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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, Microsporumgypseum, 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 20μl 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 100μl of extracts. The antimicrobial activity was evaluated by measuring the inhibition zones against the test organisms in each method.

Methanol extractand chloroform fraction (at 40 mg/ml concentration) demonstratedstronger (20 and 15 mm inhibitory zones) and broader spectrum of antimicrobial activity as compared to other fractions of Parkinsoniaaculeatebut 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 ± 0.3 and 13 ± 0.1mm respectively. In well-diffusion assay, the best results were attributed to Aspergillus niger and Staphylococcus aureus with the inhibitory zone of 30 ± 0.2 and 16 ± 0.1 mm.

It is concluded thatPseudomonas aeruginosa and Staphyloccusspecieswere more susceptible to the Parkinsoniaaculeata extracts and Prosopiskoelzianagave 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

ParkinsoniaaculeataL., is a large spinous shrub or small ornamental tree from the family Leguminosae, subfamilyCaesalpinaceae (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 includeJerusalem thorn andRatama. 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 'DarmanAghrab' in south of Iran. Parkinsoniaaculeatagrows to 4 m and is aprickle bushes.The smooth, green stems are slender and tend to droop. Parkinsoniaaculeataleaves consist of a flat, green leaf stalk up to 30cm long with numerous small (4-5 mm) green oblong leaflets staggered along both sides.The leafbase is protected by sharp, recurved spines. Theflowers have four yellow petals withred spotted area in centerwith thinpeduncle up to 7-15 mm long. Buds are ovate, Integrated in the central raceme.Ovary glabrous, thin. Fruits areOpaque green with the length of 7.5-10 cmand width of 7mm(Ghahreman, 1980; Mozaffarian, 2013).In Iranian traditional medicines, it is used as an antipyretic, and analgesic(Zargari, 1992). It istraditionally described to treat fever and malaria,abortifacient, hepathopathy, bacterial diseases, diabetes and trypanosomiasis(Hassan, Umar, Ebbo, Akpeji, & Matazu, 2008; Sharma & Vig, 2013). In recent studies the areal parts ofParkinsoniaaculeatahave been used to diabetes-related complications. The plant antioxidant, free radical scavenging andantibacterial activities have also been proven (Kamba & Hassan, 2010; Leite et al., 2007; Sharma &Vig, 2014).

Prosopiskoelziana B. is a spiny shrub belonging to Leguminosaeand sub-family of Mimosoideae.Itgrows to 2.5 m slowly. Thesmooth 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).Prosopiskoelziana is indigenous of dry and semi-dry areas of America, Asia, and Africa.The first records of Prosopisintroduction 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 Africabefore 1900. There have been, however, many other unrecorded introductions beforeand since, evident by the fact that Prosopisis now found in dry regions of most Africanand Asian countries (Fig. 3). Three species of Prosopis growing in Iran including Prosodies koelziana, Prosopis cineraria and Prosopisfarcta.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 highly significant role in drug discovery and development. Their crucial role 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 foodpoisoning. 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. aeruginosahas 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 infection and have beenisolated 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 commonlyused in the treatment of infectious diseases(Martínez, 2008). This situation forced scientists for searching new antimicrobial substances from various sources, like medicinal plants, which arethe 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 ParkinsoniaaculeataandProsopiskoelzianagrowing 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.

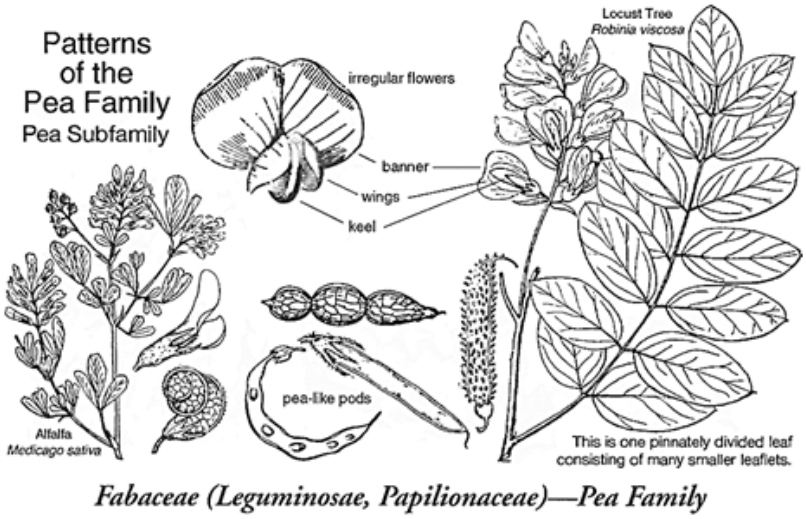

Figure 1: Patterns of Leguminosae: The 5 petals form a distinctive "banner, wings and keel".

