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Original Article

Antimicrobial Activities of Three Medicinal Plants and Investigation of Flavonoids of *Tripleurospermum disciforme*

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Abstract

Rosa damascena, *Tripleurospermum disciforme* and *Securigera securidaca* were used as disinfectant agents and for treatment of some disease in folk medicine of Iran. The antimicrobial effects of different fractions of seeds extract of *S. securidaca*, petals extract of *R. damascena* and aerial parts extract of *T. disciforme* were examined against some gram positive, gram negative and fungi by cup plate diffusion method. The petroleum ether and chloroform fractions of *S. securidaca* showed antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while its methanol fraction had no antibacterial effects. *R. damascena* petals extract demonstrated antibacterial activities against *Bacillus cereus*, *Staphylococcus epidermidis, S. aureus* and *Pseudomonas aeruginosa*. *T. disciforme* aerial parts extract exhibited antimicrobial effects only against *S. aureus* and *S. epidermidis*. None of the fractions had any antifungal activities. Therefore, present study confirmed utility of these plants as disinfectant agents. Six flavonoids were isolated from *T. disciforme*: Luteolin, Quercetin-7-O-glucoside, Kaempferol, Kaempferol-7-O-glucoside, Apigenin and Apigenin-7-O-glucoside. The flavonoids and the antimicrobial activity of *T. disciforme* are reported for the first time.

Keywords: *Securigera securidaca*; *Rosa damascena*; *Tripleurospermum disciforme*; antimicrobial activity; Flavonoids.

Introduction

There are growing interests in use of plants as natural antimicrobial agents because they do not induce antibiotic resistance which is common in the synthetic antibiotics. *Securigera securidaca* (L.) Deg. & Dorf. (Fabaceae) is one of three species of this genus which grows in Iran (1). *Rosa damascena* Mill. (Rosaceae) is a small plant with aromatic flower which appears in spring (2). Nowadays, *R. damascena* is the principle species cultivated for Rose water and attar in central part of Iran (Kashan), India and Bulgaria (3). *Tripleurospermum disciforme* (C.A. Mey) Schultz Bip., a genus of Asteraceae, is one of the native plants of Europe and western Asia (4). It was grown in many parts of Iran. These three plants had many traditional and folk uses in Iran but there were a few reports about

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antimicrobial effects of them.

The people in the south of Iran used oral administration of the seeds of *S. securidaca* for hypoglycemic effects. *S. securidaca* extract significantly reduced glucose level in diabetic animals by a mechanism different from sulfonylurea agents (5, 6).

R. damascena has some benefits such as cooling, soothing, astringent and anti inflammatory effects (7). Its extract and essential oil showed antioxidant and antibacterial properties (8-10). Rose water is a natural healer for various skin problems and a skin care in folk medicine of Iran. It is an important ingredient in many body creams and cosmetics in the world due to its pleasant fragrance and useful properties.

T. disciforme was used as anti inflammatory, anti spasmodic, anti septic, carminative and as a hair color (11, 12).

The objective of present research is to evaluate antimicrobial effects of *S. securidaca*, *R. damascena* and *T. disciforme* extracts and isolation and identification of compounds of *T. disciforme*.

Experimental

Plant material

The seeds of *S. securidaca*, petals of *R. damascena* and top flowered of *T. disciforme* were collected in September, May and July 2011 around the Fars, Gilan and Tehran Provinces of Iran, respectively. The plants were dried in shade and powdered. A voucher specimen of each plant is deposited at Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Preparation of extracts

The powder of dried seeds of *S. securidaca*, petals of *R. damascena* and top flowered of *T. disciforme* (400 g of each sample) were macerated separately with 80% methanol at room temperature in a percolator, then solvents concentrated in vacuum to give gummy residue (crude extract). The crude extract of *S. securidaca* was re-extracted with petroleum ether, chloroform and methanol to achieve different fractions. The concentrated extracts and fractions were kept at 4 ºC prior to antimicrobial tests.

