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In Vitro Biological Activities of the Components from *Silene wallichiana*

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ABSTRACT

Methanol, butanol, chloroform and water extracts and individually six phytoecdysteroids (viticosterone E, 20-hydroxyecdysone-22-benzoate, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, 20-hydroxyecdysone and integristerone A) isolated from a *Silene wallichiana* Klotsch. plant were evaluated for their antibacterial and antiproliferative properties. The methanolic extract inhibited the growth of *Acinetobacter* sp., *Enterococcus faecalis*, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Proteus rettgeri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at an MIC of 2.5 mg/ml, while *Escherichia coli* and *Klebsiella pneumoniae* were inhibited at an MIC of 1.25 mg/ml. The proliferation of cancer cells was potently inhibited by the chloroform extract (IC_{50} 28.53 ± 1.98 µg/ml in HeLa, 26.34 ± 2.34 µg/ml in HepG-2 cells). Aqueous and butanol extracts exhibited good antioxidant activity with IC_{50} values of 24.83 ± 2.82 and 46.32 ± 3.73 µg/ml.

Keywords: antibacterial, antioxidant, cytotoxic activity, phytoecdysteroids, plant extract

INTRODUCTION

The *Silene* genus (*Caryophyllaceae*) comprises more than 700 different species and its taxonomy appears to be very complex (Grauter 1995). In Central Asia, 84 species plants of the *Silene* genus grow (Bondarenko 1971). Chemical investigations of this genus have led to the isolation of ecdysteroids (Zibareva 1999), triterpene saponins (Glensk *et al*. 1999; Lacaille-Dubois *et al*. 1999), flavonoids (Zemtsova and Dzhumyrko 1976), polysaccharides (Ovodova *et al*. 2000), amino acids (Terrab *et al*. 2007), terpenes, nitrogen-containing compounds (Dotterl *et al*. 2007), organic acids and microelements (Eshmirzayeva *et al*. 2005; Arnetoli *et al*. 2008). *Silene* spp. Plants are characterized by both a large number of ecdysteroid-containing species and by the highest diversity of ecdysteroid derivatives, such as acetates, benzoates, glucosides, galactosides, xylosides, sulfates, etc. (Lafont *et al*. 2002). Given the complexity of ecdysteroid cocktails existing in many *Silene* species, it has been proposed that ecdysteroids have a chemotaxonomic value in this genus (Zibareva *et al*. 2009). The qualitative and quantitative composition of ecdysteroid cocktails depends considerably on the plant species, but possibly also on soil climatic conditions and on the developmental stage of the plant. Detailed investigations on biological and pharmacological activities of ecdysteroids isolated from the genus *Silene* have been carried out and anabolic (Syrov 1984), adaptogenic, tonic (Syrov and Kurmukov 1977), cardiotonic (Kurmukov and Yermishina 1991), antioxidant (Kuzmenko *et al*. 1997), antifeedant and insect growth-inhibition (Kubo and Klocke 1983) properties of phytoecdysteroids have been reported.

However, the *in vitro* biological actions of *Silene* plants are not yet fully studied and in order to enlarge our knowledge on the biological and pharmacological properties of this family of chemical compounds, we decided to carry out investigations in order to evaluate possible *in vitro* biological activities of phytoecdysteroids and extracts of *Silene wallichiana* Klotzsch. Previous studies (Saatov *et al*. 1987; Mamadalieva *et al*. 2000) reported that *S. wallichiana* is an ecdysteroid-rich resource and the following phytoecdysteroids were isolated from this plant: viticosterone E, 20-hydroxyecdysone-22-benzoate, 2-deoxyecdysone-22-benzoate, viticosterone E-22-benzoate, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, 20-hydroxyecdysone, 3-benzoate-2-deoxy-20-hydroxyecdysone, 22-benzoate-2-deoxy-20-hydroxyecdysone, integristerone A and 25-acetate-2-deoxy-20-hydroxyecdysone.

In the present study we report on the antimicrobial, antioxidant and cytotoxic activities of the aerial parts of several extracts (methanol, chloroform, butanol and water) from *S. wallichiana* in comparison with six major isolated phytoecdysteroids, namely viticosterone E (**1**), 20-hydroxyecdysone-22-benzoate (**2**), 2-deoxy-20-hydroxyecdysone (**3**), 2-deoxyecdysone (**4**), 20-hydroxyecdysone (**5**), and integristerone A (**6**) (**Fig. 1**).

