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IN VITRO AND IN SILICO ANTIBACTERIAL ACTIVITY OF PRANGOS *FERULACEA* (L.) Lindl AND *PRANGOS ULOPTERA* DC, AND THEIR MUTAGENICITY IN THE AMES TEST

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ABSTRACT

The present study was conducted to study antibacterial activity of different extracts of *Prangos ferulacea* and *Prangos uloptera*. The antibacterial activity was measured by disc diffusion and micro-broth dilution methods at different concentrations (250, 500, 750, 1000, 1500, 2000, 2500 and 3000µg/ml).The studied bacteria were *Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis, Serratia marcescens, Escherichia coli, Salmonella enterica*.The in sillico antibacterial activity of pinens and coumarins was performed by Autodock 4 software. The molecular docking between phytochemicals and six target proteins (DNAgyrase subunit B, pinicilin binding protein, D-alanin D-alanin syntase, dihydrofolate reductase, and dihydropteroate synthetase and isoleucyl-tRNA sinthetase) has been investigated. The mutagenicity of these extracts at different concentrations (500, 1000, 1500, 2000, 2500, 3000µg/ml) were also investigated on *Salmonella typhimurium* strain TA98. The results confirmed that all tested extracts have modest to weak antibacterial activity against studied bacteria without any mutagenicity effect.The root and seed extracts of both species respectively had highest and lowest antibacterial effects.The antibacterial activity of pinens of these plants was significantly more than coumarins. DNA gyrase subunit B and penicilin binding proteins (PBP) were the main targets of tested coumarins. DNA gyrase subunit B was also the main target of studied pinens.Our study found that *P.ferulacea* and *P.uloptera* displayed a great potential of antibacterial activity.

Keywords: Antibacterial activity, Molecular docking, Mutagenicity, *Prangos ferulacea, Prangos uloptera*

INTRODUCTION

In recent decades the activity of conventional antibiotics against pathogenic bacteria has decreased due to the expansion of bacterial resistance (**Adwan *et al.*., 2010**).However,for some decades there was an increasing interest to screen plants constituents which have antimicrobial activities (**Al-Akeel *et al.*., 2014**).These compounds act on the cell wall, protein synthesis, and DNA replication during the bacterial division cycle (**Alves *et al.*., 2014**).Some plants constituents have been reported to possess potent mutagenic effect (**Eren and Özata, 2012; Akintonwa *et al.*, 2009; Dos-Santos *et al.*., 2011**).So it is necessary to investigate the mutagenic effect of natural compounds. In silico methods have also been used to identify drug targets. Molecular docking is one of the best bioinformatics tools for drug design that used extensively by scientists.Molecular docking could determine the binding affinity of a ligand for a target protein.This technique has been used extensively for discovery of plant phytochemicals with antimicrobial activity (**Kroemer, 2007; Zoete *et al.*., 2009**).Therefore we became eager to assess different constituents of prangos genus with respect to their antibacterial activity.The genus *Prangos* that known Djashir in Iran belongs to the Smyrneae tribe from the Apiaceae family.These plants widely used in folk medicine to treat external bleeding, anti-worm, healing scars,digestive disorder and leukoplakia (**Rahimi *et al.*., 2014; Razavi *et al.*., 2011**).*Prangos ferulacea* and *Prangos uloptera* are two species of this genus that are distributed from east Europe to central and eastern Asia (**Razavi, 2012; Abolghasemi and Piryaei, 2012**).So far, many studies have been done on the medicinal properties of these plants including anti-bacterial and anti viral, anti fungal, ani cancer and anti diabetic activity (**Kafash-Farkhad *et al.*., 2013**).However the mechanism of antibacterial activity of Jashir constituents has been not investigated yet. Some coumarins and pinens have been reported to be present in the root extracts of *P.ferulacea* and *P.uloptera* (**Baser *et al.*., 1996; Sefidkon and Najafpour, 2001**).In the present study for first time antibacterial and mutagenesis effects of methanolic extracts from different parts of *P.ferulacea* and *P.uloptera* and probable antibacterial mechanism(s) of dominant reported phytochemicals consist of coumarins and pinens from these plants have been studied.

MATERIAL AND METHOD

Plant materials

Plant materials were collected from Kurdistan province in Iran, during the period between May and June 2014. The identities of the plants were confirmed by botanist at the Herbarium of the University of Isfahan, Iran.

