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Rosa foetida Herrm. flowers as a future natural antibacterial agent against the main cause of skin burn wound infections, *Pseudomonas aeroginosa*.

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ABSTRACT

Background & Aim: Belongs to Rosaecea family, *Rosa foetida* is one the Persian native plants which has not been investigated biologically. As it is traditionally used topically as poultice to treat infectious skin burns, the present paper focused on the assessment of the antibacterial activities of different extracts of *R. foetida* flowers against the main cause of skin burn wounds infections, *Pseudomonas aeruginosa*.
Experimental: The antibacterial activity and MIC value determination were investigated by cup plate method and micro plate dilution method respectively.
Results: All *R. foetid* extracts had inhibition activity on the growth of *P. aeruginosa* of which the aqueous and methanol extracts exhibited the strongest activities. Inhibition zone diameter and MIC values of the concentration of 125 mg/ml of both extracts were found to be somehow the same as those of the standard drug, Imipenem/Cilastatin (8/8 µg/ml).
Recommended applications/industries: Results demonstrated that the plant is effective against the standard and pathogenic strains of *P. aeruginosa* and could be a potential source of effective natural antibacterial compounds to be applied in further phytochemical and invivo biological studies.

1. Introduction

Burn wound infections are a serious complication of thermal injury and there are about 12000 deaths per year due to thermal injury. Although presently more patients with burns die of pneumonia than of burn wound infection, burn wound sepsis remains an important infectious complication in this population (Weinstein and Mayhall, 2003). Since the most common causes of burn wound infections were bacteria, burn wounds were treated by the exposure method, with application of topical antimicrobials and antibiotics to the burn wound

surface. *Pseudomonas aeruginosa* plays a prominent role as an etiological agent involved in serious infections in burned patients (Sutter and Hurst, 1966; Kominos and Charles, 1972).

It is well-recognized as a pathogen which exhibits inherent drug resistance and represents a leading cause of morbidity due to burn wound infection (Eckert *et al*., 2006). Reports of *P. aeruginosa* infection analyses in Burn Centers and Hospitals of Tehran to estimate their frequency and antibiotic susceptibility showed that the frequency of *P. aeruginosa* resistance to most of antibiotics including gentamicin, ceftizoxime, carbenicillin, cephaothin and ceftazidime was over 90% (Rastegar Lari *et al*., 1998). Reports from another study in a major burn center in Tehran showed that all *P. aeruginosa* strains were multi drug resistant. The percentage of resistance to tested antibiotics was: imipenem 97.5%, amikacin 90%, piperacillin 87.5%, ceftizoxime 72.7%, gentamicin 67.5%, ciprofloxacin 65%, ceftriaxone 60%, and ceftazidime 57.5% (Ranjbar *et al*., 2011). According to the previous reports, there is a pressing need for alternative therapeutics against this pathogen and additional studies are required to find antibacterial prophylaxis and new substitutes for current bacterial-resistant antibiotics.

Iran is one of the countries having endemic *Rosa* species and *Rosa foetida* Herrm. is one of the species widely spread throughout the country from Western to central and southern parts (Amin, 2005). *Rosa* species are well known for their medicinal and cosmetic properties. *Rosa* species are reported to have antibacterial, anti-inflammatory and diuretic properties (Mahmood *et al*., 1996; Brinkworth *et al*., 1992; Frederick *et al*., 2002). The commercial significance of *R. damascena* lies particularly in rose oil which has a high international demand, and Iran is one of the major producers of this oil and the related aromatic water called “Golab” in Persian (Loghmani-Khouzani *et al*., 2007). Rose hips which are rich in Vitamin C (300-4000 mg/100 g) (Ercisli, 2007) and antioxidants also have market demand for their role in enhancing the body immune system against infections and particularly the common cold.