MATERIALS AND METHODS

Chemicals and reagents

Cell culture media, supplements, dimethyl sulfoxide (DMSO), quercetin, MTT, and doxorubicin (≥ 98%) were purchased from Gibco, Invitrogen (Italy) and Sigma (Milan, Italy).

HPLC analysis

Authentical phytoecdysteroids **1-6** were obtained from the Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan. The purity of the compounds **1-6** were > 95%, as determined by HPLC using a high performance liquid chromatograph LC-10ATvp connected to a UV-VIS detector SPD-10Avp (Shimadzu Co, Kyoto, Japan). Samples were diluted to 1 mg/ml, filtered through a 0.22 µm filter and 20 µl was injected into a column (Nucleosil 100-5 C18, 250 mm × 4 mm in size (Macherey-Nagel GmbH & Co, KG)). Elution was carried out by mobile phase A

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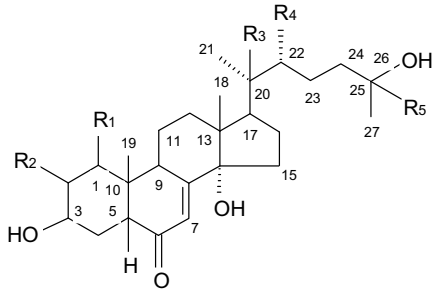

Phytoecdysteroid	R_1	R_2	R_3	R_4	R_5
1	H	OH	OH	OH	$COOCH_3$
2	H	OH	OH	$COOC_6H_5$	OH
3	H	H	OH	OH	OH
4	H	H	H	OH	OH
5	H	OH	OH	OH	OH
6	OH	OH	OH	OH	OH

Fig. 1 The chemical structure of the phytoecdysteroids studied *in vitro* tests.

(water) and solvent B (acetonitrile) and the gradient profile was as follows: from 0% B to 5% B in 8 min, from 5% B to 85% B from 8-30 min, from 95% B to 100% B% from 30-35 min and at 100% B% until 40 min. Flow rate was 1 ml/min and detection was at 247 nm and 200 nm (Abdukadyrov *et al*. 2005).

Plant material

S. wallichiana was obtained from the Syrkhandaryo region of Uzbekistan in June 2008 and identified by Dr A.M. Nigmatullaev and deposited in the Laboratory of Herbal Plants, Institute of the Chemistry of Plant Substance, Tashkent (voucher specimen No. 2006231).

Extract preparation for bioassays

S. wallichina (syn. *S. vulgaris*, *Oberna wallichiana* Klotzsch) is a hairless, perennial forb that grows up to 91 cm tall from a woody rootstock. Stems are branched from the base, smooth, and swollen at the nodes. Leaves are sessile, smooth, ovate or lanceolate, glaucous, pale green, 31 to 82 mm long, and 12 to 31 mm wide. Flowers are 12 mm in diameter and are borne in terminal clusters of 5 to 30. They are composed of 5 united and deeply notched petals, 10 stamens, and 3 styles. Calyxes are initially slender but develop into greatly inflated, often purplish, papery, sac-like structures that surround the bulbous fruits. Fruits open at the toothed tops of the calyxes. Seeds are numerous, small, and grayish (Douglas and MacKinnon 1998; Royer and Dickinson 1999; Whitson *et al*. 2000; Klein 2011).

The aerial parts of *S. wallichiana* were dried at room temperature and reduced to a coarse powder. After grinding, 10 g of plant material was extracted separately with 50 ml of different solvents (methanol, chloroform and water) for 24 h in the dark and at room temperature. Solvents were evaporated under reduced pressure to provide 0.71 g of the methanolic (7.0% of air-dried weight of the plant), 0.41 g of the chloroform (4.1%) and 0.29 g of the aqueous (2.9%) extracts. The butanol extract was prepared as reported by Saatov *et al*. (1987). The obtained residues were than used for *in vitro* screening of antibacterial, antioxidant or antiproliferative tests.

