

# Original Article

**Antimicrobial activities of Parkinsoniaaculeata and Prosopiskoelziana extracts against pathogenic fungi and bacteria (Staphylococcus aureus, S. epidermidis, S. pyogenes, Pseudomonas Aeruginosa, Escherichia Coli, Aspergillusniger, A. flavus, A. fumigatus, F. solani, Microsporungypseum, M. mcanis, Trichophyton verrucosum, T. rubrum and Candidaalbicans)**

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## Abstract

Parkinsoniaaculeata and Prosopiskoelziana are two spinous ornamental plant from Leguminosae family. Besides their traditional uses, many pharmacological activities have been reported from family members, although little studies have been done about their antimicrobial properties. Natural products especially plant sources are under great considerations of public and medical experts. Excessive drug resistance and ineffectiveness of some antimicrobial drugs have led to exploitation of natural sources especially plant materials for treatment of infection diseases. Present study was conducted to investigate the antimicrobial properties of methanol extract and different fractions of Parkinsoniaaculeata and Prosopiskoelziana growing in south of Iran against *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *M. gypseum* and *C. albicans*.

Methanol extract and three fractions of each Parkinsoniaaculeata and Prosopiskoelziana including ethyl acetate, chloroform and aqueous fractions had been assayed separately against microorganisms. The antimicrobial activities of the extract and fractions were measured by standard agar diffusion Methods (disc-diffusion and well-diffusion methods). For the first method, the impregnated disks with 20  $\mu$ l of the extracts placed on the inoculated agar and for well-diffusion method the culture plates with test organisms were punched to make open wells and filled with 100  $\mu$ l of extracts. The antimicrobial activity was evaluated by measuring the inhibition zones against the test organisms in each method.

Methanol extract and chloroform fraction (at 40 mg/ml concentration) demonstrated stronger (20 and 15 mm inhibitory zones) and broader spectrum of antimicrobial activity as compared to other fractions of Parkinsoniaaculeata but for Prosopiskoelziana, just ethyl acetate fraction was effective (30 and 12 mm inhibitory zones in disk and well-diffusion methods respectively). In disc-diffusion method the highest bacterial and fungal inhibitory zones were related to *Pseudomonas aeruginosa* and *Aspergillus niger* by inhibition zones of  $20 \pm 0.3$  and  $13 \pm 0.1$  mm respectively. In well-diffusion assay, the best results were attributed to *Aspergillus niger* and *Staphylococcus aureus* with the inhibitory zone of  $30 \pm 0.2$  and  $16 \pm 0.1$  mm.

It is concluded that *Pseudomonas aeruginosa* and *Staphylococcus* species were more susceptible to the Parkinsoniaaculeata extracts and Prosopiskoelziana gave best response against *Candida albicans* and *Aspergillus* species. These results support the notion that the two plant extracts and fractions may have a role as pharmaceuticals for antimicrobial treatments. It need more extensively studies to explore its potential role in the treatment of infectious diseases.

