

Original Article

Evaluation of the Antimicrobial Activity of Alkaloid Extracts of Four Papaver Species

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Abstract

Background and Aim: The use of bioactive compounds of medicinal plants in prevention, control and treatment of human diseases has a long history. Most of plant bioactive compounds have highly complex chemical structures, and their chemical syntheses are often uneconomical. Moreover, their production is still dependent on plants. Plants of Papaveraceae family contain a variety of bioactive compounds that have many uses in traditional and modern medicine. In the present study, we evaluated the antimicrobial activity of alkaloid extracts of four plant species of Papaveraceae family against some human pathogens.

Materials and Methods: Crude alkaloid compounds of *Papaver macrostomum*, *Roemeria refracta*, *Papaver somniferum* and *Glaucium grandiflorum* plants were extracted using cain method. The antimicrobial activity of plant alkaloid extracts against *Pseudomonas aeruginosa* PTCC 1310, *Listeria monocytogenes* PTCC 1297, *Staphylococcus aureus* PTCC 1189, *Klebsiella pneumoniae* PTCC 1290 and *Candida albicans* PTCC 5027 pathogens were assessed using agar diffusion method.

Results: The results of this study indicated that the alkaloid extracts of tested plants were effective against fungal and bacterial pathogens. All plant alkaloid extracts exhibited more inhibitory effect against fungal pathogen than the bacterial pathogens. Comparison of MIC and MBC values for bacterial pathogens revealed that all plant alkaloid extracts showed more inhibitory effects against the gram-positive bacteria than gram-negative bacterial pathogens. The results also showed that the alkaloid extracts of *R. refracta* have stronger inhibitory effect against fungal and bacterial pathogens compared with other plants.

Conclusion: The antimicrobial compounds present in the plant species of Papaveraceae family are suitable candidates to produce new antibiotics. The results of this study demonstrated that antimicrobial compound in *R. refracta* plant could be used in the treatment of infectious diseases caused by *C. albicans* and *S. aureus* pathogens.

Keywords: Papaveraceae, medicinal plants, antimicrobial compounds, MIC, MBC

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Introduction

Nowadays, the inappropriate and prolonged use of antibiotic medications may lead to drug resistance in bacteria and causes many side effects and clinical problems in patients (1, 2). Hence, the need for research to discover new antimicrobial compounds from plants and other living organisms might be necessary to reduce unwanted effects. Due to the wide range of bioactive compounds as potential sources of new pharmaceuticals, medicinal plants are particularly attractive to modern medicine (3, 4). To prevent the side effects of many synthetic pharmaceuticals, the bioactive molecules of plant extracts are used for disease treatment. Due to the lack of accumulation of these compounds in the human body, their side effects are very limited, and they have remarkable advantages in comparison with synthetic pharmaceuticals (3).

The plant bioactive compounds that are called plant secondary metabolites, are a large group of chemical compounds produced by plant species exposed to biotic and abiotic stresses. Secondary metabolites are divided into three major groups of chemical compounds called alkaloids, phenolics and terpenes, and each group of these metabolites contains thousands of distinct compounds (5, 6).

Alkaloids are a group of plant secondary metabolites that are classified as amino acid derivative basic compounds. Alkaloids contain one or more heterocyclic ring with nitrogen atoms and have been found in bacteria, fungi, plants and animals (7). Alkaloids are produced in 300 plant families and are mainly formed from the phenylalanine, tyrosine, tryptophan and lysine amino acids. They have certain physiological effects on animals and show a wide range of pharmacological properties. Analgesics, cardiovascular drugs, psychotropics and sedatives, muscle relaxants, vasodilators, anti-spasms, antimalarials, antimicrobials and purgatives are examples of alkaloid drugs. Due to these different characteristics, alkaloids have always been used in both traditional pharmacy and modern medicine. In fact, alkaloids are the skeletal framework of about 60% of the modern pharmaceuticals (7, 8).