Isolation of phytoecdysteroids

Air-dried material (2.5 kg) of *S. wallichiana* was exhaustively extracted with ethanol (10 L). The extract was concentrated and diluted with water. The resulting precipitate was removed. The ethanol was evaporated. The aqueous solution was treated first with $CHCl_3$ and then with ethyl acetate. The solvents were evaporated under vacuum. A solvent system of $CHCl_3$ - CH_3OH [15:1 (A), 9:1 (B), 4:1 (C)] was used for TLC and column chromatography.

The ethyl acetate extract (15 g) was chromatographed on an Al_2O_3 column (0.5 kg) with elution by system A and 125 mg of compound **1** was isolated. The yield of this phytoecdysteroid was 0.005%. Yield was calculated based on the air-dried weight of plant material, $C_{29}H_{46}O_8$, mp 194-196°C, using acetone (Saatov *et al*. 1987).

Further elution of the column with system A gave **2** (1 g, 0.04%), $C_{34}H_{48}O_8$, mp 203-205°C (MeOH-H_2O). Elution of the column with system B gave compounds **3** (2 g, 0.08%, $C_{27}H_{44}O_6$, mp 254-256°C (EtOH-H_2O)) and **4** (4 g, 0.16%, $C_{27}H_{44}O_5$, mp 234-235°C (EtOH-H_2O)) (Mamadalieva *et al*. 2000). Elution of the column with system C gave **5** (2 g, 0.08%), $C_{27}H_{44}O_7$, mp 241-242°C (methanol-acetone). Subsequent elution of the column with the same system gave **6** (425 mg, 0.017%), $C_{27}H_{44}O_8$, mp 246-248°C (ethyl acetate-methanol) (Mamadalieva *et al*. 2000). The isolated phytoecdysteroids were used for biological assays.

Antibacterial activity

Microorganisms. The methanol and butanol extracts were individually tested against the following clinical microorganisms: *Klebsiella oxytoca* 6653, *K. pneumoniae* 40602, *K. aerogenes* NCTC8172, *Citrobacter freundii* 82073, *Staphylococcus aureus* MRSA16, *Enterococcus faecalis* NCTC775, *Proteus rettgeri* NCIMB9570, *Pseudomonas aeruginosa* NCTC6749, *Escherichia coli* NCTC9001, *Enterobacter hormaechei* T2, *Acinetobacter* sp. T132, *Pantoea agglomerans* T26, and *Bacillus cereus* T80. Reference strains and clinical isolates were obtained from the Department of Microbiology, Manchester Metropolitan University, UK, the National Culture Type Collection (NCTC), UK, and from the culture collection of National University of Uzbekistan.

Strains were maintained at 37°C on Columbia agar slants supplemented with 5% horse blood (v/v) (Oxoid, Basingstoke, UK). The antibacterial activity of the extracts and individual phytoecdysteroids was carried out by the disc diffusion test (Kim *et al*. 1995). Microorganisms were grown overnight at 30°C in Mueller-Hinton Broth (Oxoid), supplemented with 5% horse blood and 100 µl of suspension containing 10^6 CFU/ml of bacteria spread on the surface of agar plates. Plant extracts (5 mg/ml) and compounds (1 mg/ml) were first dissolved in dimethylsulfoxide (DMSO). Sterile filter discs (6 mm in diameter) were impregnated with 15 µl of each extract (0.6-5 mg/ml) and of each individual phytoecdysteroid concentrations (0.06-1 mg/ml) and placed on the surface of inoculated plates. The plates were incubated at 37°C for 24 h. The assessment of antibacterial activity was based on the measurement of inhibition zones formed around the discs. Five discs per plate were used and each test was run in triplicate. The diameter of the zone was measured and recorded. Ampicillin (10 µg/ml) and tetracyclin (30 µg/ml) were used as positive controls.

Cytotoxic activity

1. Cell cultures

HeLa (cervical cancer) and HepG-2 (hepatic cancer) cell lines were supplied by the Laboratory of Plant Cytology and Biotechnology, Tuscia University, Italy. Cancer cells were maintained in DMEM complete media (L-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen, Italy) in addition to 10 mM of non-essential amino acids (Invitrogen, Italy). Cells were grown at 37°C in a humidified atmosphere of 5% CO_2. All experiments were performed with cells in the logarithmic growth phase.