**Keywords:** Antimicrobial, Parkinsoniaaculeata, Prosopiskoelziana, extracts, Bacteria, Fungi

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## Introduction

*Parkinsonia aculeata* L., is a large spinous shrub or small ornamental tree from the family Leguminosae, subfamily Caesalpinaceae (Fig 1 and 2). Worldwide, there are 600 genera and 13,000 species in the Leguminosae family. This is the third largest family of plants after the Orchid and Aster families (Elpel, 2004). The plant common names include Jerusalem thorn and Ratama. It is native to tropical area of America and can be cultivated to different parts of the world such as Iran. It is locally named 'Darman Aghrab' in south of Iran. *Parkinsonia aculeata* grows to 4 m and is a prickly bush. The smooth, green stems are slender and tend to droop. *Parkinsonia aculeata* leaves consist of a flat, green leaf stalk up to 30 cm long with numerous small (4-5 mm) green oblong leaflets staggered along both sides. The leaf base is protected by sharp, recurved spines. The flowers have four yellow petals with a red spotted area in center with a thin peduncle up to 7-15 mm long. Buds are ovate, integrated in the central raceme. Ovary glabrous, thin. Fruits are opaque green with the length of 7.5-10 cm and width of 7 mm (Ghahreman, 1980; Mozaffarian, 2013). In Iranian traditional medicines, it is used as an antipyretic, and analgesic (Zargari, 1992). It is traditionally described to treat fever and malaria, abortifacient, hepatothy, bacterial diseases, diabetes and trypanosomiasis (Hassan, Umar, Ebbo, Akpeji, & Matazu, 2008; Sharma & Vig, 2013). In recent studies the aerial parts of *Parkinsonia aculeata* have been used to diabetes-related complications. The plant antioxidant, free radical scavenging and antibacterial activities have also been proven (Kamba & Hassan, 2010; Leite et al., 2007; Sharma & Vig, 2014).

*Prosopis koelziana* B. is a spiny shrub belonging to Leguminosae and sub-family of Mimosoideae. It grows to 2.5 m slowly. The smooth stems are gray. Branches are whitish green, squishy with scattered thorns with 3-6 mm long. Leaves are compound and almost tomentose. Flowers are yellow, with 5 mm length and

a short peduncle (Ghahreman, 1980; Mozaffarian, 1996). *Prosopis koelziana* is indigenous of dry and semi-dry areas of America, Asia, and Africa. The first records of *Prosopis* introduction are those to West Africa and Pacific islands in or before the 1820s, to India and Pakistan in the 1870s, and to Australia and South Africa before 1900. There have been, however, many other unrecorded introductions before and since, evident by the fact that *Prosopis* is now found in dry regions of most African and Asian countries (Fig. 3). Three species of *Prosopis* growing in Iran including *Prosopis koelziana*, *Prosopis cineraria* and *Prosopis farcta*. Recently many pharmacological activities were reported for *Prosopis* species including the treatment of gastric ulcers, miscarriage, dysentery, rheumatism, laryngitis, chest pain, bronchitis, asthma, skin lesions, and scorpion sting (Al-Quran, 2008; Direkvand-Moghadam et al., 2015; Mozaffarian, 2005; Pasiecznik, Harris, & Smith, 2004). Natural products and plants have been used as medicines for thousands of years, playing a highly significant role in drug discovery and development. Their crucial role is particularly evident for treatment of infectious diseases, where over 60% of antimicrobial agents are of natural origin (Kariminejad et al., 2014; Newman & Cragg, 2007). It is estimated that infectious diseases are directly responsible for 26% of annual deaths worldwide (Morens, Folkers, & Fauci, 2008). *Staphylococcus aureus* is one of the most common gram-positive bacteria causing food poisoning. *Pseudomonas aeruginosa* is a gram-negative nonsporulating rod. In many epidemiological studies, it has been prompted it is potentially epidemic among compromised hospital patients and the colonization, increases risk to subsequent infection. In hospital environment, *P. aeruginosa* has been isolated from a wide variety of sources including vegetables, flower vases, sinks, drains and the floor. *Escherichia coli*, is a gram-negative bacteria which belongs to the normal flora of humans. *Aspergillus* spp. are responsible for different human infections and have been isolated from numerous food sources. A typical opportunist, *Candida*

*albicansis* the microbe responsible for most clinical yeast infections, e.g. in mouth infections (Kariminejad et al., 2014; Rezghi et al., 2014; Cryz Jr, 1984).

In recent years, multiple drug resistance in human pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Martínez, 2008). This situation forced scientists for searching new antimicrobial substances from various sources, like medicinal plants, which are the good sources of novel antimicrobial chemotherapeutic agents (Balunas & Kinghorn, 2005). According to some researches two-thirds of the

world's plant species have medicinal potential value (Krishnaiah, Sarbatly, & Nithyanandam, 2011). It is estimated that 10–100 million species or organisms living on earth. Higher plants contain 250,000-500,000 species that only 6% of them has been investigated for biological activities and 15% for their chemical constituents (Gurib-Fakim, 2006).