A native plant to Iran, *Rosa foetida* Herrm. (Rosaceae family) has yellow flowers without scent which some find objectionable. Since there were no yellow rose native to Europe, its introduction from Persia was an important addition to the cultivation of roses, and it is now an important contributor to the stock of cultivated roses (Forsyth, 2007). Its common name is Persian yellow rose and is wildly distributed in Kurdistan Mountains, west of Iran. The decoction of the petals is used in Iranian traditional medicine as anti-diarrhea and for treatment of stomach disorders. It is topically used as a poultice to treat burns especially the burn wound infections. It is also used traditionally in some foods as a yellow color agent. Reports on *Rose foetida* flowers volatile oil composition have been published and hydrocarbons including n-nonadecane, 1-heptadecene and n-dodecanoic acid were the main group of constituents in the oil (Asgarpanah *et al*., 2014). Since there have been no attempts to study the antibacterial activity of *R. foetida* flowers against the main cause of burn wound infections, *P. aeruginosae* up to now, we prompted to evaluate the antimicrobial properties of methanol, chloroform and aqueous extracts of *R. foetida* flowers against *P. aeruginosa* standard strain and five pathogen clinical strains isolated from patients suffering burn wound infection. This investigation is done to confirm the biological basis for the folkloric use of these flowers as natural antibacterial agent for treatment of burn wound infections.

2. Materials and Methods

2.1. Plant material

Fresh flowers of *R. foetida* were collected in June 2012 from Divandarreh, in Kurdistan Province, Iran: (N35°54´, E 32°46´, 1850 m). Specimen was identified by Dr. G.H. Amin and voucher was deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran under code number 7042 AUPF.

2.2. Extraction Procedure

2 kg of chopped fresh petals were separately extracted by percolator apparatus using methanol, chloroform and water. Each extraction repeated for 3 times. The methanol and chloroform extracts were concentrated by rotary evaporator apparatus and the solvent removed to produce a dark brown gummy solid. The aqueous extract concentrated and dried by leaving it in room temperature. All extracts were kept in sterile vials in a dark and cool place for further tests.

Table1. The inhibition zone diameter of *Rosa foetida* flowers extracts[a].

	Diameter of zone of inhibition (mm)															
	MTE (mg/ml)					ATE (mg/ml)					CTE (mg/ml)					Imi./Cil. (8/8 µg/ml)
	1000	500	250	125	62.5	1000	500	250	125	62.5	1000	500	250	125	62.5	
P. aeruginosa[b]	20.6	18.6	16.0	13.8	10.3	20.3	19.5	17.6	14.3	10.1	13.3	12.5	8.5	-	-	15.6
Sample 1[c]	20.0	18.1	15.5	13.0	10.1	20.8	19.3	17.3	13.5	9.5	13.0	12.3	10.0	-	-	17.3
Sample 2	21.3	19.5	17.0	15.8	12.0	20.8	19.0	16.8	14.0	11.1	12.3	8.6	-	-	-	17.0
Sample 3	27.6	25.5	24.0	22.1	20.3	26.1	23.6	20.8	18.0	14.0	11.1	10.5	8.5	-	-	13.2
Sample 4	17.6	16.3	14.6	12.8	10.8	19.5	17.1	15.8	13.0	11.6	10.5	9.6	8.1	-	-	14.3
Sample 5	21.6	19.3	16.1	13.8	10.0	21.5	19.3	16.3	13.6	10.0	10.0	8.3	-	-	-	17.4

[a]Zone of inhibition, including the diameter of the well (6mm); mean value of three independent experiments.
MTE=Methanol total extract; ATE=Aqueous total extract; CTE=Chloroform total extract
Imi./Cil.=Imipenem/Cilastatin
[b]Standard *P. aeruginosa* strains
[c]Samples 1-5 are 5 different clinical isolates of *P. aeruginosa*

2.3. Test Organisms

One Gram-negative bacterium *Pseudomonas aeruginosa* (PTCC25823) obtained from Persian type culture collection (PTCC) of Iranian Research Organization for Science and Technology accompany with five clinical pathogen *P. aeruginosa* strains isolated from the patients suffering burn wound infections taken for this study.

2.4. Antibacterial activity

Antibacterial activities of *R. foetida* flowers extracts of were investigated against 6 bacterial strains by the cup plate method (Fazly-Bazzaz *et al.*, 2005). An overnight bacterial culture equal to 0.5 McFarland standard (1.5 x 108 CFU/ml) was used to culture on Muller-Hinton agar plates. The wells were made on agar plates with 5mm diameter. 1000, 500, 250, 125 and 62.5 mg of the each extract were separately dissolved in 1 ml water (for aqueous extract) and DMSO 10% (for methanol and chloroform extracts) and then filtered and 80 µl of each solution was added to each well. Imipenem/Cilastatin (8/8 mg/ml) was used as positive control for tested microorganisms. 80 µl of DMSO 10% served as negative control. The plates incubated at37ºC for 24h. The diameter of zone of inhibitions was detected in each plate. The experiments carried out 3 times and the results were presented as mean±SD.