Some members of Papaveraceae family including *Papaver somniferum*, *Papaver bracteatum*,

Eschscholzia californica, *Argemone mexicana* and other valuable plants are the most important medicinal plants in the world (9). All members of this family produce and store different groups of benzyloisoquinoline alkaloids. The Iranian poppy (*Papaver bracteatum*) is a commercial source for codeine and thebaine alkaloids. California poppy (*Eschscholzia californica*) produces sanguinarine, reticuline, protopine, scoulerine and californidine. Mexican prickly poppy (*Argemone mexicana*) stores sanguinarine, dihydrosanguinarine, protopine and berberine alkaloids in its tissues (9, 10). The antimicrobial alkaloids produced by members of Papaveraceae family are considered as promising compounds in the treatment of infectious diseases. Up to now, different researchers have investigated the antimicrobial activity of some Papaveraceae species. The extracts of four annual poppy species showed strong antibacterial activity against *Staphylococcus aureus* pathogen (11). The same study proved that *Papaver rhoeas* extracts could exhibit high inhibitory effect against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella abony* and *Candida albicans* pathogens (12). The extracts of aerial parts of *Chelidonium majus* indicated high antibacterial activity against methicillin-resistant *Staphylococcus aureus* pathogen (13). In another study, seed extract of *Argemone mexicana* demonstrated significant inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* pathogens (14). The aim of this study was to investigate the antimicrobial activity of alkaloid extracts of *Papaver somniferum*, *Papaver macrostomum*, *Roemeria refracta* and *Glaucium grandiflorum* plants against some human pathogens.

Materials and Methods

Plant Sample Collection

Plant family of Papaveraceae contains important pharmaceutical alkaloids, especially benzyloisoquinoline ones. In the present study, plant samples were prepared from 4 species of Papaver. *Papaver macrostomum*, *Roemeria refracta* and *Glaucium grandiflorum* were collected from Lorestan province and identified in morphological analyses in herbarium of Faculty of Agriculture of Lorestan

University. Moreover, seeds of *Papaver somniferum* species were purchased from Research Institute of Forests and Rangelands (Iran) and grown in greenhouse. The collected tissues samples were dried for one week at 30°C. The dried tissues were ground into powder and used for alkaloid extraction.

Alkaloids Extraction

Alkaloids extraction was performed according to cain method (15). For this purpose, 50 g of the powdered tissues were soaked in methanol (Merck, Germany) and placed in dark bottles and shaken for 72 h. Then, the resulting extracts were filtered through Whatman papers (Whatman Int. Ltd., Maidstone, UK) and air dried. Twenty milliliters of 1N HCl (Merck, Germany) were added to 1 g of dried extracts, and then shaken for 1 h and kept at room temperature for 2 h. After 2 h, the resulting solutions were centrifuged at 13000 rpm for 15 min and supernatant was removed. Then, the precipitants were dried, dissolved in water and then filtered. Due to the basic nature of the alkaloids, addition of acids causes the precipitation of these compounds (15).

Microbial Strains

Microbial strains, including *Pseudomonas aeruginosa* PTCC 1310, *Listeria monocytogenes* PTCC 1297, *Staphylococcus aureus* PTCC 1189, *Klebsiella pneumoniae* PTCC 1290 and *Candida albicans* PTCC 5027, were purchased from Iranian Research Organization for Science and Technology (Tehran, Iran). Lyophilized microbial strains were dissolved in sterile water and plated on nutrient agar. After 16 h, colonies were grown in liquid LB and used for subsequent assays.

Antimicrobial Activity Assay

The antimicrobial activity of plant alkaloid extracts were evaluated using agar diffusion method (16). For this purpose, fresh cultures of microbial strains were separately prepared in liquid LB. After 16 h, the cultures were centrifuged at 5000 rpm for 10 min, supernatants was removed and pellets were dissolved in physiological saline without any adjuvant to reach 1.5×10^8 cfu.ml⁻¹ (0.5 McFarland). Subsequently, using sterile swabs cultures that were lawn cultured on sterile Mueller-Hinton agar plates and wells were cut using a sterile cork borer, and then agar plugs were removed. The Alkaloid extracts at a concentration of 15 mg.ml⁻¹ were added to wells and plates were

incubated at 37° C for 16 h. After incubation, the inhibition zones were recorded by caliper.

Minimum inhibitory concentration (MIC) was determined using serial dilution assay (17, 18). Ninety-six well plates were used for the serial dilution assay. Ten wells were used for each alkaloid extract, eight wells for different dilutions of each extract and two wells for negative and positive controls. Fifty microliter of microbial suspension (0.5 McFarland) was added to each well, except negative control and plates were incubated at 37°C for 16 h. After incubation, the optical density of the wells were measured using Stat Fax-2100 microplate reader (Awareness Technology, USA), and the lowest concentration prior to color change was considered as the MIC (17).