The present study was conducted to investigate antimicrobial properties of methanol extract and fractions of *Parkinsoniaaculeata* and *Prosopiskoelziana* growing in south of Iran against a wide range of bacteria, fungi, and yeast species which have not been evaluated in the previous studies in Iran.

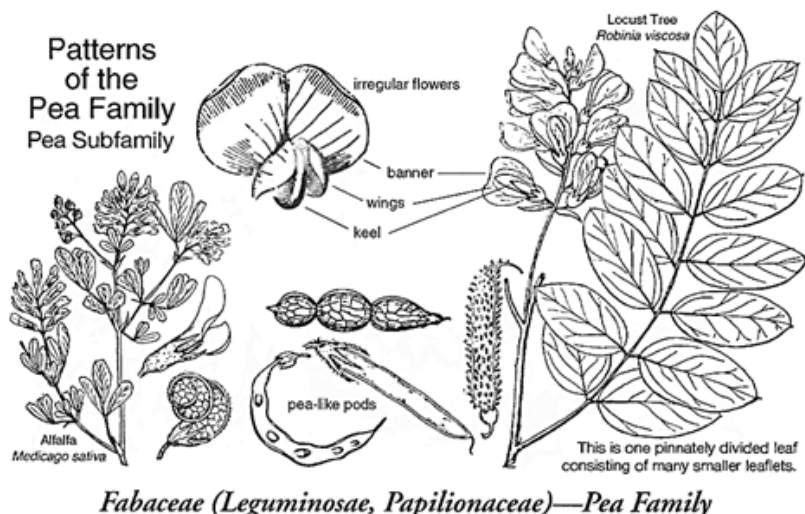


Figure 1: Patterns of Leguminosae: The 5 petals form a distinctive "banner, wings and keel".

