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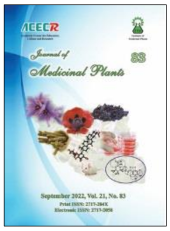

Research Article

Antibacterial activity of aqueous and lipid extracts of five common allergenic pollens

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ABSTRACT

Background: Different parts of plants are rich sources of bioactive ingredients and have received much attention during recent decades. **Objective:** The aim of this study was to assess the antibacterial properties of five common allergenic pollens extracts. **Methods:** The aqueous and lipid extracts were prepared from allergenic plant pollens including *Amaranthus retroflexus*, *Chenopodium album*, *Artemisia vulgaris*, *Cupressus arizonica*, and *Ailanthus altissima*. Antibacterial activity of the extracts was determined using the broth microdilution method against ATCC bacteria strains. **Results:** Total protein content of aqueous extracts was form 453 to 2772 µg/ml, but for all lipid extracts it was less than 0.2 µg/ml. Aqueous pollen extracts of *C. album*, *C. arizonica* and *A. altissima* (MIC between 38.8 to 562 µg/ml), and the lipid pollen extracts of *C. album*, *C. arizonica*, *A. altissima*, *A. retroflexus*, and *A. vulgaris* (MIC between 0.89 to 21.9 µg/ml) had a significant antibacterial activity on *E. faecalis*. Moreover, the aqueous pollen extract of *A. altissima* (MIC = 8.7 µg/ml), and the lipid pollen extracts of *C. arizonica*, *A. altissima*, *A. retroflexus*, and *A. vulgaris* (MIC between 1.78 to 21.9 µg/ml) showed a significant antibacterial activity against *S. aureus*. The only extract with antibacterial effect on *K. pneumoniae* was the lipid pollen extract of *A. vulgaris* (MIC value of 3.72 µg/ml). **Conclusion:** The results of this study showed the significant antibacterial activity of some plants pollen extracts. More studies are needed to further assess the active compounds in the pollen extracts and their cytotoxic effects.

1. Introduction

Infectious diseases are still an important cause of morbidity and mortality worldwide, despite improvements in control, prevention and treatments. In recent decades, with the advancement of pharmaceutical science, a wide range of antibiotics have entered the market and improve the course of infectious diseases

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Abbreviations: MDR, Multidrug-resistant Bacteria; PBS, Phosphate-buffered Saline; MIC, Minimum Inhibitory Concentration; CLSI, Clinical and Laboratory Standards Institute; AMR, Antimicrobial Resistance

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fundamentally [1, 2]. In parallel, widespread use of antibiotics has caused resistant forms of pathogenic bacteria. Currently, antibiotic resistance and the emergence of multidrug-resistant bacteria (MDR) in hospitals and the community are relatively common and put a significant economic burden on the healthcare sector. Therefor new medicinal agents and strategies are needed in the control and treatment of infectious diseases [3, 4].

Recently, traditional and ancient methods based on the use of natural elements, especially medicinal plants, in the treatment of infections have been reconsidered. Plants are rich sources of various substances which some of them are well known for their therapeutic properties and have been used for thousands of years. Today, attention to plants as a potential source for the discovery and development of new or complementary therapies has increased [5, 6]. Pollen grains are a rich source of bioactive ingredients and their role in triggering allergic symptoms is well known. Noteworthy, plant pollens are a rich source of biological substances including carbohydrates, proteins, fats, vitamins and minerals, and polyphenol compounds, especially flavonoids, which are necessary for plant growth and reproduction and have long been used to strengthen the body and treat some infections [7, 8]. The chemical composition of plant pollen varies mainly based on the plant species, and pollen chemical compounds may have various biological functions such as anti-tumor, anti-diabetic, and antimicrobial effects. In addition, pollen has been reported to have beneficial effects on health and diseases such as prevention of prostate problems, atherosclerosis, gastroenteritis, respiratory diseases, as well as delaying aging [9, 10].

Given the presence of various biological compounds in plant pollens and the limited study of the antimicrobial effects of pollen from common plants, the aim of the present study was to assess the antibacterial effects of aqueous and lipid extracts of five common allergenic pollens which are abundant in the East of Iran, against different bacteria.

2. Materials and Methods

2.1. Pollen Sampling

In the present study, five selected plant pollens including from *Amaranthus retroflexus* L., *Chenopodium album* L., *Artemisia vulgaris* L., *Cupressus arizonica* Greene, and *Ailanthus altissima* (Mill.) Swingle were collected during the pollination season from several plants in different regions of the South Khorasan province, Iran. After drying, the pollens were sieved sequentially using micron filters to achieve a purity of over 90 %. The purity and plant species were confirmed by a botanist using microscopic examination of stained pollens and the pollen atlas [11]. The protocol of this study was reviewed and approved by the ethics committee of Birjand University of Medical Sciences with code: IR.BUMS.REC.1399.078.