2. MTT assay

Sensitivity to drugs was determined in triplicate using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay (Mosmann 1983). The extracts and phytoecdysteroids were dissolved in DMSO and diluted in the medium at final concentrations ranging from 0.977 to 500 µg/ml for extracts and from 0.977 to 500 µM for phytoecdysteroids, in 96-well plates (Greiner Labortechnik). Wells containing the solvent and wells without the solvent were included in the experiment. Cells (2×10^4 cells/well of exponentially growing cells of each individual HeLa and HepG-2 cell lines) were seeded in a 96-well plate (Grei-

Table 1 Minimum inhibitory concentrations (MIC) of the extracts and phytoecdysteroids of *S. wallichiana* against different pathogens using the disc diffusion test.

Bacterial strain	MIC (mg/ml)											
	Methanol extract	Butanol extract	Chloroform extract	Water extract	Viticosterone E (**1**)	20-Hydroxyecdysone-22-benzoate (**2**)	2-Deoxy-20-ydroxyecdysone (**3**)	2-Deoxyecdysone (**4**)	20-Hydroxyecdysone (**5**)	Integristerone A (**6**)	Ampicillin	Tetracyclin
Acinetobacter sp. T132	2.5	2.5	-	-	-	-	-	-	-	-	2.5	0.25
B. cereus T80	-	-	-	-	-	0.25	-	-	-	-	0.25	0.25
C. freundii 82073	-	-	2.5	-	-	-	-	-	-	-	2.5	0.5
E. coli NCTC9001	1.25	2.5	2.5	2.5	0.25	0.5	0.5	0.5	0.5	-	1.25	1.25
E. faecalis NCTC775	2.5	-	-	-	-	-	-	-	-	-	0.5	1.25
E. hormaechei T2	-	-	-	-	-	-	-	-	-	-	-	0.5
K. oxytoca 6653	2.5	-	-	-	-	-	-	-	-	-	-	1.25
K. pneumoniae 40602	1.25	2.5	-	-	0.25	0.5	-	-	0.5	-	-	1.25
K. aerogenes NCTC8172	-	-	-	-	0.25	0.25	-	-	-	0.5	-	1.25
P. agglomerans T26	2.5	2.5	-	-	-	-	-	-	-	-	1.25	1.25
P. rettgeri NCIMB9570	2.5	1.25	-	-	0.5	-	0.5	0.5	0.25	-	2.5	2.5
P. aeruginosa NCTC6749	2.5	2.5	1.25	1.25	-	-	-	-	-	-	-	1.25
S. aureus MRSA16	2.5	-	-	-	0.5	-	-	-	-	-	2.5	1.25

ner Labortechnik), cultivated for 24 h then incubated with various concentrations of tested samples at 37°C for 24 h and then with 0.5 mg/ml MTT for 4 h. The formazan crystals that formed were dissolved in 100 µl DMSO. The absorbance was detected at 595 nm with a TECAN Sunrise Reader. The cell viability rate (%) of three independent experiments was calculated by the following formula (Zeytinoglu *et al*. 2008):

Cell viability rate (%) = ((OD of treated cells − OD of media (blank) / (OD of control cells − OD of media (blank)) × 100 %

DPPH radical-scavenging (antioxidant) activity

The antioxidant and radical scavenging activities of the isolated compounds and extracts were evaluated according to Brand-Williams *et al*. (1995) using diphenyl picryl hydrazyl (DPPH). Equal volumes of sample solutions containing 0.02-10 mg/ml of sample and 0.2 mM methanolic solution of DPPH were pipetted into 96-well plates. The absorbance was measured against a blank at 517 nm using a TECAN Sunrise Reader after incubation in the dark for 30 min at room temperature and compared with DPPH control after background subtraction. Quercetin was used as a positive control. The percent inhibition was calculated from three different experiments using the following equation:

$$RSA\ (\%) = [(Abs_{517\ control} - Abs_{517\ sample})/ Abs_{517\ control}] \times 100$$

where RSA = radical scavenging activity; Abs_{517} = absorption at 517 nm; control = non-reduced DPPH.

Statistical analysis

All experiments were carried out three times unless mentioned in the procedure. Continuous variables were presented as mean ± SD. IC_{50} values were calculated using Student's *t*-test followed by Dunn's *post-hoc* multiple comparison test when the significance value was < 0.05 using the same significance level.