2.5. Minimum Inhibitory Concentration (MIC)

After confirmation the antibacterial activity in the extracts MIC of each was determine by testing 10 concentrations of the extracts against sensitive tested bacteria by the micro plate dilution method. The reconstituted extracts were separately diluted to give concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml, 3.90 mg/ml, 1.95 mg/ml and 0.97 mg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC (Mehregan *et al.*, 2008). As the same, Imipenem/Cilastatin and DMSO 10% were used as positive and negative controls, respectively.

3. Results and discussion

According to Table 1, all *R. foetida* extracts had inhibition effect on the growth of *P. aeroginosa* among

which the aqueous and methanol extracts exhibited significant activities. Observed antibacterial activity by 125 mg/ml concentrations of methanol and aqueous extracts were somehow equal to the antibacterial activity of the standard drug Imipenem/Cilastatin (8/8 mg/ml). MIC values of the extracts determined and as the *Tab. 2* the lowest MIC values observed by methanol and aqueous extracts.

Occurrence of bacterial infections especially infections caused by *P. aeruginosa* in skin burns is a serious problem. Regarding to development of antibacterial drug resistance of pathogen *P. aeruginosa* and side effects exhibited by the drugs used for skin burn infections, there is a great demand alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of *P. aeruginosa* infections is an old practice in many parts of the world. Plants contain a spectrum of secondary metabolites that their importance as antimicrobial agents against this bacterial strain has been emphasized by several works.

As the flowers of the native species *R. foetida* is used traditionally as a disinfectant and antibacterial agent in dermatitis, skin disorders and especially in skin burn wounds, we prompted to evaluate the antibacterial activities of methanol, aqueous and chloroform extracts of the flowers against the standard *P. aeruginosa* strain and five pathogenic strains isolated from different patients suffered skin burn wounds. All the extracts had inhibition activity on the growth of all six studied strains among which the methanol and aqueous ones with dose of 125 mg/ml had the most significant effects by the same inhibition zone diameters to those of the standard drug. The MIC values were also determined as the same.

As the results, it is concluded that the flowers possess polar constituent which were extracted in aqueous and methanol solvents. It is confirmed by weak antibacterial activity observed by chloroform extract against all studied *P. aeruginosa* strains. This extract contained non/semi polar compounds which had low antimicrobial activity in this study.

Phytobiological evaluations of *Rosa* species have found that the most possess moderate to strong antibacterial activities against human pathogenic bacterial strains including *P. aeruginosa* (Tambekar and Mankar, 2006) and colorful petals including red, yellow and orange ones possess natural resistance to microbial attack. (Zhang *et al*., 2011). Literature survey revealed that these properties were maybe due to complex mixtures of polar organic compounds especially the flavonoids (Velioglu and Mazza, 1991) and lack of violet, yellow or red flowers in some *Rosa* species was due to the absence of these compounds (Katsumoto *et al*., 2007).

Table 2. Minimum inhibitory concentration (MIC) of *Rosa foetida* flowers extracts[a].

	MIC (mg/ml)		
	MTE	**ATE**	**CTE**
P. aeruginosa[b]	62.5	62.5	500
Sample 1[c]	62.5	125	250
Sample 2	31.25	31.25	500
Sample 3	31.25	31.25	500
Sample 4	62.5	31.25	500
Sample 5	62.5	62.5	1000

[a]All determinations were done in triplicate.
MTE=Methanol total extract; ATE=Aqueous total extract; CTE=Chloroform total extract
Imi./Cil.=Imipenem/Cilastatin
[b]Standard *P. aeruginosa* strains
[c]Samples 1-5 are 5 different clinical isolates of *P. aeruginosa*

4. Conclusion

Results show that *R. foetida* possess reliable antibacterial activities against the main cause of skin burn wounds infections, *P. aeruginosa* (the standard and pathogenic strains). This plant could be a good choice for further invivo biological investigations especially on the infectious skin burns and future phytochemical studies, identification and structure elucidation of the responsible compounds is suggested.

5. Acknowledgments

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