Microorganisms and media

The various organisms were used as standard strains in this study, include *Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC12229, *Bacillus subtilis* ATCC6633 and *Bacillus cereus* ATCC1274 as Gram positive bacteria; *Pseudomonas aeruginosa* ATCC9027, *Escherichia coli* ATCC8739 and *Klebsiella pneumoniae* ATCC1003 as Gram negative bacteria; *Candida albicans* ATCC1023 and *Aspergillus niger* ATCC16404 as fungi, which were obtained from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. Soybean Casein Digest Agar (Merck, Germany) and Saburouad Dextrose Agar (Merck, Germany) were used as medium for the growth of bacterial and fungal strains, respectively.

Antimicrobial assay

The antibacterial and antifungal activity of the different extracts and fractions of the plants were studied by cup plate diffusion method as described by Warnock DW (13). Each organism was separately suspended in normal saline solution which was equal to 10^8 CFU/mL. For preparing base plates, 25 mL of cooled media was poured in to the sterile Petri dishes and inoculated with one of the microorganisms by spreading microbial suspension over the plate with a sterile cotton swab. Then in each plate, holes of 7 mm in diameter were made at equal distances using sterile cork borer. Different concentrations of fractions (100, 50, 25, 12.5, 6.25, 3.125 and 1.562 mg/mL) were prepared and DMSO (dimethyl sulfoxide) with 1% w/v concentration was used as a solvent. 100 µL of each extracts and fractions were added to each hole on the medium. The plates containing bacteria and fungi were incubated at 35 °C for 24 h and 25 °C for 48 h, respectively. The diameter of zone of inhibition was measured in millimeters after incubation as an indication of activity and compared with the solvent as negative control. Gentamycin and Nystatin were used as positive control. All the tests were repeated in triplicate.

Elucidation of compounds of T. disciforme

Since there was few reports about phytochemical investigation of *T. disciforme*

extract, it was selected for isolation and purification of compounds. Crude extract (313.61 g) from 1.5 Kg of *T. disciforme* was fractionated with petrol ether (PE) and chloroform (CH) yield 50.11 and 13.5 g respectively. Remaining gummy residue which was soluble in methanol called methanol fraction (ME; 250 g).

ME fraction (5 g) was applied to reverse phase silicagel column chromatography (2.5×13.5 cm) and eluted with gradient mobile phase H_2O-MeOH (80:20 → 0: 100, V/V) to afford 5 subfractions. M_3 subfraction (564 mg) was selected for chromatography on Sephadex LH-20 column (2.1×67 cm) eluted with MeOH. Compounds 1 (5.5 mg), 2 (4.3 mg) and 3 (12 mg) were isolated and purified. M_4 subfraction (435 mg) subjected to SEC on Sephadex LH-20 column (2.1×67 cm) and MeOH: EtOAC (2:1) was used as solvent to obtain compound 4 (6.5 mg), 5 (3.8 mg) and 6 (9.5 mg). For further purification all compounds were applied to a Sephadex-LH20 CC (1.2×55 cm) eluted with methanol separately.

Spectral data of isolated compounds

Luteolin 1: UV λ_{max} nm MeOH: 345.5, 308, 284, 260sh; + AlCl3: 422, 307sh, 286; + AlCl3/HCl: 384, 350, 318sh, 307, 286; + NaOAC : 390, 307, 289 ; + NaOAC/H3BO3 : 430sh, 367, 370, 289; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.46 (1H, *dd*, *J*=8.4, 2.0 Hz, H-6′), 7.00 (1H, *d*, *J*=8.4 Hz, H-5′), 7.52 (1H, *d*, *J*=2.0 Hz, H-2′), 6.57 (1H, *s*, H-3), 6.54 (1H, *d*, *J*=2 Hz, H-8), 6.25 (1H, *d*, *J*=2 Hz, H-6): ^{13}C NMR(DMSO-d_6): δ 182.9 (C-4), 165.2 (C-7), 165.4 (C-2), 162.3 (C-5), 158.5 (C-9), 150.1 (C-4′), 146.1 (C-3′), 122.8 (C-6′), 121.4 (C-1′), 115.9 (C-5′), 113.2 (C-2′), 102.9 (C-3), 102.9 (C-10), 99.2 (C-6), 94.1 (C-8).