2.2. Pollen Extracts

In the study, two aqueous and lipid extracts were prepared from the plant pollens. The preparation of the aqueous extracts was performed by mixing 1 gram of the desired pure pollen with 20 ml acetone and magnetic stirring for 4 hours at room temperature. The acetone was separated using filter paper, and after drying the pollen was mixed with phosphate-buffered saline (PBS, pH = 7) (1:10 w/v) and placed on a shaker for 24 hours at 4 °C. Then, the mixture was centrifuged at 5000 rpm for 5 minutes, and the supernatant was sterilized using a 0.45-micron syringe filter. The total protein of the aqueous and lipid pollen extracts was measured by the Bradford method.

Furthermore, the Folch method was used to prepare the lipid extracts [12]. Briefly, 1 gram of the pollen was mixed with 20 ml of solvent (a 2:1 chloroform-methanol (v/v) mixture) in a glass container with a Teflon lid and then the mixture was shaken for 5 minutes and sonicated for 20 minutes. The mixture was centrifuged and the obtained supernatant was washed twice with normal saline. Solvent evaporation was performed using nitrogen gas flow. Finally, the jelly extract was weighted and dissolved in DMSO solvent (DMSO with the final concentration of < 1 %). The extracts were stored away from light and air at 80°C until the test time.

2.3. Antibacterial activity of extracts

The antibacterial effects of the extracts was investigated by determination of the minimum inhibitory concentration (MIC) using the microdilution broth method and according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Bacteria experimented were *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 9997), *E. faecalis* (ATCC 29212), and *S. aureus* (ATCC 25923). The procedure involves preparing twofold serial dilutions of the extracts into wells of 96-well plates and adding the bacterial suspension with the final concentration of 5×10^5 CFU/ml to each well of the microtiter plate. Finally, plates were incubated at 37 °C under aerobic conditions for 18-24 hours. Plant pollen extracts with MICs < 100 µg/ml were identified as highly active antimicrobial compounds, MICs ranging from 100 to 500 µg/ml as active, MICs ranging between 500 and 1000 µg/ml as moderately active, MICs ranging from 1000 to 2000 µg/ml as low activity, and MICs > 2000 µg/ml as inactive [14, 15]. Gentamicin was used as a positive control, and all experiments were performed with three replicates.

3. Results

The total protein content of aqueous and lipid extracts presents in table 1. The MIC values of the aqueous pollen extracts ranged between 8.7 and 2770 µg/ml (Table 2). The best antibacterial activity of the aqueous pollen extracts was found for *A. altissima* to *S. aureus* with a MIC value of 8.7 mg/ml and for *A. altissima* to *E. faecalis* with a MIC value of 38.8 mg/ml. Furthermore, there was a good inhibitory effect for *C. arizonica* (classified as active) and *C. album* (classified as moderately active) against *E. faecalis*.

The antibacterial activities of the lipid pollen extracts against various Gram-positive and Gram-negative bacteria are shown in table 3. The lipid pollen extracts MIC values were ranged from 0.52 to >277 µg/ml. All the lipid pollen extracts were as highly active against *E. faecalis* as the positive control Gentamicin. Moreover, there was found a significant antibacterial activity for the lipid extract of *C. arizonica*, *A. altissima*, and *A. retroflexus* against *S. aureus*; and *A. vulgaris* against *K. pneumoniae* and *S. aureus*.

Table 1. Total protein content of aqueous and lipid pollen extracts (µg/ml)

Pollen extracts	***C. album***	***C. arizonica***	***A. altissima***	***A. retroflexus***	***A. vulgaris***
Aqueous	1124	453	1115	1530	2772
Lipid	0.194	0.117	0.184	0.120	0.125

Table 2. Antibacterial activity of the aqueous pollen extracts against different bacterial species

Bacteria	**MIC values (µg/ml)**					
	C. album	*C. arizonica*	*A. altissima*	*A. retroflexus*	*A. vulgaris*	Gentamicin
E. faecalis (ATCC 29212)	562	113	38.8	> 1530	> 2770	0.48
K. pneumoniae (ATCC 9997)	> 1124	> 453	> 1110	> 1530	> 2770	< 0.48
P. aeruginosa (ATCC 27853)	> 1124	> 453	> 1110	> 1530	> 2770	0.48
E. coli (ATCC 25922)	> 1124	> 453	> 1110	> 1530	> 2770	0.48
S. aureus (ATCC 25923)	> 1124	> 453	8.7	> 1530	> 2770	< 0.48

Table 3. Antibacterial activity of the lipid pollen extracts against different bacterial species.