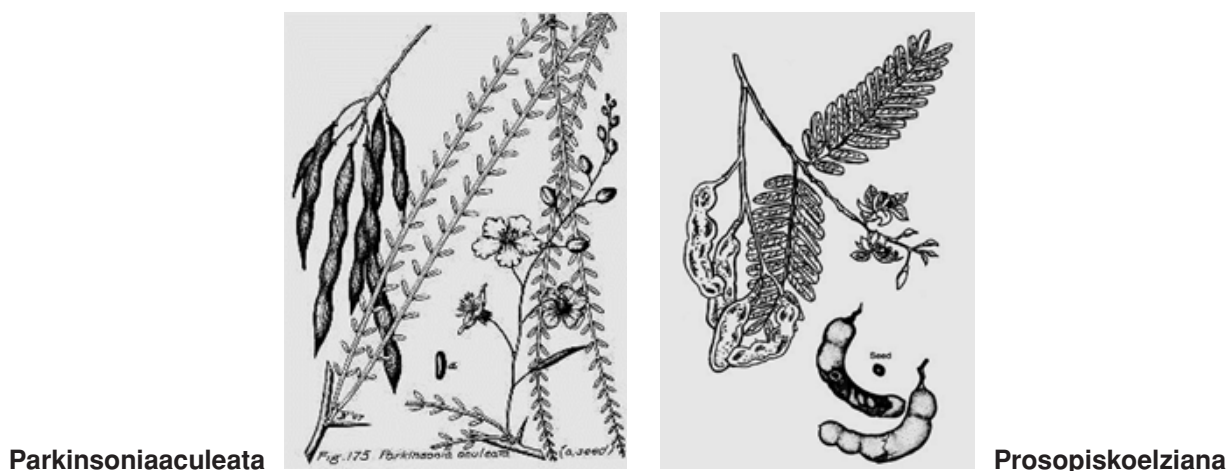


Figure 2: Parkinsoniaaculeata and Prosopiskoelziana

## Material and methods

### Plant materials and Extraction procedure

The plants were collected from Village of Hajiabad located in the region of Bandar abbas, south of Iran. Samples were authenticated by voucher specimen at the herbarium of Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. Plant samples was dried in the shade, and the leaves were separated from the stem, and ground in a grinder to reach fine powder. The proper amount of the air-dried and powdered plant material was submitted to solid-liquid extraction by maceration with methanol for 48h at ambient temperature, the extract was collected. This procedure was repeated for tree times. After concentration of collected extractions under vacuum, distilled water 20% (v/v) was added and liquid-liquid extraction was performed with chloroform, ethyl acetate and water respectively at room temperature. After each step, the extracts were filtered and air-dried and the solvents were removed under vacuum until dry extracts were obtained (Okpekon et al., 2004).

### Bacteria and Fungi Culturing

The bacterial strains used to assess the antibacterial properties of the extracts included five Gram-positive bacteria (*S. aureus*, *S. epidermidis* and *S. pyogenes*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*). Nine fungal strains consist of four mold (*A. niger*, *A. flavus*, *A. fumigatus* and *F. solani*), four dermatophytes (*M. gypseum*, *M. canis*, *T. verrucosum* and *T. rubrum*) and *C. albicans*). All microorganisms-species were obtained from Pasteur institute and Islamic Azad University, Tehran, Iran. The organisms were confirmed first and maintained on nutrient agar and sub-cultured before use.

### Antimicrobial activity (Disc-diffusion assay)

The dried plant extracts were dissolved in methanol to a final concentrations of 10mg/ml, 20mg/ml and 40 mg/ml. Antimicrobial tests were then carried out by disc-diffusion method (Shadomy et al., 1991) using 100  $\mu$ l of suspension containing 108 CFU/ml of bacte-

ria, 106CFU/ml of yeast, and 10<sup>4</sup> spore/ml of fungi spread on nutrient agar (NA), sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The discs (Hi-Media, 6.0 mm in diameter) were impregnated with 20 $\mu$ l of the extracts (200 $\mu$ l/disc, 400  $\mu$ l/disc and 800  $\mu$ l/disc) and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Vancomycin (30  $\mu$ g), Itraconazole (10  $\mu$ g), Amphotericin B (10 $\mu$ g) and Ketoconazol (10  $\mu$ g) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains, 48 h for yeast, and 72 h for filamentous fungi. Plant-associated microorganisms were incubated at 27 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

### Antimicrobial activity (well- diffusion assay)

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer dipped in alcohol and flamed to make open wells (6.0 mm diameter). Different cork borers were used for different test organisms. Ketoconazol and vancomycin were as used in comparison with antibacterial and antifungal activity of other test organisms. The wells were filled with 100  $\mu$ l of methanolic extraction and each fractions (40 mg/ml). Then, the plates were left at room temperature for 2 hours to allow diffusion of test sample and incubated face upwards at 37°C for overnight. The diameter of the zones of inhibition was measured with scale (Irshad, Mahmood, & Perveen, 2012).

## Results

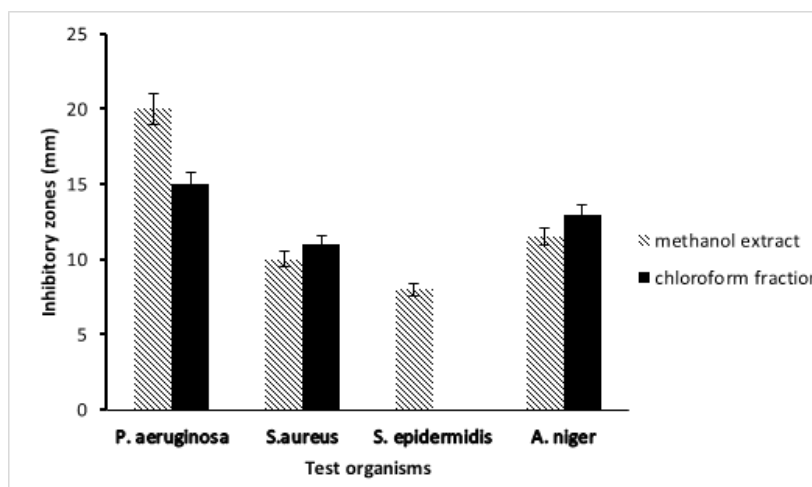
In the present study, the antimicrobial compounds from the leaves of *Parkinsonia aculeata* and *Prosopis koelziana* collected from Hajiabad, one of the village of Bandar abbas in Iran, were extracted against wide range of microorgan-