Parkinsoniaaculeata

Prosopiskoelziana

Figure 2: Parkinsoniaaculeataand 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 sampleswas 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 extractionby 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. mcanis, T.verrucosumand 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 μl of suspension containing 108 CFU/ml of bacteria, 106CFU/ml of yeast, and 104 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 20μl of the extracts (200μl/disc, 400 μl/disc and 800 μl/disc) and placed on the inoculated agar. Negative controls were prepared usingthe same solvents employed to dissolve the plant extracts. Vancomycin (30 μg), Itraconazole (10 μg), Amphotericin B (10μg) and Ketoconazol (10 μg) 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 measuringthe 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 borerdipped in alcohol and flamed to make open wells (6.0 mmdiameter).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 μl of methanolic extraction and each fractions (40 mg/ml). Then, the plates were left at room temperature for 2 hours to allowdiffusion of test sample and incubated face upwards at 37ºC 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 koelzianacollected 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 ofP. aculeata andP. 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 (µg/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**
Pseudomonas aeruginosa	P. aculeata	10 ± 0.2	20± 0.3	Vancomycin (30 μg): 25± 0.3
Staphyloccus aureus	P. aculeata	NA	10 ±0.5	Vancomycin (30 μg): 21± 0.3
Staphyloccus epidermidis	P. aculeata	NA	8 ±0.2	Vancomycin (30μg): 20 ± 0.2
Aspergillus niger	P. aculeata	7± 0.1	11.5 ± 0.6	Itraconazole (100 μg): 19 ± 0.1
Microorganisms	**plant**	Inhibition zone diameter (mm) around test disc for chloroform fraction		
		200	**400**	**Standard antibiotic discs**
Pseudomonas aeruginosa	P. aculeata	8 ± 0.1	15 ± 0.3	Vancomycin (30 μg): 25 ± 0.3
Staphyloccus aureus	P. aculeata	7 ± 0.2	11 ± 0.4	Vancomycin (30 μg): 21 ± 0.3
Aspergillus niger	P. aculeata	7 ± 0.2	13 ± 0.1	Vancomycin (30 μg): 25 ± 0.3
Microorganisms	**plant**	Inhibition zone diameter (mm) around test disc for ethyl acetate fraction		
		200	**400**	**Standard antibiotic discs**
Aspergillus niger	P. koelziana	NA	12 ± 0.4	Amphotericin-B (10μg):13.5

NA: no activity

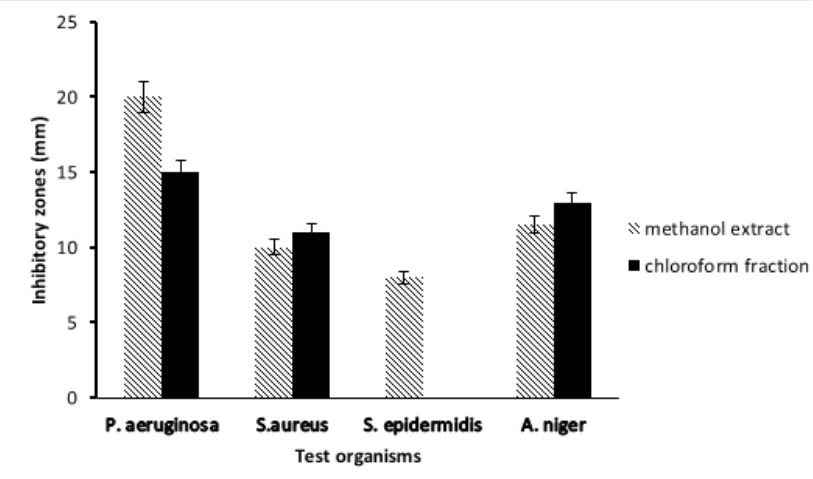

Figure 3: Inhibition zones (mm) of methanol extract and chloroform fractions (400 µg/disk) of P. aculeata against test organism by disk- diffusion method

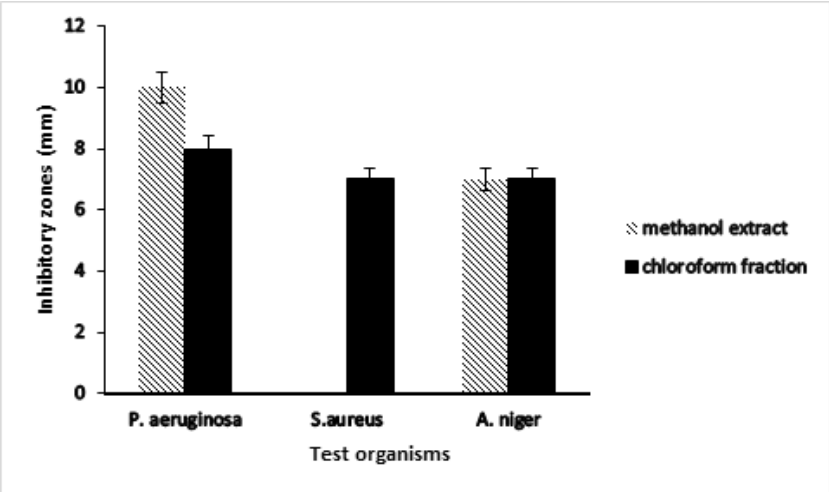

Figure 4: Inhibition zones (mm) of methanol extract and chloroform fractions (200µg/disk) of P. aculeata against test organism by disk- diffusion method