Preparation of plant extracts

The samples were separated into flower, leaf, stem, seed and root parts.The plant parts were dried in shadow and powdered. The methanol extracts were prepared by macerating 100 g of powdered plant material in 300 ml of methanol, for 72 h and filtered using Whatman filter paper. The extraction was done thrice at room temperature.The collected solvents were concentrated by rotary vacuum evaporator (Stero glass, Italy) at 45°C and then dried using a freeze dryer (Zirbus, Germany). All extracts were dissolved in dimethyl sulphoxide (DMSO) and diluted to give concentrations of 250, 500, 1000, 1500, 2000, 2500, 3000 µg/ml (**Behbahani and Sadeghi-aliabadi, 2013**)

Bacterial strains

Bacterial strains were purchased from the Iranian Biological Resources Center and Bio Reliance Corporation (Rockville,MD,USA).The strains used in this study were *Streptococcus pyogenes* (ATCC:1447), *Staphylococcus aureus* (ATCC:25923), *Bacillus subtilis* (ATCC:6633), *Serratia marcescens* (ATCC:1111), *Escherichia coli* (ATCC:25922), *Salmonella enterica* (ATCC:14028) and *Salmonella typhimurium* TA98. The bacterial strains were grown on Nutrient Broth medium at 37˚C for 8 h.

In vitro antibacterial activity

Inhibition of bacterial growth by the plant extracts was evaluated by disk diffusion assay (**Dunkelberg, 1981**).The sterile Whatman filter papers No.1 were prepared and soaked separately in each of the extracts for 5 min.The filter papers

placed on the plate. After 24 h of incubation at 37 °C, the zone of inhibition around the each disc was measured. The MIC values were also determined by micro-dilution method **(Eloff, 1998).** Briefly, the plant extracts were serially diluted and added to a 96-well plate. 100 µl of an appropriate medium (*Mueller-Hinton Broth*) and 20 µl of the inoculums (containing about 6×10^4 colony) were dispensed into each well of a 96-well plate. After 24 hour incubation period at 30°C, plates were read at 620 nm. MIC value is defined as the lowest concentration which inhibits the growth or fewer than 3 discrete colonies were detected.On the other hand MBC value was defined as the lowest concentration of the plant extracts to kill the microorganisms.Plates were read in triplicate, and the average MIC value was recorded.

Docking study

Ten active compounds in *P.ferulacea* root has been selected from previous reports including: α-pinen, β–pinen, Gosferol, Terpinolen, Myrcene, P-cymene, δ-3-caren, Pesoralen, Osethole and Isoimperatorin.All these compounds were subjected to molecular docking studies for inhibition of antibiotic target proteins. In the present study 6 target proteins consist of DNAgyrase subunit B (DGSB with PDB entry 3TTZ), Pinicilin binding protein (PBP1a with PDB entry 3UDI), D-alaninD-alanin ligase (DdL with PDB entry 2ZDQ), dihydrofolate reductase (DHFR with PDB entry 3SRW),dihydropteroate synthetase (DHPS with PDB entry 2VEG) and isoleucyl-tRNA sinthetase (IARS with PDB entry 1JZQ) have been chosen for docking study.Also performed molecular docking between six standard antibiotics(Ciprofloxasin,Benzylpenicillin, Sulfadiazine, Trimethoprim, D-cycloserine, Mupirocin) with mention targets as the positive control.The 3D structure of mentioned compounds and standard antibiotics was obtained from Pub Chem (http://pubchem.ncbi.nlm.nih.gov) database as SDF format.The 3D structure of mentioned target proteins was also obtained from protein data bank as PDB format. Molecular docking was performed using Autodock4 (version 4.2) with the Lamarckian genetic algorithm.Docking parameters which selected for AutoDock4 runs were as follows:100 docking runs, population size of 200, random starting position and conformation, translation step ranges of 2A, mutation rate of 0.02, cross-over rate of 0.8, local search rate of 0.06 and 2.5 million energy evaluations. Docked conformations were clustered by a tolerance of 2 Å root mean square deviations (RMSD).

Mutagenicity assay

The Mutagenic effect of flower, leaf, stem, seed and roots of *P.ferulacea* and *P.uloptera* on S.typhimurium TA98 were studied by plate incorporation assay procedure at different concentrations (250,500,1000,1500,2000,2500 and 3000 µg/ml). Briefly, 100 µl of an overnight grown culture (10^7 CFU/ml) added in sterile screw capped tubes. Then, 2 ml of top agar and 100 µl of each extract were added to the tubes and the tubes were vortexed. Then the solution was poured onto a minimal glucose agar plate. Then plates were incubated at 37 °C and the number of His+ revertant colonies was counted after 48h.The positive and negative controls in this assay were sodium azide and 1-3% DMSO respectively. The minimal glucose agar plate contained 1.5% agar, 2.0% glucose, and 2.0% Vogel-Bonner medium.The top agar was consisted of 0.6% agar and 0.5%NaCl. The triplicate plating was used for each extract. The mutagenic effect of different compounds was estimated using the twofold rule according to the following formula. The substance is considered mutagen if the QM higher than 2**(Nosrati and Behbahani, 2015).**