RESULTS AND DISCUSSION

Antibacterial activity

Powder extracts (methanol, chloroform, butanol and water) and individual phytoecdysteroids **1**-**6** of *S. wallichiana* were tested in order to evaluate their antibacterial activity. The plant extracts were tested at various concentrations ranging from 0.6 – 5 mg/ml and the evaluated MIC values are reported in **Table 1**. The *Acinetobacter sp*, *Enterococcus faecalis*, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Proteus rettgeri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were inhibited by the methanol extract of *S. wallichiana* at MIC = 2.5 mg/ml, while *Escherichia coli* and *Klebsiella pneumoniae* was inhibited at MIC = 1.25 mg/ml. The butanol extract of *S. wallichiana* showed activity against the pathogenic bacterium *Acinetobacter* sp., *E. coli*, *K. pneumoniae*, *P. agglomerans*, *P. aeruginosa* (MIC = 2.5 mg/ml), and *P. rettgeri* (MIC =1.25 mg/ml), although with weaker action respect to the methanol extract. The chloroform extract had minimum activity against all bacterial strains and only inhibited *Citrobacter freundii*, *E. coli* (MIC = 2.5 mg/ml) and *P. aeruginosa* (MIC = 1.25 mg/ml). The *S. wallichiana* aqueous extract also showed low antimicrobial activity against two strains only, *E. coli* and *P. aeruginosa* (MIC = 2.5 and 1.25 mg/ml, respectively).

Pure phytoecdysteroids exhibited very low activity against the bacteria. Among the tested compounds, phytoecdysteroid **1** showed *in vitro* activity against *E. coli*, *K. pneumoniae*, *K. aerogenes*, *P. rettgeri* (MIC = 0.25 mg/ml), and *S. aureus* and *P. rettgeri* (MIC = 0.5 mg/ml). Compound **2** showed antibacterial activity against *B. cereus*, *K. aerogenes* (MIC = 0.25 mg/ml) and *E. coli* and *K. pneumoniae* (MIC = 0.5 mg/ml). Phytoecdysteroids **3** and **4** showed low antimicrobial activity against *E. coli* and *P. rettgeri* (MIC = 0.5 mg/ml). Phytoecdysteroid **6** only inhibited the growth of *K. aerogenes* NCTC8172. In contrast, phytoecdysteroid **5** promoted the growth of *P. rettgeri* (MIC = 0.25 mg/ml), *E. coli* and *K. pneumoniae* (MIC = 0.5 mg/ml) (**Table 1**). From these results we observed that phytoecdysteroids show weak antibacterial activity compared medicinal plant extracts tested. In addition, this finding was consistent with the previous results of Ahmad *et al*. (1996) and Shirshova *et al*. (2006), who claimed that most likely such compounds are not the major molecules responsible for the antibacterial activity of the plant extracts. Ahmad *et al*. (1996) and Shirshova *et al*. (2006) reported that some natural phytoecdysteroids, including 20-hydroxyecdysone, inokosterone, and ecdysone, did not exhibit antimicrobial activity with respect to most standard test microbe cultures. However, introduction of the acetyl group into the 20-hydroxyecdysone molecule significantly increased the antibacterial activity with respect to microbes inducing inflammatory and purulent processes (Shirshova *et al*. 2006). In our case, besides ecdysteroids, extracts exhibiting antibacterial activity were related to the chemical

Table 2 Antiproliferative activities of ecdysteroids and extracts isolated from *S. wallichiana* on HeLa and HepG-2 cell lines. The data are represented as IC_{50} values (mean ± SD).

Sample	IC_{50} of extracts (μg/ml) and compounds (μM)	
	HeLa	HepG-2
Extracts		
Methanol	70.81 ± 1.73 de	74.56 ± 2.92 de
Butanol	86.08 ± 2.30 d	80.21 ± 7.12 d
Chloroform	28.53 ± 1.98 e	26.34 ± 2.34 e
Water	103.72 ± 1.84 c	98.47 ± 3.92 cd
Phytoecdysteroids		
Viticosterone E (**1**)	98.27 ± 6.25 cd	88.96 ± 5.77 d
20-Hydroxyecdysone-22-benzoate (**2**)	127.97 ± 1.34 b	106.76 ± 7.81 c
2-Deoxy-20-hydroxyecdysone (**3**)	174.88 ± 9.10 a	195.61 ± 7.26 a
2-Deoxyecdysone (**4**)	171.25 ± 8.13 a	184.48 ± 9.49 ab
20-Hydroxyecdysone (**5**)	175.02 ± 6.34 a	130.26 ± 2.87 bc
Integristerone A (**6**)	158.75 ± 4.52 ab	142.67 ± 5.87 b
Control		
Doxorubicin (μg/ml)	1.07 ± 0.11	0.39 ± 0.04
Doxorubicin (μM/ml)	1.84 ± 0.19	0.67 ± 0.07