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**
Candida albicans	P. koelziana	30 ± 0.2	Ketoconazol (10 μg): 48 ± 0.2
Aspergillus niger	P. koelziana	18 ± 0.1	Ketoconazol (10 μg): 23 ± 0.1
Aspergillus flavus	P. koelziana	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**
Staphyloccus aureus	P. aculeata	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**
Staphyloccus aureus	P. aculeata	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**
Aspergillus fumigatus	P. aculeata	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,Staphyloccus aureus and Staphyloccus epidermidis) of 5 bacterial species and one fungal species (Aspergillus niger) (Figure 3). However, the aqueous extract of both plant specieshad 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 (Pseudomonas aeruginosa,Staphyloccus 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 acetatefraction of P. koelzianawere in the range of 30±0.2mm belongs to Candida albicans and 18±0.1mm for Aspergillus niger (Figure 5).

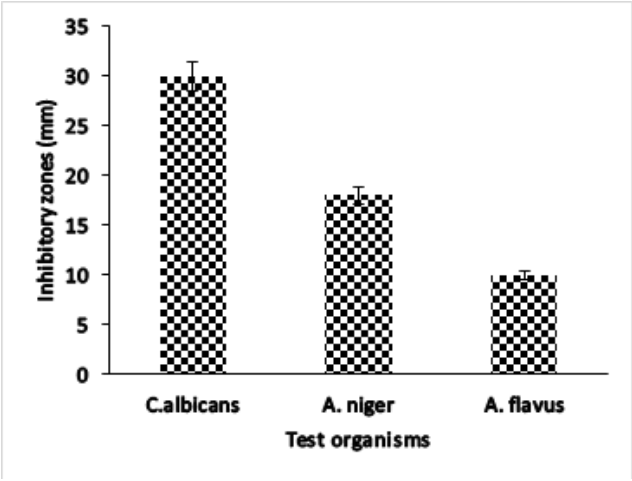

Figure 5: Inhibition zones (mm) of ethyl acetate fraction (40 mg/well) of P. koelziana against test organism by well-diffusion method

Discussion

Accurate determination of bacterial susceptibility to antibioticsis essential to the successful management of bacterial infectionsand to the comparative analysis of antimicrobial agents. Thiscan be done by a number of techniques such as agar diffusion method as we used in this study.It involves the application ofantibiotic solutions of different concentrations to cups, wells orpaper discs(Bonev, Hooper, & Parisot, 2008).

The results given in Table 1 show that Parkinsoniamethanol extract and chloroform fraction are more effective against pseudomonas aeruginosa.Staphyloccus aureus was also susceptible to the above extracts.The antimicrobial activity ofparkinsonia aculeata leavesin this study agrees with the findings of others (Al-Youssef & Hassan, 2015; Ali, Azhar, Amtul, Ahmad, & Usmanghani, 1999; Divya, Mruthunjaya, & Manjula, 2011; KAMBA & HASSAN, 2010).In an investigation about the phytochemical analysis of Parkinsonia aculeata leaves, the chloroform and alcoholic extracts contains alkaloids, flavonoids, tanins, volatile oil, saponins and steroids. Itwas reported that some phenolic compounds liketannins present in the cells of plants which are potentinhibitors of many hydrolytic enzymes such asproteolytic enzymes presented in pathogens. Other compounds likesaponins also have antifungal properties. Therefore the principleactive compounds in Parkinsonia may be responsible forthe antibacterial activity of the

testedorganisms (Kamba & Hassan, 2012).
Prosopis koelzianagave 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 Prosopisspecies,Aspergillus and Candida are very suceptibe 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 alkaloides isolated from the Prosopis leaves. It has been prooved 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, betterpatient tolerance, relatively less expensive and acceptance due to a long history of use, especiallyherbal medicines has provide rational means for the treatment of many diseases. Parkinsonia aculeata and Prosopis koelzianahas been widely used in various traditional system of medicine. According to their distributionin difficult areas like the arid regions it has been less attention paid to it. From the present study it can be concluded thatParkinsonia aculeata and Prosopis koelziana mayrepresent new sources of anti-microbials.More studies need tobe extended for future investigation into thephytochemistry and other biological actions of these plant species.

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