QM =Number of His+ revertant colonies from tested extracts/colonies from negative control

RESULTS

In vitro antibacterial assay

The results of disc diffusion method showed that all plant extracts have antibacterial activities against the mentioned bacteria (Table 1-3). The antibacterial activities of different parts of studied plants were dose dependent. The results demonstrated that the antibacterial activity of *P.ferulacea* extracts is significantly more than *P.uloptera* extracts.The root extracts of both species were found to have the higher antibacterial effect compared to the flower, leaf, stem and seed extracts.The MIC and MBC values of all extracts were further estimated and are shown in Table 4. Among these bacterial strains, E.coli was susceptible to all plant extracts with MIC value ranges of ≤250 to 3000 µg/ml. Based on the results, the root extracts of both species showed highest antibacterial activity with MIC values ranges of ≤250 to 1000 µg/ml against tested strains. The antibacterial activity of the extracts was followed by flower, leaf, stem, and seed in both species.Based on these results and in comparison to MIC and MBC values of standard antibiotics ,MIC value at ≤250µg/ml was judged to show high antibacterial activity, while 500-1000 µg/ml were considered to show moderate and ≥3000 µg/ml weak antibacterial activity.

Table 1 Antibacterial *activity* of flower, leaf, stem seed and root extracts of *P.ferulacea* and *P.uloptera* against gram positive bacteria (Inhibition zone are mean±SD)organism

	Concentration(µg/ml)	Inhibition zone(mm)									
		P.ferulacea					*P.uloptera*				
		flower	leaf	stem	root	seed	flower	leaf	stem	root	seed
S. aureus	250	-	-	-	6±0.22	-	-	-	-	7±0.21	-
	500	7±0.11	-	-	7±0.20	-	7±0.13	-	-	8±0.28	-
	750	8±0.10	-	-	7±0.28	-	7±0.18	-	-	8±0.34	-
	1000	8±0.23	6±0.22	-	9±0.40	-	9±0.52	8±0.24	7±0.13	10±0.30	-
	1500	8±0.22	7±0.24	-	9±0.36	-	9±0.21	8±0.22	9±0.12	10±0.37	-
	2000	10±0.40	9±0.28	6±0.25	11±0.26	7±0.16	11±0.13	9±0.26	9±0.16	11±0.12	6±0.22
	2500	12±0.45	11±0.10	7±0.11	13±0.20	7±0.24	13±0.24	10±0.11	10±0.32	12±0.17	7±0.12
	3000	15±0.18	13±0.30	8±0.18	15±0.18	9±0.27	14±0.28	12±0.25	10±0.20	15±0.50	8±0.18
B. subtilis	250	6±0.17	-	-	7±0.24	-	6±0.22	6±0.28	-	6±0.11	-
	500	6±o.24	-	-	7±0.26	-	6±0.32	8±0.25	-	7±0.37	-
	750	8±0.16	6±0.16	-	8±0.32	-	9±0.36	8±0.52	-	8±0.31	-
	1000	8±0.44	8±0.18	-	10±0.30	-	9±0.35	9±0.23	-	10±0.32	-
	1500	9±0.11	8±0.32	8±0.33	11±0.21	6±0.25	10±0.30	11±0.12	6±0.22	11±0.37	7±0.32
	2000	10±0.23	9±0.52	8±0.28	13±0.25	7±0.32	12±0.38	12±0.26	7±0.43	13±0.30	7±0.23
	2500	12±0.26	11±0.26	9±0.18	14±0.15	8±0.34	13±0.42	15±0.20	8±0.27	13±0.39	9±0.42
	3000	14±0.36	13±0.32	12±0.13	16±0.12	11±0.37	14±0.44	15±0.28	9±0.41	16±0.62	9±0.40
S. pyogenes	250	-	-	-	6±0.27	-	-	6±0.26	-	6±0.32	-
	500	-	-	-	7±0.30	-	7±0.30	8±0.34	-	8±0.30	-
	750	7±0.11	-	-	7±0.27	-	7±0.28	10±0.30	6±0.50	8±0.12	-
	1000	8±0.15	-	-	9±0.24	-	9±0.11	10±0.25	7±0.27	9±0.25	-
	1500	8±0.25	7±0.13	-	10±0.20	-	10±0.19	11±0.22	7±0.32	11±0.42	-
	2000	9±0.34	8±0.37	-	10±0.24	-	10±0.36	12±0.25	9±0.20	11±0.36	-
	2500	11±0.30	10±0.40	7±0.24	11±0.36	7±0.11	12±0.23	12±0.23	10±0.17	12±0.27	-
	3000	12±0.27	10±0.12	7±0.26	13±0.12	8±0.22	12±0.26	13±0.26	11±0.20	14±0.20	7±0.21