nature of the solvents which play a key role in the extraction of different chemical compounds from the powder of *S. wallichiana*.

Compared with antibiotics (positive control), the disk diffusion method showed that all plant extracts had antimicrobial properties. The aqueous extract showed weak antimicrobial activity against the tested microorganisms. The antimicrobial activity of the chloroform extract of *S. wallichiana* against bacterial strains confirmed the results reported by us and by other authors (Kucukboyaci *et al*. 2010; Mamadalieva *et al*. 2010). Both chloroform and aqueous extracts were more active only for *P. aeruginosa* than methanol and butanol extracts. The methanol and butanol extracts of *S. wallichiana* contains flavonoids and triterpene glycosides (unpublished data), and flavonoids might be responsible for part of the antimicrobial activity, as well as several other classes of alcohol-soluble plant molecules (Cowan 1999). The methanol extract some of *Silene* species contains a wider range of components, especially lipids, essential oils, flavonoids, steroids, carbohydrates, microelements, proteins and amino acids (Lacaille-Dubois *et al*. 1999; Jürgens 2004; Eshmirzayeva *et al*. 2005; Dotterl *et al*. 2007; Terrab *et al*. 2007). Its higher activity than that of the butanol extract can be explained by the fact that some polar compounds present in the methanol extract (which are removed during the preparation of the butanol extract) display a maintain effect towards the antimicrobial components. As a consequence, this raises a question about the evaluation of the activity of crude methanolic plant extracts, as used classically during screening tests, which might underscore their actual antimicrobial potency.

Antiproliferative activity

The antiproliferative activity of the extracts, six individual isolated phytoecdysteroids and doxorubicin (as the positive control) were tested against HeLa and HepG-2 cell lines. The IC_{50} values are shown in **Table 2**. Experimental results revealed that the chloroform extract of *S. wallichiana* possesses significant antiproliferative activity which potently inhibited cell growth in all cells tested (IC_{50} 28.53 ± 1.98 μg/ml in HeLa, 26.34 ± 2.34 μg/ml in HepG-2 cells). The proliferation of malignant cells was more strongly inhibited by methanol extract (IC_{50} 70.81 ± 1.73 μg/ml in HeLa, 74.56 ± 2.92 μg/ml in HepG-2 cells) than by butanol (86.08 ± 2.30 μg/ml in HeLa, 80.21 ± 7.12 μg/ml in HepG-2 cells). In this case water extract showed weak antiproliferative activities against HeLa and HepG-2 cell lines, with IC_{50} values of 103.72 ± 1.84 and 98.47 ± 3.92 μg/ml.

Viticosterone E (**1**) was most active in HeLa and HepG-2 cells (IC_{50} = 98.27 ± 6.25 and 88.96 ± 5.77 μM, respectively), while other phytoecdysteroids **2**-**6** were weakly active against human cancer cells (IC_{50} > 106.76 ± 7.81 μM). Compared to doxorubicin, the extracts and phytoecdysteroids showed moderate antiproliferative activity.

In the experiments phytoecdysteroids displayed mild antiproliferative activity against both cancer cell lines while the chloroform extract was more active. Some phytoecdysteroids isolated from *Ajuga* species showed antitumour activities in a mouse-skin model *in vivo* in a two-stage carcinogenesis trial, using 7,12-dimethylbenz[a]anthracene as initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as the promoter (Takasaki *et al.* 1999). Also, El-Mofti (1987, 1994) reported that ecdysone was able to induce neoplastic lesions in toads and mice; other researchers reported that ecdysteroid-containing extract of *Silene viridiflora* exerted antitumour activity *in vivo* in mice (Zibareva 2003). However, Lagova and Valueva (1981) reported that 20-hydroxyecdysone was mainly ineffective in preventing tumour growth in mice, but it stimulated the growth of mammary gland carcinomas. Most likely, since ecdysteroids structurally resemble sex hormones, they may bind to steroid hormone receptors in mammals and stimulate the growth of hormone-dependent tumours.