To determine the MBC (minimum bactericidal concentration) and MFC (minimum fungicidal concentration), 50 µL of microbial cultures from each well at the MBC and MFC was plated onto a sterile Mueller-Hinton agar plate. Plates were incubated at 37°C for 16 h and the colonies were then counted. The concentration that killed 99.9% of the microbes was considered the MBC and MFC (17, 18).

Penicillin, gentamicin, ciprofloxacin and vancomycin were used as positive control, and distilled water was used as negative control standards in the assays.

Statistical Analysis

All experiments were performed in three replicates, and statistical analysis was performed using the Microsoft Excel and Statistical Package for the Social Sciences (SPSS Inc, Chicago, USA) software. The Student's t-test was used to determine the significance at $P < 0.05$.

Results and Discussion

The results of antimicrobial activity showed that the alkaloid extracts of *G. grandiflorum*, *R. refracta*, *P. macrostomum* and *P. somniferum* were effective against Gram positive and Gram negative bacteria and *Candida albicans* (Figure 1-5). Alkaloid extracts of these plants had the same inhibitory effects on *L. monocytogenes*, and no significant differences were observed among them (Figure 1).

A similar trend was observed for *P. aeruginosa* and *S. aureus*, and there was no significant difference in the inhibitory activity among alkaloid extracts of *G.*

Table 1: Minimum inhibitory concentration (MIC) (mg.ml⁻¹).

	<i>L. monocytogenes</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
<i>P. somniferum</i>	4.4	4.4	4.4	2.2	1.1
<i>G. grandiflorum</i>	2.1	2.1	0.26	4.2	1.05
<i>R. refracta</i>	0.52	2.08	0.065	1.04	0.016
<i>P. macrostomum</i>	2.5	2.5	2.5	5	0.625
Ciprofloxacin	0.002	-	-	0.001	-
Vancomycin	-	-	0.002	-	-
Gentamicin	-	0.006	-	-	-

Table 2: Minimum bactericidal and fungicidal concentrations (mg.ml⁻¹).

	<i>L. monocytogenes</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
<i>P. somniferum</i>	4.4	4.4	4.4	4.4	2.2
<i>G. grandiflorum</i>	2.1	2.1	2.1	8.4	2.1
<i>R. refracta</i>	1.04	4.16	1.04	4.16	0.13
<i>P. macrostomum</i>	>5	2.5	2.5	>5	5
Ciprofloxacin	0.002	-	-	0.001	-
Vancomycin	-	-	0.002	-	-
Gentamicin	-	0.006	-	-	-

grandiflorum, *R. refracta*, *P. macrostomum* and *P. somniferum* (Figure 2, 3).

Alkaloid extracts of *P. macrostomum* and *R. refracta* showed a higher inhibitory effect than the alkaloid extracts of *G. grandiflorum* and *P. somniferum* on *K. pneumonia* (Figure 4). Furthermore, alkaloid extracts of *P. macrostomum* and *R. refracta* showed a higher inhibitory effect than the alkaloid extracts of *G. grandiflorum* and *P. somniferum* exhibited on *C. albicans* (Figure 5).

The least and most MIC values were determined for alkaloid extracts of *R. refracta* and *P. macrostomum*, respectively (Table 1). The lowest MIC value of *R. refracta* extract was calculated (0.016 mg.ml⁻¹) for *C.*

albicans (Table 1). Fungal pathogen *C. albicans* was the most sensitive pathogen to alkaloid extracts among the tested pathogens (Table 1).

The calculated MBC and MFC values had the similar trend to MIC and the least MBC and MFC values were determined for alkaloid extracts of *R. refracta* (Table 2).

Through the inhibition zone formation on growth media, the antimicrobial activity of alkaloid extracts of *G. grandiflorum*, *R. refracta*, *P. macrostomum* and *P. somniferum* were proven (Figures 1-5). However, the tested pathogens showed a different sensitivity to different plant alkaloid extracts (Table 1, 2).

In the study conducted by Kaeaniova et al. (2010),

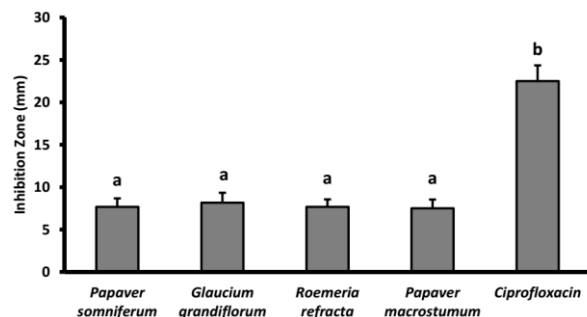


Figure 1. Antimicrobial activity of alkaloids extracts of different plants against *L. monocytogenes*. Bars represent standard errors. The different letters above the error bars indicate significant differences at the 0.05 level (Student's t-test: $P < 0.05$).

pollen extract of *Papaver somniferum* showed inhibitory activity against *Staphylococcus* sp. and Enterobacteriaceae pathogens (19). In another study, extract of *Papaver rhoeas* exhibited antifungal activity against *Candida albicans* and *Candida utilis* (20).