Table 2 Antibacterial effects of flower, leaf, stem seed and root extracts of *P.ferulacea* and *P.uloptera* against gram negative bacteria (Inhibition zone are mean±SD)

organism	concentration	Inhibition zone(mm)									
		P.ferulacea					*P.uloptera*				
		flower	leaf	stem	root	seed	flower	leaf	stem	root	seed
S.marcescens	250	-	-	-	-	-	-	-	-	-	-
	500	6±0.21	-	-	7±0.28	-	6±0.21	6±0.44	-	7±0.25	-
	750	7±0.23	6±0.21	-	8±0.26	-	8±0.25	6±0.36	-	9±0.33	-
	1000	8±0.32	8±0.16	6±0.38	8±0.14	-	8±0.31	7±0.40	-	9±0.40	-
	1500	8±0.11	9±0.31	7±0.34	9±0.12	-	9±0.43	8±0.23	-	11±0.28	-
	2000	10±0.25	9±0.16	8±0.37	10±0.18	6±013	10±0.26	8±0.62	6±0.34	13±0.47	-
	2500	12±0.22	10±0.26	10±0.52	13±0.15	8±0.14	11±0.36	9±0.32	8±0.60	14±0.55	7±0.47
	3000	13±0.27	12±0.33	13	14±0.12	9±042	13±0.19	10±0.12	9±0.52	16±0.20	8±0.35
E.coli	250	6±0.11	-	-	7±0.14	-	-	-	-	7±0.37	-
	500	7±0.27	6±0.15	-	8±0.16	-	-	7±0.27	-	9±0.40	-
	750	8±0.25	7±0.32	-	8±0.25	-	7±0.15	8±0.33	-	10±0.36	-
	1000	9±0.33	8±0.41	6±0.21	9±0.28	-	8±0.32	9±0.40	-	11±0.13	-
	1500	10±0.42	8±0.20	7±0.26	11±0.32	-	10±0.52	10±0.37	-	12±0.27	-
	2000	10±0.28	9±0.26	8±0.32	12±0.30	-	11±0.42	10±0.44	-	12±0.37	6±0.60
	2500	11±0.20	10±0.40	9±0.23	13±0.26	-	12±0.17	11±0.50	7±0.21	14±0.26	7±0.16
	3000	13±0.21	12±0.13	10±0.25	14±0.29	-	13±0.47	12±0.47	8±023	16±0.30	8±0.46
S.enterica	250	-	-	-	6±0.33	-	7±0.32	-	-	7±0.34	-
	500	7±0.28	-	-	7±0.40	-	8±0.33	-	-	9±0.50	-
	750	8±0.42	6±0.15	-	9±0.52	-	9±0.27	6±0.35	-	9±0.25	-
	1000	9±0.17	7±0.16	6±0.41	9±0.34	-	10±0.26	7±0.10	6±0.36	11±0.32	-
	1500	11±0.20	8±0.23	7±0.35	10±0.33	6±0.37	11±0.54	8±0.13	7±0.18	13±0.41	-
	2000	11±0.61	10±0.24	9±0.22	11±0.22	8±0.39	12±0.47	8±0.19	9±0.25	14±0.55	-
	2500	12±0.42	11±0.28	11±0.26	13±0.17	9±017	13±0.19	9±0.32	9±0.10	14±0.39	7±0.18
	3000	13±0.21	13±0.30	11±0.42	15±0.33	10±0.28	14±0.38	11±0.24	9±0.26	16±0.10	8±0.43

Table 3 Inhibition zone (mm) and MIC (µg/ml) values of 5 standard antibiotics (Ampicillin, Penicillin, Gentamicin, Streptomycin, and Ciprofloxacin). Ciprofloxacin and Penicillin were effective antibiotics, IZ in this table is the abbreviation of Inhibition zone

Organism	Standard antibiotic									
	Ampicillin		Penicillin		Gentamicin		Streptomycin		Ciprofloxacin	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
S.aureus	19±0.21	250	23±0.63	125	19±0.18	500	20±0.42	125	22±0.36	125
B.subtilis	22±0.33	125	21±0.47	125	17±0.40	500	21±0.58	125	24±0.28	125
S.pyogenes	20±0.40	125	20±0.36	125	21±0.38	125	18±0.36	250	21±0.25	125
S.marcescens	18±0.51	250	18±0.28	125	19±0.15	500	15±0.40	750	20±0.33	125
E.coli	16±0.27	500	18±0.30	250	17±0.10	500	18±0.68	500	19±0.25	250
S.enterica	17±0.16	250	17±0.45	250	15±0.50	750	15±0.13	500	17±0.14	250