Quercetin-7-O-glucoside 2: UV λ_{max} nm MeOH: 369, 270sh, 250; + AlCl3: 441, 340sh, 270; + AlCl3/HCl: 430, 368sh, 292sh, 266; + NaOMe: 423, 270, 267sh, 246; + NaOAC : 256, 386 ; + NaOAC/H3BO3 : 254, 420; EIMS: m/z %: 302[M-glucose]$^+$(100), 285 [M-OH]$^+$(12), 273 [M-COH]$^+$(8), 193 [M-B]$^+$(12), 153[A1+H]$^+$(27), 137[B2]$^+$(32), 105[B1-COH]$^+$(34); ^{1}H NMR (400MHz, DMSO-d_6): δ 7.56 (1H, *dd*, *J*=8.4, 2.4 Hz, H-6′), 7.74 (1H, *d*, *J*=2.4 Hz, H-2′), 6.91 (1H, *d*, *J*=8.4 Hz, H-5′), 6.77 (1H, *d*, *J*=2 Hz, H-8), 6.42 (1H, *d*, *J*=2 Hz, H-6), 5.08 (1H, *d*, *J*=7.6 Hz, H-1″), 3.5- 4.5 (5H, *m*, H-2″-6″); ^{13}C NMR(DMSO-d_6): δ 174.9 (C-4), 161.6 (C-7), 159.3 (C-5), 157.2 (C-9), 146.9 (C-4′), 146.5 (C-2), 144.0 (C-3′), 141.5 (C-3), 135.0 (C-1′), 120.7 (C-6′), 118.4 (C-2′), 114.5 (C-5′), 103.9 (C-10), 100.0 (C-1″), 98.8 (C-6), 98.0 (C-8), 76.1 (C-5″), 75.4 (C-3″), 72.1 (C-2″), 68.5 (C-4″), 59.6 (C-6″).

Kaempferol-7-O-glucoside 3: UV λ_{max} nm MeOH: 367, 298sh, 267, 255; + AlCl3: 425, 345, 293sh, 266; + AlCl3/HCl: 425, 345, 293sh, 265; + NaOMe: dec; + NaOAC : 325sh, 380, 325sh, 266 ; + NaOAC/H3BO3 : 364, 256; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.93 (2H, *d*, *J*=8.0 Hz, H-2′,6′), 6.88 (2H, *d*, *J*=8.0 Hz, H-3′,5′), 6.46 (1H, *d*, *J*=2.0 Hz, H-8), 6.20 (1H, *d*, *J*=2.0 Hz, H-6).

Kaempferol 4: UV λ_{max} nm MeOH: 365, 320sh, 295sh, 266, 255; + AlCl3: 425, 330sh, 300sh, 272; + AlCl3/HCl: 425, 330sh, 300sh, 272;+ NaOMe: dec; + NaOAC : 390, 302, 268 ; + NaOAC/H3BO3 : 370, 320sh, 295sh, 265; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.15 (2H, *d*, *J*=8.9 Hz, H-2′,6′), 6.99 (2H, *d*, *J*=8.9 Hz, H-3′,5′), 6.46 (1H, *d*, *J*=1.9 Hz, H-8), 6.27 (1H, *d*, *J*=1.9 Hz, H-6); ^{13}C-NMR(DMSO-d_6): δ 176.19 (C-4), 163.8 (C-7), 160.7 (C-5), 160.44 (C-4′), 156.75 (C-9), `149.0 (C-2), 136.19 (C-3), 129.52 (C-2′, 6′), 131.72 (C-1′), 115.45 (C-3′, 5′), 102.56 (C-10), 98.05 (C-6), 93.5 (C-8).