All plant alkaloid extracts showed more inhibitory effect against fungal pathogen than the bacterial pathogens. Alkaloid extract of *R. refracta* indicated a strong antimicrobial activity against *C. albicans* (Table 1). Moreover, alkaloid extracts of *P. macrostomum* exhibited a moderate inhibitory effect against this fungal pathogen (Table 1). The activity of alkaloid extracts of *P. somniferum* and *G. grandiflorum* against *C. albicans* was less significant than that of *R. refracta* and *P. macrostomum* (Table 1, 2).

There are different kinds of inhibitory activities of alkaloid extracts against fungal and bacterial pathogens, probably due to the different cellular structures of pathogens. One of the mechanisms of action of plant extracts is the effect on cell membranes and the disruption of the integrity of these membranes. This Lack of integrity causes leakage and impaired ion balance which leads to the cell death (21). Due to differences in the arrangement of the fatty acids, proteins, and other cell wall and membrane components in fungal and bacterial pathogens, different sensitivities to alkaloid extracts were observed for these pathogens. Such a trend was also observed in previous studies, in which antimicrobial activities of *Glaucium vitellinum*, *Papaver argemone*, *Papaver dubium*, *Papaver*

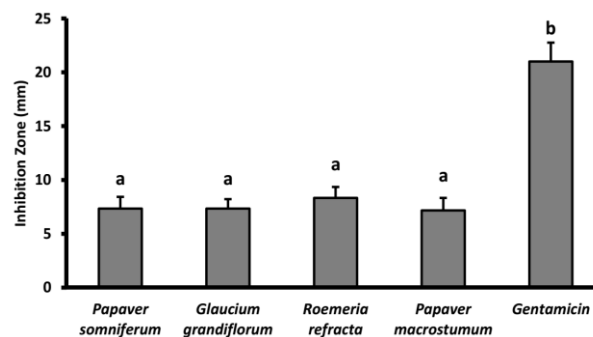


Figure 2. Antimicrobial activity of alkaloids extracts of different plants against *P. aeruginosa*. Bars represent standard errors. The different letters above the error bars indicate significant differences at the 0.05 level (Student's t-test: $P < 0.05$).

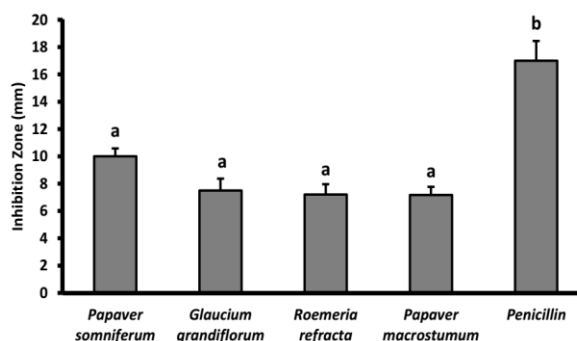


Figure 3. Antimicrobial activity of alkaloids extracts of different plants against *S. aureus*. Bars represent standard errors. The different letters above the error bars indicate significant differences at the 0.05 level (Student's t-test: $P < 0.05$).

rhoeas and *Papaver clavatum* against fungal and bacterial pathogens were investigated (22-24).

Gram-positive and gram-negative bacterial pathogens exhibited distinct reactions to the plant alkaloid

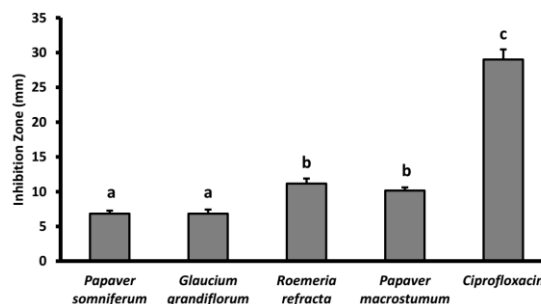


Figure 4. Antimicrobial activity of alkaloids extracts of different plants against *K. pneumonia*. Bars represent standard errors. The different letters above the error bars indicate significant differences at the 0.05 level (Student's t-test: $P < 0.05$).