Table 4 MIC (µg/ml) and MBC (µg/ml) values of flower, leaf, stem seed and root extracts of *P.ferulacea* and *P.uloptera*

Microorganism												organ	plant
S.aureus		*S.pyogenes*		*B. subtilis*		*E.coli*		*S.enterica*		*S.marcescens*			
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC		
<1000	2000	<1500	2500	<750	1500	250	500	<750	1500	750	1500	leaf	*P.ferulacea*
<500	1000	750	2000	250	500	<500	1500	<500	1500	500	1000	flower	
2000	>3000	<2500	>3000	1500	>3000	1000	2000	<1000	2500	<1000	2500	stem	
250	500	250	500	<250	500	250	500	250	500	500	1000	root	
2000	>3000	<2500	>3000	1500	3000	>3000	>3000	1500	>3000	<2000	>3000	Seed	
<1000	2000	<250	500	250	500	250	500	750	1500	500	1000	leaf	*P.uloptera*
500	1500	500	1000	250	500	500	1500	<250	750	<500	1000	flower	
1000	1500	750	2000	1500	>3000	<2500	>3000	1000	2000	<2000	>3000	stem	
<250	750	250	500	250	500	250	500	250	500	<500	1000	root	
<2000	>3000	<2500	>3000	<1500	2500	<2000	>3000	<2500	>3000	2500	>3000	Seed	

Mutagenicity assay

Mutagenicity effect of methanol extracts of *P.ferulacea* and *P.uloptera* are shown in Table 4. These extracts were tested under comparable conditions at different concentrations (250, 500, 1000,1500,2000,2500, 3000 µg ml^{-1}).The results demonstrated that both plant extracts didn't have any mutagenicity effect and the QM values of all tested extracts were calculated less than 2. However, the numbers of TA98 His+ revertant colonies in plates treated with *P.ferulacea* extracts were higher than *P.uloptera* extracts.The plates treated with leaf extract of *P.ferulacea* was also showed highest revertant colonies with QM values of 1.68.

Table 5 QM values of extracts obtained from separate parts of *P.ferulacea* and *P.uloptera* tested on TA98: The highest numbers of TA98 His+ revertant colonies was observed in plates treated with3000 µg/ml of the leaf of the *P.ferulacea*

Mean Qm of different extracts ±SD						Part of plant	Plant species
3000	2500	2000	1500	1000	500		
1.44±0.09	1.39±0.1	1.35±0.04	1.29±0.04	1.25±0.03	1.23±0.06	**leaf**	
1.48±0.1	1.43±0.06	1.39±0.04	1.37±0.2	1.36±0.06	1.34±0.08	**flower**	
1.49±0.04	1.46±0.07	1.40±0.03	1.37±0.03	1.34±0.02	1.32±0.03	**root**	***P. uloptera***
1.40±0.02	1.38±0.02	1.31±0.04	1.29±0.04	1.27±0.09	1.25±0.07	**stem**	
1.38±0.03	1.35±0.06	1.28±0.09	1.26±0.1	1.23±0.06	1.20±0.09	**seed**	
1.68±0.1	1.66 ±0.1	1.46 ±0.07	1.40 ±0.09	1.26 ±0.1	1.13 ±0.1	**leaf**	
1.48±0.1	1.43 ±0.09	1.33 ±0.09	1.26 ±0.08	1.16 ±0.09	1.10 ±0.08	**flower**	
1.50±0.08	1.46 ±0.08	1.33 ±0.06	1.26 ±0.09	1.26 ±0.08	1.20 ±0.09	**root**	***P. ferulacea***
1.58±0.05	1.53 ±0.1	1.46 ±0.07	1.40 ±0.05	1.33 ±0.09	1.26 ±0.11	**stem**	
1.64±0.1	1.60 ±0.09	1.46 ±0.07	1.40 ±0.08	1.23 ±0.09	1.10 ±0.13	**seed**	

INSILICO ANALYSIS OF ANTIMICROBIAL ACTIVITY

The results of docking study of six antibiotic target proteins with the mentioned phytochemicals and standard antibiotics have been showed in Table 5. The results demonstrated that all studied compounds had appropriate interaction to antibiotic targets.Results also showed that all compounds had RMSD less than 2.Analysis of docking results showed that both DNA gyrase subunit B and penicillin binding protein (PBP) were the main targets for tested coumarins. DNA gyrase subunit B was also the main target for studied pinens. The other targets for pinens and coumarins were respectively D-alanin D-alanin ligase(Ddl), dihydrofolate reductase (DHFR), dihydropteroate synthetase (DHPR) and isoleucyl-tRNA sinthetase (IARS).The patterns of amino acids interaction of DNAgyrase subunit B with the mentioned compounds have been shown in figur1.Molecular docking revealed that 11 amino acids of DNA gyrase subunit B interact with the mentioned compounds.Among these amino acids, Asn54, Glu58, Asp81, Gly85, Ile86 and Thr173 appeared to interact with all compounds.Based on the results, α-pinen had highest efficacy for tested target proteins with ΔGb and ki values in (-7.5 to -11.2 kcal/mol) and (21.2-32.4µM) spectra respectively. Among coumarins osethole had the highest efficacy, it was followed by pesoralen, isoimpratorin, Terpinolen, Gosferol, δ-3-caren, P-cymene and Myrcene respectively.