isms on the basis of disc-diffusion and well-diffusion assay. The antimicrobial activities of *P. aculeata* and *P. koelziana* methanol extract and different fractions against microorganisms examined in the present study and their potency were quantitatively assessed by the presence or absence of inhibition zones and zone diameters (Tables 1 and 2).

**Table1: Antimicrobial activity of *P. aculeata* and *P. koelziana* methanol extract and fractions ( $\mu\text{g}/\text{disk}$ ) against the microorganisms tested based on disc-diffusion method**

Microorganisms	plant	Inhibition zone diameter (mm) around test disc for methanol extract		
		200	400	Standard antibiotic discs
<i>Pseudomonas aeruginosa</i>	<i>P. aculeata</i>	$10 \pm 0.2$	$20 \pm 0.3$	Vancomycin ( $30 \mu\text{g}$ ): $25 \pm 0.3$
<i>Staphylococcus aureus</i>	<i>P. aculeata</i>	NA	$10 \pm 0.5$	Vancomycin ( $30 \mu\text{g}$ ): $21 \pm 0.3$
<i>Staphylococcus epidermidis</i>	<i>P. aculeata</i>	NA	$8 \pm 0.2$	Vancomycin ( $30 \mu\text{g}$ ): $20 \pm 0.2$
<i>Aspergillus niger</i>	<i>P. aculeata</i>	$7 \pm 0.1$	$11.5 \pm 0.6$	Itraconazole ( $100 \mu\text{g}$ ): $19 \pm 0.1$
Microorganisms	plant	Inhibition zone diameter (mm) around test disc for chloroform fraction		
		200	400	Standard antibiotic discs
<i>Pseudomonas aeruginosa</i>	<i>P. aculeata</i>	$8 \pm 0.1$	$15 \pm 0.3$	Vancomycin ( $30 \mu\text{g}$ ): $25 \pm 0.3$
<i>Staphylococcus aureus</i>	<i>P. aculeata</i>	$7 \pm 0.2$	$11 \pm 0.4$	Vancomycin ( $30 \mu\text{g}$ ): $21 \pm 0.3$
<i>Aspergillus niger</i>	<i>P. aculeata</i>	$7 \pm 0.2$	$13 \pm 0.1$	Vancomycin ( $30 \mu\text{g}$ ): $25 \pm 0.3$
Microorganisms	plant	Inhibition zone diameter (mm) around test disc for ethyl acetate fraction		
		200	400	Standard antibiotic discs
<i>Aspergillus niger</i>	<i>P. koelziana</i>	NA	$12 \pm 0.4$	Amphotericin-B ( $10 \mu\text{g}$ ): $13.5$

NA: no activity



**Figure 3: Inhibition zones (mm) of methanol extract and chloroform fractions ( $400 \mu\text{g}/\text{disk}$ ) of *P. aculeata* against test organism by disk- diffusion method**