extracts. The comparison of calculated MIC and MBC values for bacterial pathogens revealed that compared with gram-negative bacterial pathogens, the gram-positive bacteria are more sensitive to alkaloid extracts (Table 1, 2). Alkaloid extract of *R. refracta* with MIC of 0.065 mg.ml^{-1} showed a strong antimicrobial activity against gram-positive pathogen *S. aureus*, and alkaloid extracts of *G. grandiflorum* with MIC of 0.26 mg.ml^{-1} exhibited a moderate inhibitory effect against this pathogen (Table 1). Such a trend was observed in the study conducted by Rahman et al., in which alkaloid extracts of *Argemone mexicana* indicated stronger inhibitory effect against the gram-positive bacteria than gram-negative bacteria (25). In the same study, alkaloid extracts of *Argemone mexicana* exhibited inhibitory activity against *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* (14). In the study conducted by Morteza-Semnani et al., alkaloid extracts of three *Glaucium* species showed different inhibitory activities against gram-negative and gram-positive bacterial pathogens (26).

In another study, alkaloid fractions of *Chelidonium majus* showed strong antibacterial activity against methicillin-resistant *S. aureus* (13). Moreover, alkaloid extract of *R. refracta* with MIC of 0.52 mg.ml^{-1} showed a moderate antimicrobial activity against gram-positive pathogen *L. monocytogenes* (Table 1). The alkaloids extracts of other plants exhibited a weaker inhibitory effect on bacterial pathogens (Table 1). Gram-negative bacteria have more complex cell wall and membranes than gram-

positive, and due to the existence of outer membrane, show various resistance mechanisms against antimicrobial agents. In contrast, gram-positive bacteria have simpler cell membrane structure and do not form outer membrane. Gram-positive bacteria also form thicker peptidoglycan compared with gram-negative bacteria. However, cell membranes have greater sensitivity to antimicrobial agents than gram-negative bacteria due to their simplicity (27). In a study, extracts of *P. rhoeas* showed different antimicrobial activities against gram-negative and gram-positive bacteria and exhibited stronger activities against gram-positive bacteria (22). In another study, similar a trend was observed and extracts of aerial parts of four members of Papaveraceae family showed higher antimicrobial activities against gram-positive bacteria compared with gram-negative bacteria (24). In the present study, alkaloid extracts had a lower inhibitory activity than antibiotics, and most of plant alkaloid extracts showed their antimicrobial activity in high concentration, which might be due to low concentration of antimicrobial compounds in these crude alkaloid extracts. Using different separation and purification methods, various antimicrobial compounds can be isolated and purified from these crude alkaloid extracts and used for antimicrobial assays. Antimicrobial compounds exist in many medicinal plants. Using various methods, these compounds can be extracted and purified and introduced to the pharmaceutical industries. The antimicrobial compounds present in the plants of Papaveraceae family are suitable candidates for this purpose.

Conclusion

New antimicrobial agents can be produced and used against infectious diseases through the separation and purification of antimicrobial compounds from the plants of Papaveraceae family. The results of this study showed that antimicrobial compound in *R. refracta* could be used in the treatment of infectious diseases such as candidiasis and vaginitis caused by *C. albicans* and stye, meningitis and pneumonia caused by *S. aureus*.

Acknowledgment

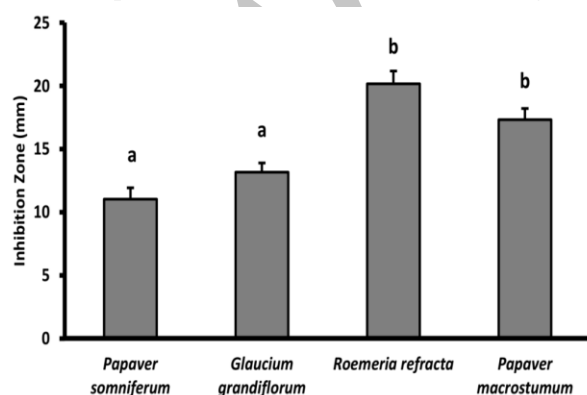


Figure 5. Antimicrobial activity of alkaloids extracts of different plants against *C. albicans*. Bars represent standard errors. The different letters above the error bars indicate significant differences at the 0.05 level (Student's t-test: $P < 0.05$).

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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