Table 6 In silico analysis of the antibacterial activity of dominant compounds in the root of *P.ferulacea* with the highest antibacterial activity, in compared to standard antibiotics.The main target for pinens and coumarins studied were DNA gyrase subunit B and penicilin binding proteins (RMSD (Å)-Ki (µM)-ΔGb (kcal/mol)).

Compound	Target proteins																	
	DGSB			PBP1a			DHFR			DHPR			Ddl			IARS		
	RMSD	Ki	ΔGb	RMSD	Ki	ΔGb	RMSD	Ki	ΔGb	RMSD	Ki	ΔGb	RMSD	Ki	ΔGb	RMSD	Ki	ΔGb
α-pinen	0.19	21.2	-11.2	0.35	21.1	-10.3	0.40	29.5	-9.4	0.47	30.2	-8.5	0.33	31.4	-8.2	0.30	32.4	-7.5
β-pinen	0.27	34.4	-10.3	0.43	23.4	-10.1	0.34	36.3	-9.1	0.51	35.6	-8.4	0.40	37.2	-7.3	0.32	34.6	-7.2
Osethole	0.33	36.3	-9.6	0.48	37.3	-9.2	0.20	37.2	-8.7	0.28	38.9	-8.2	0.43	39.1	-7.1	0.38	41.7	-6.3
Pesoralen	0.46	41.7	-9.4	0.56	43.4	-8.6	0.76	43.2	-8.3	0.18	44.1	-7.9	0.57	44.5	-6.4	0.46	45.3	-5.4
Isoimpratorin	0.52	44.3	-8.1	0.68	46.2	-8.2	0.81	47.8	-7.6	0.43	48.2	-6.4	0.63	47.3	-6.3	0.60	48.2	-5.1
Terpinolen	0.61	48.2	-7.4	0.90	51.2	-7.1	0.93	51.8	-6.4	0.76	52.3	-6.1	0.74	54.2	-5.5	0.71	57.2	-4.8
Gosferole	0.74	51.3	-6.2	0.93	54.9	-6.3	1.05	53.9	-6.3	0.87	54.6	-5.3	0.86	56.8	-5.3	0.75	58.3	-4.3
δ-3-caren	0.80	55.7	-5.3	0.98	57.4	-5.2	1.09	57.4	-5.9	0.94	58.9	-4.4	0.90	59.2	-4.2	0.93	63.1	-3.8
P-cymene	0.91	57.3	-5.2	1.06	59.3	-5.1	1.12	59.2	-5.5	1.14	61.2	-4.2	0.95	62.9	-3.7	0.97	65.2	-3.2
Myrcene	1.06	68.9	-4.7	1.11	71.6	-4.6	1.27	71.6	-5.3	1.20	73.4	-3.7	1.21	75.6	-3.2	1.12	80.2	-31
Ciprofloxasin	0.23	16.2	-13.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-2.8
Benzylpenicillin	-	-	-	0.17	14.7	-12.5	-	-	-	-	-	-	-	-	-	-	-	-
Sulfadiazine	-	-	-	-	-	-	-	-	-	0.14	19.3	-11.8	-	-	-	-	-	-
Trimethoprim	-	-	-	-	-	-	0.18	20.5	-11.3	-	-	-	-	-	-	-	-	-
D-cycloserine	-	-	-	-	-	-	-	-	-	-	-	-	0.07	15.7	-11.2	-	-	-
Mupirocin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	19.2	-12.3