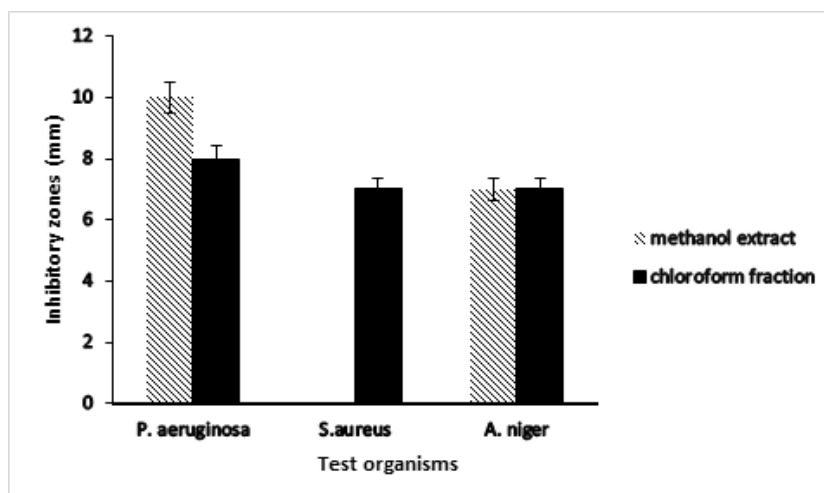


Figure 4: Inhibition zones (mm) of methanol extract and chloroform fractions (200µg/disk) of *P. aculeata* against test organism by disk- diffusion method

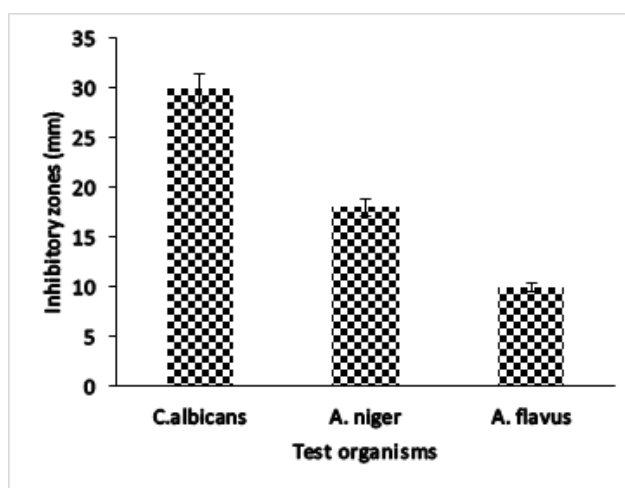
Table2: Antimicrobial activity of *P. aculeata* and *P. koelziana* methanol extracts and fractions in concentration of 40 mg/well against the microorganisms tested based on well-diffusion method

Microorganisms	plant	Inhibition zone diameter (mm) around test disc for ethyl acetate fraction	
		40 mg/well	Standard antibiotic discs
<i>Candida albicans</i>	<i>P. koelziana</i>	30 ± 0.2	Ketoconazol (10 µg): 48 ± 0.2
<i>Aspergillus niger</i>	<i>P. koelziana</i>	18 ± 0.1	Ketoconazol (10 µg): 23 ± 0.1
<i>Aspergillus flavus</i>	<i>P. koelziana</i>	10 ± 0.3	Ketoconazol (10 µg): 17 ± 0.3
Microorganisms	plant	Inhibition zone diameter (mm) around test disc for chloroform fraction	
		40 mg/well	Standard antibiotic discs
<i>Staphylococcus aureus</i>	<i>P. aculeata</i>	16 ± 0.1	Vancomycin (30 µg): 33 ± 0.
Microorganisms	plant	Inhibition zone diameter (mm) around test disc for methanol fraction	
		40 mg/well	Standard antibiotic discs
<i>Staphylococcus aureus</i>	<i>P. aculeata</i>	14 ± 0.3	Vancomycin (30 µg): 33 ± 0.3
Microorganisms	plant	Inhibition zone diameter (mm) around test disc for chloroform fraction	
		40 mg/well	Standard antibiotic discs
<i>Aspergillus fumigatus</i>	<i>P. aculeata</i>	8 ± 0.1	Ketoconazol (10µg): 18 ± 0.1