Apigenin 5: UV λ_{max} nm MeOH: 336, 284, 267.5; + AlCl3: 388, 345sh, 301, 276, 219; + AlCl3/HCl: 387, 343, 300, 276, 217; + NaOMe: 394, 318, 274, 214; + NaOAC : 359, 305, 272; + NaOAC/H3BO3 : 336, 268; EIMS: m/z %: 270[M-glucose]$^+$(100), 152[A1](25), 121[B2](36), 118[B1](25); ^{1}H NMR (400MHz, DMSO-d_6): δ 7.76 (2H, *d*, *J*=8.4 Hz, H-2′,6′), 6.95 (2H, *d*, *J*=8.4 Hz, H-3′,5′), 6.51 (1H, *s*, H-3), 6.48 (1H, *s*, H-8), 6.24 (1H, *s*, H-6).

Apigenin-7-O-glucoside 6: UV λ_{max} nm MeOH: 332, 268; + AlCl3: 385, 347, 299, 276; + AlCl3/HCl: 382, 341, 299, 277; + NaOMe: 386, 300, 279, 265; + NaOAC : 397sh, 341, 267; + NaOAC/H3BO3 : 336, 266, 256sh; EIMS: m/z %: 270[M-glucose]$^+$(100), 152[A1](18), 120[B2-H](25), 117[B1-H](18); ^{1}H NMR (400MHz, DMSO-d_6): δ 7.84 (2H, *d*, *J*=8.0 Hz, H-2′,6′), 6.94 (2H, *d*, *J*=8.0 Hz, H-3′,5′), 6.72 (1H, *s*, H-3), 6.66 (1H, *s*, H-8), 6.44 (1H, *s*, H-3),

Table 1. Antimicrobial activity of *S. securidaca* seed different fractions by cup-plate method.

Sample	Concentration mg/mL	Inhibition zone diameter(mm)					
		SA	PA	EC	KP	BS	CA
Petroleum ether Fraction	100	10	12	-	-	-	-
	50	8	10	-	-	-	-
	25	7.5	9.5	-	-	-	-
	12.5	-	-	-	-	-	-
Chloroform Fraction	100	12	-	-	-	-	-
	50	10	-	-	-	-	-
	25	9.5	-	-	-	-	-
	12.5	-	-	-	-	-	-
Methanol Fraction	100	-	-	-	-	-	-

SA: *S. aureus,* PA: *P. aeruginosa,* EC: *E. coli,* KP: *K. pneumoniae,* BS: *B. subtilis,* CA: *C. albicans,* -: no effect

5.00 (1H, *d*, *J*=7.2 Hz, H-1″), 3.4- 4.5 (5H, *m*, H-2″-6″).

Results and Discussion

The antimicrobial effects of different fractions of *S. securidaca* seeds was demonstrated in Table 1. The Petroleum ether fraction only inhibited the growth of *S. aureus* and *P. aeruginosa* with inhibition zone diameter of 7.5 -12 mm. The chloroform fraction showed inhibitory effect only against *S. aureus* with inhibition zone of 9.5 -12 mm diameter. The methanol fraction showed no antimicrobial activity. The largest zones of inhibition were observed for petroleum ether fraction against *P. aeruginosa* and chloroform fraction against *S. aureus* (each 100 µg/mL). All fractions exhibited no antifungal activities.

The phytochemical analysis of *S. securidaca* showed existence of flavonoids, coumarins and cardiac glycosides (14-16). Some flavonoids of *S. securidaca* have been shown potent cytotoxicity by MTT assay against three Human cancer cell lines: colon carcinoma (HT-29), breast ductal carcinoma (T47D) and colorectal adenocarcinoma (Caco-2) (17).