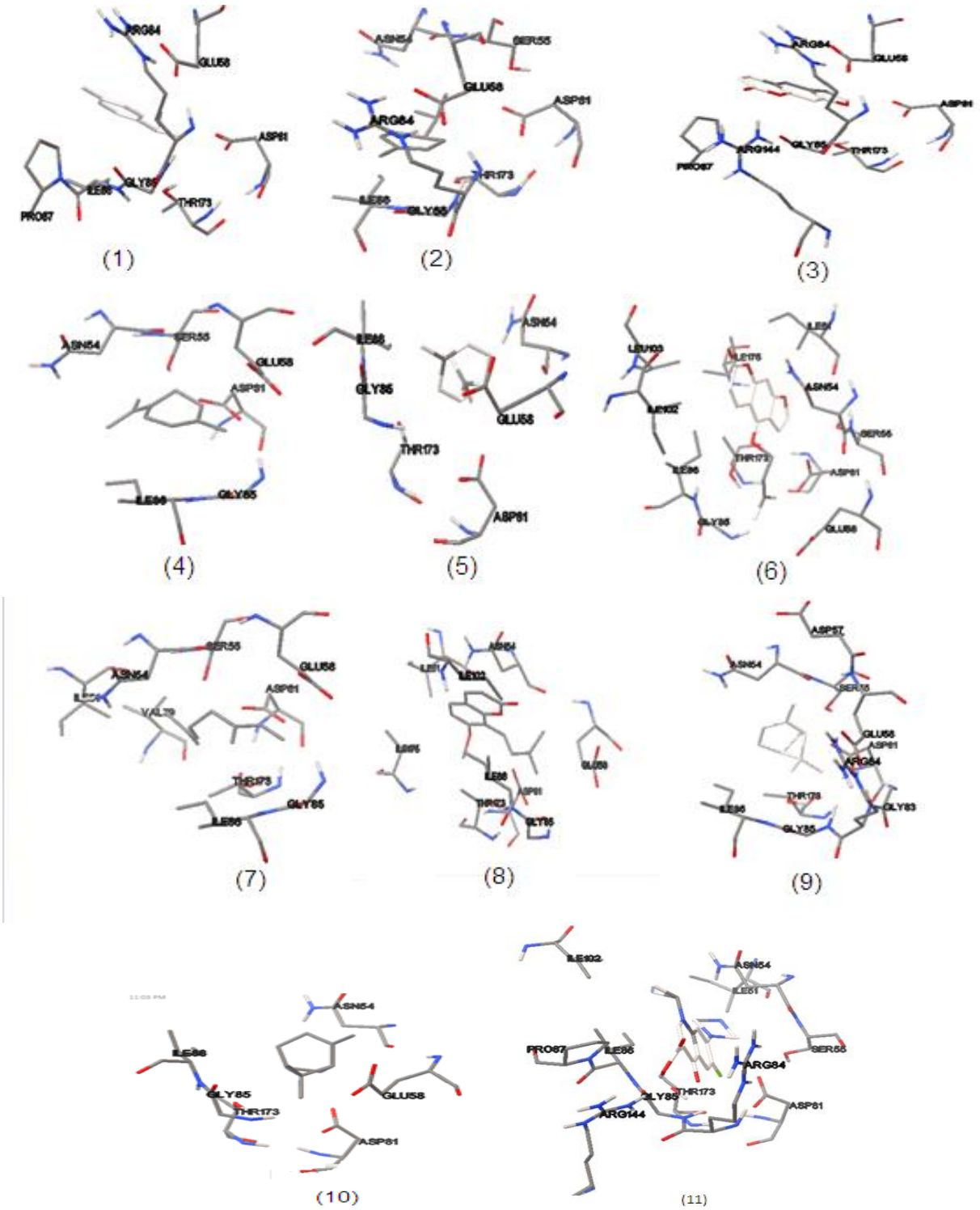

Figure 1 Molecular docking study between 10 chemical compounds and ciprofloxasin with DNAgyrase subunit B us mainly target for all compounds.1: p-cymen and DNAgyrase subunit B, 2:gosferol ,3:psoralen ,4:terpinolen , 5:δ-3-caren, 6:isoimpratorin, 7:myrecen ,8:osthole ,9:α-pinen ,10:β-pinen ,11:ciprofloxasin

DISCUSSION

The results of the present study demonstrated that methanol extracts of different parts of *P.ferulacea* and *P.uloptera* have modest to weak antibacterial activity against *S.aureus, B.subtilis, S.pyogenes, S.marcescens, E.coli and S.enterica.*The results also showed that antibacterial activity of *P.ferulacea* was significantly more than *P.uloptera*. Several researches have been done on antibacterial activity of aerial parts of *Prangos* species against Pathogenic bacteria such as: *B.cereus, B.subtilis, M.luteus and S.*aureus (**Durmaz *et al*, 2006; Massumi *et al.*, 2007**).Previous studies have also reported that α-pinens and coumarins are dominant constituents in essential oils of different parts of *P.ferulacea* and have significant antibacterial activity (**Baser *et al.*, 1996**).Some studies demonstrated that pinens and coumarins are main phytochemicals in roots of prangos species (**Sefidkon *et al.*, 1998; Sajjadi *et al.*, 2011**).In the present study, the root extracts of prangos species have the most antibacterial effect compared to leaf, stem and root extracts.It may be due to the accumulation of coumarins in root extracts of these two species in comparison with stem and leaf extract. Previous studies demonstrated that coumarins of *Prangos pabularia* and *P.uloptera* have significant anti bacterial and anti fungal activity (**Razavi *et al.*, 2008; Tada *et al.*, 2002**). Razavi *et al* **(2010)** also showed that dichloromethane (DCM) extract and different coumarins derivative from *P.uloptera* root collected from Ardebil province of Iran has high antibacterial properties against Staphylococcus aureus and Bacillus subtilis, whereas our results showed that methanolic extract from *P.uloptera* root exhibited modest antibacterial activity on mentioned strains. So the location of plant growth and extract type can affect the antibacterial properties of this plant. Although the antibacterial potential of *P.ferulacea* and