The results showed that the methanol extract has inhibition effect on the growth of 3 (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) of 5 bacterial species and one fungal species (*Aspergillus niger*) (Figure 3). However, the aqueous extract of both plant species had no antimicrobial activity against any of the bacterial or fungal tested in the present study. The chloroform fraction was inhibited the growth of 2 bacterial (*Pseudomo-*

*nas aeruginosa*, *Staphylococcus aureus*) and 2 fungal species (*Aspergillus niger*, *Microsporom gypseum*) (Figure 4). The controls did not show any antimicrobial activity.

Maximal inhibition zones, in well-diffusion method were related to the microorganisms sensitive to the ethyl acetate fraction of *P. koelziana* were in the range of  $30 \pm 0.2$  mm belongs to *Candida albicans* and  $18 \pm 0.1$  mm for *Aspergillus niger* (Figure 5).



**Figure 5:** Inhibition zones (mm) of ethyl acetate fraction (40 mg/well) of *P. koelziana* against test organism by well-diffusion method

## Discussion

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques such as agar diffusion method as we used in this study. It involves the application of antibiotic solutions of different concentrations to cups, wells or paper discs (Bonev, Hooper, & Parisot, 2008).

The results given in Table 1 show that *Parkinsonia* methanol extract and chloroform fraction are more effective against *Pseudomonas aeruginosa*. *Staphylococcus aureus* was also susceptible to the above extracts. The antimicrobial activity of *Parkinsonia aculeata* leaves in this study agrees

with the findings of others (Al-Youssef & Hassan, 2015; Ali, Azhar, Amtul, Ahmad, & Usmanjani, 1999; Divya, Mruthunjaya, & Manjula, 2011; KAMBA & HASSAN, 2010). In an investigation about the phytochemical analysis of *Parkinsonia aculeata* leaves, the chloroform and alcoholic extracts contain alkaloids, flavonoids, tanins, volatile oil, saponins and steroids. It was reported that some phenolic compounds like tannins present in the cells of plants which are potent inhibitors of many hydrolytic enzymes such as proteolytic enzymes presented in pathogens. Other compounds like saponins also have antifungal properties. Therefore the principle active compounds in *Parkinsonia* may be responsible for the antibacterial activity of the

tested organisms (Kamba & Hassan, 2012). *Prosopis koelziana* gave best response against *Candida albicans*, *Aspergillus flavus* and *A. niger* by producing nearly about 30 and 18 and 10 mm inhibition zones. There are only a few investigation about *P. koelziana*. According to the antimicrobial studies of different *Prosopis* species, *Aspergillus* and *Candida* are very susceptible to *Prosopis juliflora* and *Prosopis cineraria* (Henciya et al., 2016; Napar et al., 2012; Sheikh, Malik, Meghavanshi, & Mahmood, 2012; Zainal, Abdel-Rahim, Abu-Ali, & Radwan, 1988). Juliprosopine, prosoflorine and juliprosine are related alkaloids isolated from the *Prosopis* leaves. It has been proved that the antimicrobial activities of the plant is related to the mentioned alkaloids (Aqeel, Khursheed, Viqaruddin, & Sabiha, 1989; dos Santos et al., 2013).

## Conclusion

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines has provide rational means for the treatment of many diseases. *Parkinsonia aculeata* and *Prosopis koelziana* has been widely used in various traditional system of medicine. According to their distribution in difficult areas like the arid regions it has been less attention paid to it. From the present study it can be concluded that *Parkinsonia aculeata* and *Prosopis koelziana* may represent new sources of anti-microbials. More studies need to be extended for future investigation into the phytochemistry and other biological actions of these plant species.

## 6. Acknowledgements

We would like to offer our special thanks to Department of Mycology, Pasteur Institute of Iran and the research council of Islamic Azad University Pharmaceutical Sciences Branch, for their support of this study.

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