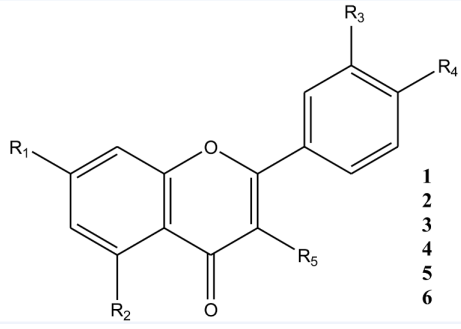
There were reports for antimicrobial effects of some cardenolides (18, 19), and the antibacterial activity of *S. securidaca* may be due to existence of this class of compounds.

Rosa damascena extract showed good antibacterial activities against *B. cereus*, *S. aureus*, *and S. epidermidis* as Gram positive bacteria and *P. aeruginosa* as Gram negative bacteria with MICs (Minimum Inhibitory Concentration) 70, 140, 560 and 140 µg/mL, respectively. It was inactive against other microorganisms with MICs of >1000 µg/mL. The inhibition zone diameter of *R. damascena* extract against *S. aureus* and *S. epidermidis* is more than Gentamycin as positive control (5 µg/mL) (Table 2).

A previous investigation showed the MIC of butanol extract of *R. damascena* receptacles against *Salmonella typhimurium* and *Bacillus cereus* were 62.5 and 250 µg/mL, respectively.

Aqueous extract of *R. damascena* receptacles were inhibited *Candida albicans* and Methicillin-resistant *S. aureus* with MIC of 125 and 500 µg/mL (20). It is obvious that antimicrobial potential of crude extract of *R. damascena* against *B. cereus* was more than that of butanol extract. Another study demonstrated fresh and spent flower extracts of *R. damascena* showed the strongest effects against *Salmonella enteritidis* and *Mycobacterium smegmatis*. Both extracts were not effective against *E. coli* (9).

Tripleurospermum disciforme extract showed antimicrobial effects only against *S. aureus* and *S. epidermidis* with MICs 112 and 224 µg/mL, respectively. It was inactive against the other microorganisms (Table 2). Another study reported the essential oil of *T. disciforme* was effective on *Staphylococcus subtilis* and *Bacillus cereus* with MICs 4 µL/mL and on *Citrobacter amalonaticus* with MIC 22 µL/mL (21). Methanol extract of *T.*



1 R_1, R_2, R_3, R_4=OH; R_5=H
2 R_1=O-glucoside; R_2, R_3, R_4, R_5=OH
3 R_1=O-glucoside; R_2, R_4, R_5=OH; R_3=H
4 R_1, R_2, R_4, R_5=OH; R_3=H
5 R_1, R_2, R_4=OH; R_3, R_5=H
6 R_1=O-glucoside; R_2, R_4=OH; R_3, R_5=H

Figure 1. Chemical structure of isolated compounds of *Tripleurospermum disciforme.*

disciforme were not exhibited antiproliferative activity by using the MTT assay against: A549, human lung adenocarcinoma; MCF7, human breast adenocarcinoma; HepG2, hepatocellular carcinoma; HT-29, human colon carcinoma and one normal cell line MDBK, bovine kidney (22).

There was only one report about phytochemical investigation on flowers extract of *T. disciforme* which demonstrated isolation of a new dioxaspiran derivative (23). In our study, six flavonoids were isolated from *T. disciforme*: Luteolin, Quercetin-7-O-glucoside, Kaempferol, Kaempferol-7-O-glucoside, Apigenin and Apigenin-7-O-glucoside. The isolated compounds were identified using different spectroscopic methods (Figure 1).

Flavonoids act as antimicrobial agents in different ways including direct antibacterial activity, synergism with antibiotics and suppression virulence (24). Many researchers investigated the antibacterial activity of flavonoids (25), for example, it can be mentioned the antibacterial activities against *Propionibacterium acnes* by kaempferol and quercetin (26), inhibitory effects of apigenin

Table 2. Antimicrobial activity of *R. damascena* and *T. disciforme* extract by cup-plate method.