Flavonoids, triterpene saponins, ecdysteroids, and polysaccharides are common constituents in the genus *Silene*. In particular, saponins may be responsible for the antiproliferative effects of the extracts. The saponins jenisseensosides C and D from *Silene fortunei* stimulated the proliferation of Jurkat tumor cells at low concentrations (10^{-3} – 10^{-1} μM); at high concentrations (10 μM) they were cytotoxic and apparently induced apoptosis (Gaidi *et al*. 2002). Our results suggest that the chemical contents of chloroform extract and the mechanism of these substances as they affect tumor cells needs to be further elucidated.

Antioxidant activity

The antioxidant and radical scavenging activities of the isolated compounds, extracts and quercetin (as a positive control) are summarized in **Table 3**. The activity of the positive control was 3.37 μg/ml. Maximum scavenging activity of DPPH was observed with the aqueous extract at IC_{50} = 24.83 μg/ml, followed by the activity of the butanol, methanol, and chloroform extracts with IC_{50} = 46.32, 131.55, and 153.31 μg/ml, respectively. All ecdysteroids had weak DPPH radical scavenging activity with IC_{50} values greater than 100 μM. The effectiveness of phytoecdysteroids as DPPH radical scavengers ranged in the following ascending order: 2-deoxyecdysone (137.68 μg/mL) > 20-hydroxyecdysone (144.75 μg/mL) > 2-deoxy-20-hydroxyecdysone (157.29 μg/mL) > 20-hydroxyecdysone-22-benzoate (168.33 μg/mL) > integristerone A (178.98 μg/mL) > viticosterone E (181.23 μg/mL).

Our results coincide with what was found in another study (Miliauskas *et al*. 2005). In their study identified

Table 3 Antioxidant activity of pure isolated phytoecdysteroids and extracts of *S. wallichiana* using the DPPH* radical scavenging assay. The data are represented as IC_{50} values (mean ± SD).

Sample	IC_{50} (µg/ml)
Extracts	
Methanol extract	131.55 ± 9.43 d
Butanol extract	46.32 ± 3.73 e
Chloroform extract	153.31 ± 12.65 bc
Water extract	24.83 ± 2.82 e
Phytoecdysteroids	
Viticosterone E (**1**)	181.23 ± 15.34 a
20-Hydroxyecdysone-22-benzoate (**2**)	168.33 ± 11.02 ab
2-Deoxy-20-hydroxyecdysone (**3**)	157.29 ± 16.72 b
2-Deoxyecdysone (**4**)	137.68 ± 3.56 cd
20-Hydroxyecdysone **(5)**	144.75 ± 11.53 c
Integristerone A (**6**)	178.98 ± 11.51 a
Control	
Quercetin (positive control)	3.37 ± 0.77

radical scavenging compounds in extracts of *Rhaponticum carthamoides* which tested against DPPH radical showed weak radical scavenging activity. Our TLC investigations showed that polar extracts of *S. wallichiana* such as methanol, water and butanol contained a high amount of phytoecdysteroids (unpublished data). And this seemed that presence of phytoecdysteroids in these extracts might reduce their antioxidant activity. It is known that the presence of the ortho arrangement of two hydroxyl groups on the aromatic ring and 2,3-double bond in conjugation with 4-oxo function is essential for the antiradical activity of flavonoids. More effective is the ortho-arrangement of hydroxyl groups on the aromatic ring B (quercetin) (Harborne and Williams 2000). Ecdysteroids are polyhydroxylated steroids that contain a 7,8-double bond and a 6-oxo function. This explanation seems to be a more forceful argument since the structure of ecdysteroid molecules is unlikely to exert an antioxidant effect compared to common antioxidative flavonoids.

In conclusion, we suggest that further studies should be performed on the isolation and identification of the active nonpolar compounds of the chloroform extract of *S. wallichiana*. Also, the aqueous extract should be further studied in detail to isolate individual chemical constituents responsible for the antioxidant activity. These results may provide a starting point for investigations to exploit new natural antimicrobial, cytotoxic and antioxidant substances.

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