Bacteria	**MIC values (µg/ml)**					
	C. album	*C. arizonica*	*A. altissima*	*A. retroflexus*	*A. vulgaris*	Gentamicin
E. faecalis (ATCC 29212)	4.32	0.52	21.9	0.89	0.93	0.48
K. pneumoniae (ATCC 9997)	> 277	> 66.8	> 175	> 114	3.72	< 0.48
P. aeruginosa (ATCC 27853)	> 277	> 66.8	> 175	> 114	> 119	0.48
E. coli (ATCC 25922)	> 277	> 66.8	> 175	> 114	> 119	0.48
S. aureus (ATCC 25923)	> 277	8.53	21.9	1.78	7.44	<0.48

4. Discussion

Antimicrobial resistance (AMR) and the emergence of multidrug-resistant bacteria (MDR) is one of the most serious global public health concerns. Plants are rich sources of various substances and many of them have medicinal uses and have been used for thousands of years. In this context, plant pollens could represent an appropriate option for finding new, safer, and more effective

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antibacterial compounds that can substitute for traditional antibacterial agents [2, 9].

In the present study, the antibacterial activity of aqueous and lipid extracts of five common plant pollens including *A. retroflexus*, *C. album*, *A. vulgaris*, *C. arizonica*, and *A. altissima* were assessed against different species of bacteria. The best antibacterial activity of the aqueous pollen extracts was found for *A. altissima* to *S. aureus* and *E. faecalis*. There were found good inhibitory effects for the aqueous extracts of *C. arizonica* (classified as active) and *C. album* (classified as moderately active) against *E. faecalis*. Furthermore, all the lipid pollen extracts were highly active against *E. faecalis*. There was found a significant antibacterial activity for the lipid extract of *C. arizonica*, *A. altissima*, and *A. retroflexus* against *S. aureus*; and *A. vulgaris* against *K. pneumoniae*. The antimicrobial effects of the *A. retroflexus*, *C. album*, *A. vulgaris*, *C. arizonica*, and *A. altissima* pollen extracts have not been widely studied. However, there are studies on the antimicrobial effects of different parts of these plants.

In the current study, we found a very good antibacterial effect (highly active) of the aqueous and lipid pollen extracts of *A. altissima* against *S. aureus* and *E. faecalis*, while these extracts had no significant effect against gram-negative bacteria. A similar result was reported by Albouchi *et al.* with methanol extracts of *A. altissima* (Mill.) Swingle leave was effective against Gram-positive bacteria, but not active towards Gram-negative bacterial strains [16]. In another study, ethanol extract from the fruits of *A. altissima* Swingle was weakly active against *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhimurium* [17]. In the Rahman *et al.* study, methanolic extracts of *A. altissima* Swingle leaves were reported to have significantly higher antibacterial activities against some foodborne pathogens such as *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *B. subtilis*, and *E. coli*, and overall Gram-positive bacteria were proved to be more sensitive to plant extracts [18].

In our study, there was found a good inhibitory effect for the aqueous (active) and lipid (highly active) pollen extracts of *C. album* against *E. faecalis*. In addition, a similar antibacterial effect was reported for the aqueous extract of *C. arizonica* (active) against *E. faecalis* and its lipid extract (highly active) against *S. aureus* and *E. faecalis*. The results of the study of Amjad *et al.* showed that bark and fruit extract of *C. album* seems to be more effective against *S. aureus* than other bacterial strains including *B. subtilis* and *P. aeruginosa* [19]. However, in another study, flower and leaf methanolic and ethanolic extracts of *C. album* had no activity against selected bacterial strains [20]. It should be noted that no study on the antibacterial effects of the *C. arizonica* extracts has been found in the literature.

Finally, in the present study, the lipid pollen extracts of *A. retroflexus* were highly active against *E. faecalis* and *S. aureus*. Moreover, there was a significant antibacterial activity for the lipid extract of *A. vulgaris* (highly active) against *E. faecalis*, *K. pneumoniae*, and *S. aureus*. In one of the rare studies assessing antimicrobial effects of lipid extracts of vegetative organs of *A. retroflexus*, Marinas and colleagues found that the extracts had the highest antibacterial effect against *B. subtilis* and *K. pneumoniae* with no activity against *S. aureus* [21]. In the Addo-Mensah *et al.* study, the acetone extract of *A. vulgaris* exhibited high antimicrobial activity against *B. subtilis*, methicillin-resistant *S. aureus* (MRSA), and *S. aureus*, and the lipid extract showed very moderate activity against *S. aureus*, and *B. subtili*, and no activity against MRSA [22].