	Concentration mg/mL	Inhibition zone diameter (mm)							
		BC	**BS**	**SA**	**SE**	**EC**	**PA**	**AN**	**CA**
R. damascena Extract	64	14	-	24	18	-	12	-	-
	32	13.4	-	23	16	-	11	-	-
	16	13.3	-	18	15	-	10.3	-	-
	8	13.2	-	16	13	-	10	-	-
	4	13	-	13	-	-	9.6	-	-
	2	11	-	10	-	-	9	-	-
	1	10	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
T. disciforme Extract	64	-	-	14	12	-	-	-	-
	32	-	-	10.2	10	-	-	-	-
	16	-	-	10	-	-	-	-	-
	8	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
Gentamycin	5	25	18	18	12	18	19	-	-
Nystatin	50	-	-	-	-	-	-	23	25

BC: *B. cereus*, BS: *B. subtilis,* SA: *S. aureus,* SE: *S. epidermidis*, EC: *E. coli,* PA: *P. aeruginosa,* AN: *A. niger,* CA: *C. albicans,* -: no effect

against *S. typhi*, *Proteus mirabilis* and *P. aeruginosa* (27) and selective toxicity of apigenin and luteolin against *S. aureus* including the MRSA and methicillin-sensitive *S. aureus* strains (28, 29).

Conclusion

In conclusion, *Rosa damascena* and *Tripleurospermum disciforme* have shown antimicrobial effects against *Staphylococcus* strains. These results confirmed the folklore consumption of distilled water of *R. damascena* as tonic and face cleanser and fume of *T. disciforme* as tonic and disinfectant for treatment of acne. Because of antibiotic resistance of *S. aureus*, these two herbs can be used in health and beauty products for treatment of skin disorders especially acne in teenagers.

