracts of *Astragalus* spp revealed intriguing findings (27). Specifically, it was observed that *A. boeticus* exhibited elevated levels of total phenols, flavonoids, and tannins, indicative of its significant potential for antioxidant and antimicrobial activities, a trend that aligns with the findings of the present study. Moreover, the aqueous extract of *A. boeticus* demonstrated notable antibacterial activity, while its methanolic extract exhibited prominent antifungal and antioxidant properties. These observations underscore the diverse pharmacological potential inherent in different species of *Astragalus* and highlight the importance of exploring the phytochemical profiles and biological activities of these plants. The consistent findings between Jaradat et al.'s study and our investigation reinforce the notion of *Astragalus* species as valuable sources of bioactive compounds with therapeutic potential, thereby warranting further exploration and utilization in pharmaceutical and medical applications.

In the study conducted by Jahangir et al., a comprehensive phytochemical analysis of *A. psilocentros* methanol extract confirmed the presence of various bioactive compounds, including tannins, flavonoids, sugars, alkaloids, terpenoids, phenolics, and saponins (28). This extensive phytochemical profile underscores the rich chemical composition of *A. psilocentros* and suggests the potential therapeutic relevance of these compounds. Moreover, the investigation of the

antimicrobial properties of various extracts against a range of microorganisms, including *Bacillus subtilis* and *Pasteurella multocida*, produced significant results. Notably, at a concentration of 10 mg/ml, chloroform extracts exhibited the highest degree of inhibition, suggesting their potential as potent antimicrobial agents against a broad range of pathogens. These results shed light on the diverse pharmacological properties inherent in *A. psilocentros* extracts and underscore the significance of further research to elucidate their mechanisms of action and explore their potential therapeutic applications in combating microbial infections. The findings of Jahangir et al.'s study complement and expand upon our understanding of *Astragalus* species as valuable reservoirs of bioactive compounds with promising antimicrobial properties, thereby highlighting their potential for pharmaceutical and medical utilization.

Albayrak et al. meticulously identified and reported the total phenolic and flavonoid contents of methanolic extracts obtained from *A. gummifer*, *A. microcephalus*, *A. talasseus*, and *A. acmophyllus* (29). Subsequently, after assessing the antimicrobial activity of these extracts, their cytotoxic effects on MCF-7 (human breast cancer cell lines) were determined using the MTT assay. Interestingly, the results unveiled ferulic acid as the predominant component of the extracts. Despite the extensive phytochemical profile, the extracts exhibited no discernible antibacterial activity against a broad spectrum of pathogens including *Escherichia coli*, *Mycobacterium smegmatis*, *Staphylococcus* spp, and *Candida albicans*. Notably, *A. talasseus* exhibited the highest cytotoxic activity against MCF-7 cells over 48 hours. The results of this study offer significant insights into the phytochemical composition and biological activities of various *Astragalus* species. This highlights the necessity for additional research to clarify their mechanisms of action and explore potential therapeutic applications. The results of Albayrak et al.'s study complement our understanding of *Astragalus* extracts and highlight their potential as sources of bioactive

compounds with cytotoxic properties, thus warranting continued exploration of their pharmaceutical and medical potential.

The variations observed in the results regarding the antimicrobial properties of extracts derived from different species of *Astragalus* can be attributed to several factors (30). Firstly, the inherent diversity among plant species, including genetic variations and phytochemical profiles, may influence the efficacy of antimicrobial compounds present in the extracts. Additionally, differences in methodologies used to assess antimicrobial properties, such as variations in experimental conditions, microbial strains employed, and extraction techniques, can contribute to discrepancies in outcomes. The source of plant materials, their preparation methods, growth phases, and types of protection during extraction are also influential factors that may impact the bioactivity of the extracts. Furthermore, variations in culture media composition, as well as incubation duration and temperature, can introduce additional complexities, potentially affecting the observed antimicrobial effects. Therefore, the multifaceted nature of these factors underscores the importance of standardizing experimental protocols and conducting comprehensive investigations to better understand the antimicrobial potential of *Astragalus* extracts and facilitate their optimal utilization in pharmaceutical and medical applications.

The antimicrobial properties elucidated in the present study hold significant promise for guiding future research endeavors and clinical trials aimed at harnessing the therapeutic potential of *Astragalus* plant compounds. The robust antimicrobial efficacy demonstrated by the extracts underscores their viability for various industrial applications, including food, pharmaceuticals, and medicine. By elucidating the antimicrobial effects of *Astragalus* extracts, this study contributes valuable insights that could inform the development of novel antimicrobial agents and therapeutic interventions. Furthermore, the identification and characterization of effective compounds within

the plant extracts pave the way for further investigations aimed at elucidating their mechanisms of action and exploring their potential applications in diverse industrial sectors. The findings of this study serve as a foundational framework for future research endeavors aimed at harnessing the antimicrobial properties of *Astragalus* extracts for the development of innovative products and therapeutic modalities, thereby addressing critical challenges in healthcare and industry. Based on the findings of this study, it is evident that the methanolic extract derived from *A. baba-alliar* exhibits notable antimicrobial and anti-biofilm properties against tooth root canal pathogens in vitro. The extract demonstrated robust activity against these microbial strains, highlighting its potential as a valuable antimicrobial agent in the pharmaceutical industry for both the prevention and treatment of dental root canal infections. However, it is important to note that the extract also exhibited a toxic effect on the KB cell line, indicating the need for further investigation into its cytotoxic profile and potential side effects. These results underscore the importance of exploring natural sources, such as *A. baba-alliar*, for their antimicrobial properties, particularly in combating dental infections where conventional treatments may be insufficient. The antimicrobial and anti-biofilm efficacy demonstrated by the extract suggests its potential utility in developing novel therapeutic interventions for dental care. Moving forward, it is recommended that additional research be conducted under in vivo conditions to further elucidate the therapeutic applications of the methanolic extract of *A. baba-alliar*. Such studies would provide valuable insights into the extract's efficacy, safety profile, and potential clinical applications, facilitating its wider adoption and utilization in dental practice. In conclusion, the findings of this study highlight the promising antimicrobial properties of the methanolic extract of *A. baba-alliar*, suggesting its potential as a natural alternative for combating dental root canal pathogens. Further exploration and validation

of its therapeutic efficacy in vivo are warranted to fully harness its potential benefits in dental healthcare.

Conflict of interest

The authors declare no conflict of interest in this study.

Author Contributions

PS and MR designed and supervised the study and writing the draft; MDM, AH, SB, and AS do experiments, obtained data, review and edited the manuscript; all authors agreed the final version to be published.

Ethical Approval

This study was approved by the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number of IR.LUMS.REC.1402.245.

Data Availability

No datasets were produced or examined in the course of the present study.

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