Overall, in our study, the antibacterial activity of aqueous and lipid pollen extracts of *A. retroflexus*, *C. album*, *A. vulgaris*, *C. arizonica*, and *A. altissima* were very significant against Gram-positive bacteria, but these extracts were not active against most Gram-negative bacterial strains. A similar result was obtained in previous studies, which reported that Gram-negative bacteria are more resistant to the pollen action than Gram-positive bacteria [9, 10, 23, 24]. These results can be explained by the difference in the composition of the bacterial cell wall and the presence of the additional outer layer membrane in Gram-negative bacteria, which is consists of phospholipids, lipopolysaccharides, and proteins, and is impermeable to most molecules [9, 10].

It is noteworthy that our study is one of the first studies to investigate the antibacterial activity of *A. retroflexus*, *C. album*, *A. vulgaris*, *C. arizonica*, and *A. altissima* pollen extracts. However, a limitation of the present study was the inability to assess the antibacterial effect of some lipid pollen extracts at higher concentrations due to the low efficiency of the lipid pollens extraction.

5. Conclusion

Our findings highlight a significant antibacterial activity of some plants pollen extracts, especially lipid extracts. The results showed that pollen extracts were more potent at inhibiting the growth of gram-positive bacteria than gram-negative bacterial strains. More studies are needed to further assess the active compounds in the pollen extracts and their cytotoxic effects.

Author contributions

Conceptualization: MF; Data collection and laboratory analysis:

ZB, MY; Interpretation of the results: MY, MF; Writing of original draft: ZB, MY;

Writing, reviewing and editing: MY, SGR, MF. All authors read and approved the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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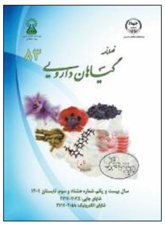

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گرده گیاهان

چکیده

مقدمه: بخش‌های مختلف گیاهان منابع غنی از مواد فعال زیستی هستند و در دهه‌های اخیر مورد توجه قرار گرفته‌اند. **هدف:** مطالعه حاضر با هدف بررسی فعالیت ضدباکتریایی عصاره‌های پنج گرده آلرژی‌زا شایع انجام شد. **روش بررسی:** عصاره‌های آبی و لیپیدی گرده گیاهان آلرژی‌زا شامل تاج خروس، سلمک، برنجاسف، سرو سیمین و درخت عرعر تهیه شد. فعالیت ضدباکتریایی عصاره‌ها با استفاده از روش میکرودایلوشن براث برای سویه‌های استاندارد باکتریایی تعیین گردید. **نتایج:** میزان پروتئین تام عصاره‌های آبی از ۴۵۳ تا ۲۷۷۲ میکروگرم بر میلی‌لیتر متغیر بود. پروتئین تام همه عصاره‌های لیپیدی کمتر از ۰/۲ میکروگرم بر میلی‌لیتر بود. عصاره آبی گرده سلمک، سرو سیمین و درخت عرعر (MIC بین ۳۸/۸ تا ۵۶۲ میکروگرم در میلی‌لیتر) و همچنین عصاره لیپیدی گرده سلمک، سرو سیمین، درخت عرعر، تاج‌خروس و برنجاسف (MIC بین ۰/۸۹ تا ۲۱/۹ میکروگرم در میلی‌لیتر) فعالیت ضدباکتریایی قابل توجهی بر روی انتروکوکوس فکالیس داشت. علاوه بر این، عصاره آبی گرده درخت عرعر با MIC برابر با ۸/۷ میکروگرم در میلی‌لیتر و عصاره لیپیدی گرده سرو سیمین، درخت عرعر، تاج خروس و برنجاسف (MIC بین ۱/۷۸ تا ۲۱/۹ میکروگرم در میلی‌لیتر) فعالیت ضدباکتریایی قابل توجهی را علیه استافیلوکوکوس اورئوس نشان دادند. تنها عصاره با اثر ضدباکتریایی روی کلبسیلا نومونیه عصاره لیپیدی گرده برنجاسف با MIC برابر با ۳/۷۲ میکروگرم در میلی‌لیتر بود. **نتیجه‌گیری:** نتایج این مطالعه نشان داد که عصاره گرده برخی از گیاهان دارای فعالیت ضدباکتریایی قابل‌توجهی هستند. مطالعات بیشتری برای ارزیابی ترکیبات فعال در عصاره گرده و اثرات سیتوتوکسیک آنها مورد نیاز است.

مخفف‌ها: MDR، مقاومت آنتی بیوتیکی چندگانه؛ PBS، بافر فسفات نمکی؛ MIC، حداقل غلظت مهاری رشد؛ CLSI، موسسه استانداردهای آزمایشگاه و بالین؛ AMR، مقاومت ضد میکروبی

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