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References

(1) Rechinger KH. *Flora Iranica*. Akademische Druck-u. Verlagasanstalt, Graz-Austria (1984) 157: 343.
(2) Mozaffarian V. *A Dictionary of Iranian Plant Names*. Farhang Moaser Publication, Iran (1995) 462.
(3) Groom N. *The Perfume Handbook*. Blackie Academic Professional, UK (1997) 292.
(4) Bakhtiarian A, Ejtemaimehr Sh, Strobl S, Pournaghash-Tehrani S, Partoazar A, Ghamami G and Yassa N. Inhibition of carrageenan-induced edema by *Tripleurospennum disciforme* extract in rats. *Pak. J. Biol. Sci*. (2007) 10: 2237-2240.
(5) Hosseinzade H, Ramezani M and Danaei AR. Antihyperglycaemic effect and acute toxicity of *Securigera securidaca* L. seed extract in mice. *Phytother. Res.* (2002) 16: 745-747.
(6) Porchezhian E and Ansari SH. Effect of *Securigera securidaca* on blood glucose levels of normal and Alloxan-induced diabetic rats. *Pharm. Biol.* (2001) 39: 62-64.
(7) Ody Mnimh P. *The Herbs Society's Complete Medicinal Herbal*. Dorling Kindersley, London (2001) 995: 162-168.
(8) Yassa N, Masoomi F, Rohani Rankouhi SE and Hadjiakhoondi A. Chemical composition and antioxidant activity of the extract and essential oil of *Rosa damascena* from Iran, Population of Guilan. *Daru* (2009) 17: 175-180.
(9) Ozkan G, Sagdic O, Baydar NG and Baydar H. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Int.* (2004) 10: 277-281.
(10) Shahriari S, Yasa N, Mohamrnadirad A, Khorasani A and Abdollahi M. *In-vivo* antioxidant potentials of *Rosa damascene* petal extract from Gilan, Iran, comparable to α-tocopherol. *Int. J. Pharmacol.* (2007) 3: 187-190.
(11) Ghassemi-Dehkordi N, Amin GhR, Rahiminejad R, Salehi MH and Jafarpisheh A. Morphological and phytochemical study of *Tripleurospermum disciforme* (C.A. Mey) Schultz Bip. *Pajouhesh & Sazandegi* (2003) 58: 42-46.
(12) Grainger B and Wichtl M. *Herbal Drugs and phytopharmaceuticals*. Medpharm, Stuttgart Scientific Publishers (2004) 322.
(13) Warnock DW. Methods with antifungal drugs. In: Evans EG and Richrdson MD (eds.) *Medical Mycology: A Practical Approach*. IRL Press, Oxford University Press (1991) 179-200.
(14) Komissarenko AN and Kovalev VN. Hydroxycoumarins and flavones of *Securigera securidaca*. *Chem. Nat. Compd.* (1987) 23: 252.
(15) Zamula VV, Maksyutina NP and Kolesnikov DG. Cardenolide of *Securigera securidaca* L. *Khim. Prir. Soedin.* (1965) 1: 153-156.
(16) Behbahani M, Shanehsazzadeh M, Shokoohinia Y and Soltani M. Evaluation of anti-herpetic activity of methanol seed extract and fractions of *Securigera securidaca In-vitro*. *J. Antivir. Antiretrovir.* (2013) 5: 72-76.
(17) Tofighi Z, Asgharian P, Goodarzi S, Hadjiakhoondi A, Ostad SN and Yassa N. Potent cytotoxic flavonoids from Iranian Securigera securidaca. *Med. Chem. Res.* (2014) 23: 1718-1724.
(18) Abbassy MA, Kadous EA, Abd-Allah EAM and Marei GIK. Isolation and Identification of cardenolide compounds of *Gomphocarpus sinaicus* and their fungicidal activity against soil borne and post harvest fungi. *J. Life Sci.* (2012) 6: 985-994.
(19) Akhtar N, Malik A, Noorali S and Urooj Kazmit Sh. Proceragenin, an antibacterial cardenolide from Calotropisprocera. *Phytochem.* (1992) 31: 2821-2824.
(20) Wamidh, Talib H and Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of jordanian plants used in traditional medicine. *Molecules* (2010) 15: 1811-1824.
(21) Chehregani A, Mohsenzadeh F, Mirazi N, Hajisadeghian S and Baghali Z. Chemical composition and antibacterial activity of essential oils of *Tripleurospermum disciforme* in three developmental stages. *Pharm. Biol.* (2010) 48: 1280-1284.
(22) Behzad H, Pirani A and Mosaddegh M. Cytotoxic activity of some medicinal plants from hamedan

district of iran. *Iran. J. Pharm. Res.* (2014) 13: 199-205.
(23) Souri E, Sarkhail P, Kaymanesh P, Amini M and Farsam H. Antioxidant activity of extract and a new isolated dioxaspiran derivative of *Tripleurospermum disciforme*. *Pharm. Biol.* (2005) 43: 620-623.
(24) Cushnie TPT and Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. *Int. J. Antimicrob. Agents* (2011) 38: 99-107.
(25) Cushnie TPT and Lamb AJ. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* (2005) 26: 343-356.
(26) Lim YH, Kim IH and Seo JJ. *In-vitro* activity of Kaempferol isolated from the *Impatiens balsamina* alone and in combination with Erythromycin or Clindamycin against *Propionibacterium acnes*. *J. Microbiol.* (2007) 45: 473-477.
(27) Basile A, Giordano S, Loapez-Saez JAA and Castaldo Cobianchi R. Antibacterial activity of pure flavonoids isolated from mosses. *Phytochem.* (1999) 52: 1479-1482.
(28) Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H and Higuti T. Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol.* (2000) 72: 483-488.
(29) Cushnie TPT, Hamilton VES and Lamb AJ. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. *Microbiol. Res.* (2003) 158: 281-289.

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