P.uloptera has been demonstrated in several studies but the antibacterial mechanisms of the active constituents of these plants have not well defined. Molecular docking is one of best bioinformatic tools for drug design and determination the mechanism of antimicrobial agents (**Kumalo *et al*, 2015**).In the present study, the docking between 10 known compounds from *P.ferulacea* and bacterial proteins has been done.The results showed that the antibacterial activity of pinens of this plant was significantly more than coumarins. DNAgyrase subunit B was the main target proteins of pinens.The α-pinen was more effective than β-pinen with lowest and highest Ki and ΔGb values respectively.Pinens (α-pinene & β-pinene) are hydrocarbon compounds that, well known chemicals having antimicrobial activity (**Dorman and Deans, 2000**). The previous study confirmed that enantiomers of α-pinene, β-pinene have antibacterial activity (**Da-Silva *et al.*, 2012**).Several results also have been presented the effectiveness of pinenes against molds and pathogen yeasts and bacteria (**Moreira *et al.*, 2007; Leite *et al.*, 2007**).DNA gyrase subunit B and penicilin binding proteins (PBP) were the main target proteins of tested coumarins.Among the tested coumarins,osethole was most effective which followed by Pesoralen,Isoimpratorin, Terpinolen,Gosferol, δ-3-caren, P-cymene, and Myrcene respectively.Coumarins are secondary metabolites that occur naturally in several plant families and possess important pharmacological properties, including inhibition of oxidative stress and use as the fragrance in food and cosmetic products (**Borges *et al.*, 2014**).The antibacterial activity of some coumarins such as osthol,*imperatorin*, isoimpinellin.arbutin,baicalin and naringin have been reported previously (**Widelski *et al.*, 2009; Ng *et al.*, 1996**).The mechanisms of antibacterial effect of these compounds have not well defined and the present study is the first investigation of the mechanism of antibacterial activity of coumarins and pinens.Despite the therapeutic advantages possessed by medicinal plants but some constituents of medicinal plants have been shown to be potentially mutagenic,toxic, teratogenic and carcinogenic (**Gadano *et al.*, 2006; Akinboro and Bakare, 2007**).Therefore, these plants should be evaluated to better understand their safety.The Ames test is commonly used with plant extracts for possible gene mutation determination (**Mortelmans *et al.*, 2000**).In this study the Ames test was carried out using methanolic extracts of flower, leaf, stem, root and seeds from *P.ferulacea* and *P.uloptera* obtained results have not shown any mutagenicity to TA98 for both studied plants. Some studies demonstrated that different pinens and coumarins have not any mutagenicity effect. Gomes-Carneiro *et al* confirmed that beta-myrcene, alpha-terpinene, (+,-) alpha-pinene have not mutagenic effect in the Ames test **(Gomes-Carneiro *et al*, 2005)**. Also, another study *revealed* that different coumarin derivatives have not any mutagenic effect on peripheral blood, liver, bone marrow and testicular cells of Swiss albino mice by the comet assay **(de Souza Marques *et al*, 2015)**. On the other hand some study confirmed the mutagenic and co-mutagenic effect of coumarins while as yet mutagenic effect induced by pinens not reported. In this regards some studies reported that Coumarin modulates the mutagenic effects of other chemicals such as aflatoxin B1 and heterocyclic amines **(Sanyal *et al*, 1997; Goeger *et al*, 1999; Kim *et al*, 2005).**

CONCLUSION

The results of this study confirmed that the methanolic extracts of different parts of *P.ferulacea* and *P.uloptera* especially roots extracts present potential antibacterial activity without any mutagenic effect.In silico analysis of antibacterial effect also showed that pinens and coumarins of mentioned plants play key roles in appearance antibacterial activity with inhibition of DNA gyrase subunit B and penicilin binding proteins respectively.Based on these results *P.ferulacea* and *P.uloptera* are good candidates for discovering bioactive compounds in the form of the antibacterial agents and may serve for the development of new pharmaceuticals.

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