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SHORT COMMUNICATION

Phytochemical investigation and antimicrobial activity of *Derris scandens*

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Abstract Different fractions of root and stem of *Derris scandens* demonstrated good antibacterial (*Escherichia coli*, and *Bacillus megaterium*), antialgal (*Chlorella fusca*), and antifungal (*Microbotryum violaceum*) activities. Phytochemical investigation resulted in isolation of scandenin, scandenin A, betulinic acid, lupeol, β-amyran-3-one, β-amyrin, β-sitosterol and ß-sitosterol glucopyranoside. Study showed that scandenin has strong antibacterial activity against *B. megaterium* and good antifungal and antialgal properties. Scandenin A showed good antibacterial, antifungal and antialgal properties.

1. Introduction

Derris scandens Benth. (family Leguminosae) is distributed in South East Asia and North Australia (Mahabusarakam et al., 2004). Its stem is widely used in traditional medicine as an anti-tussive, diuretic, expectorant and anti-dysentery agent and for treatment of muscle pains (Mahabusarakam et al., 2004), cough and diarrhea (Sreelatha et al., 2010). Insecticidal constituents, viz., rotenone and lonchocarpic acid, were reported also from the roots of *D. scandens*, and these com-

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pounds demonstrated insect anti-feedant activity (Rani et al., 2013).

The use of *D. scandens* as a folk medicine and the antimicrobial activity of the crude extract prompt us to investigate phytochemical investigation. Previous studies indicated the presence of coumarins, isoflavones, flavones, isoflavone glycosides and phenyl coumarins as chemical constituents from *D. scandens* (Sreelatha et al., 2010; Mahabusarakam et al., 2004). Now we are reporting two coumarins (**1** and **2**), four triterpenes (**3**–**6**), and two steroids (**7** and **8**) from the roots and stems extracts of *D. scandens*. Working as anti-dysentery agent and in curing diarrhea lead us to propose the hypothesis that plant parts may have bacteriocidal, antifungal, anti-protozoa/antialgal properties. For the purpose we exploited phytochemical separation and analysis of different fractions and bioassay for testing of bacteriocidal/antifungal/antialgal activities.

2. Materials and methods

2.1. General experimental procedure

UV (MeOH) and IR (KBr) spectra were measured on Hitachi U-3200 and Shimadzu-8900 spectrophotometers, respectively. EI-MS and HR-EI-MS were carried out using MAT 8200 and Micromass LCT mass spectrometers, in *m*/*z*. The ^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer using TMS as an internal reference. The chemical shifts are reported in ppm (δ) while the coupling constants (*J*) are in Hertz. The ^{13}C NMR spectra were recorded at 125 MHz on the same instrument. Column chromatography (CC) was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany) and Aluminum sheets precoated with silica gel 60 F 254 (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as the spray reagent.

2.2. Plant material

D. scandens plants were collected from Karachi, Pakistan, and identified through comparison with earlier identified herbarium specimen (voucher specimen, No. ICP-201) present in Herbarium, Department of Botany, University of Peshawar, Pakistan.

2.3. Compound isolation and identification

The dried and powdered roots and stem of *D. scandens* were extracted with methanol at r.t. for two weeks. The crude extract of roots (6.5 g) and stem (5.3 g) of *D. scandens* was subjected to column chromatography (*n*-hexane, *n*-hexane/CH_2Cl_2 and CH_2Cl_2/MeOH, in the order of increasing polarity) to separate different fractions. Two coumarins viz., scandenin (**1**, 5.4 mg) (Pelter and Stainton, 1964) and scandenin A (**2**, 5.7 mg) (Rao et al., 2007) were purified from fraction DSR-3 by silica gel column chromatography (CC) and with *n*-hexane/ethyl acetate (10:1) as the eluent. Fraction DSR-1 was further separated by silica gel column chromatography and eluted with *n*-hexane–EtOAc (9:1) to give triterpene named betulinic acid (**3**, 2.2 mg) (Ikuta et al., 1995). Similarly, another triterpene viz., lupeol (**4**, 3.0 mg) (Carvalho et al., 2001) was also isolated from fraction DSR-1, after elution with a mixture of *n*-hexane–EtOAc (8.5:1.5). Repeated column chromatography of fraction DSS-1 using *n*-hexane–acetone (9.5:0.5) as the eluent afforded β-amyran-3-one (**5**, 2.5 mg) (Krishnaveni and Rao, 2000). Moreover fraction DSR-4 gave one triterpene named β-amyrin (**6**, 8.0 mg) (Heupel, 1985) upon elution with *n*-hexane–EtOAc (9:1) and one steroid viz., ß-sitosterol (**7**, 3.7 mg) (Rubinstein et al., 1976) was isolated from fraction DSS-4 with *n*-hexane–EtOAc (8:2) as eluent. Finally DSS-7 gave ß-sitosterol glucopyranoside (**8**, 7.8 mg) (Seo et al., 1978) using *n*-hexane–EtOAc (2.5:7.5) as the eluent. All the above compounds (Fig. 1) were identified by an intensive comparison of their NMR spectral data reported in the literature.

2.4. Bioactivity test – agar diffusion test

Tests for antifungal, antibacterial, and antialgal activities were performed as previously described (Schulz et al., 1995; Höller et al., 2000). The test organisms for the agar diffusion and screening tests were the bacteria *Bacillus megaterium* de Bary (Gram positive) and *Escherichia coli* (Migula) Castellani & Chambers (gram negative), the fungi *Microbotryum violaceum* [formerly known as *Ustilago violacea* (Pers.) Roussel (Ustomycetes), *Mycotypha microspora* Fenner (Zygomycetes)], and the alga *Chlorella fusca* Shih Krauss (Chlorophyceae). The crude extract of roots and stem of plant *D. scandens* was subjected to column chromatography and resulted in 12 fractions (DSR 1–12) for roots and 10 fractions (DSS1–10) for stems tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties. Reference compounds were *penicillin*, *nystatin*, *actidione*, and *tetracycline*.

The extracted compounds were dissolved in acetone at a concentration of 1 mg/mL. Fifty microliters of the solutions (50 µg) was pipetted onto a sterile filter disk (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism (Schulz et al., 1995).

3. Results and discussion

All tested fractions of root and stem showed noticeable antibacterial (*E. coli*, and *B. megaterium*), antialgal (*C. fusca*), and antifungal (*M. violaceum*) activities (Table 1). Phytochemical investigation of roots resulted in the isolation two known coumarins, viz. scandenin and its methyl ether scandenin A. It is interesting to note that scandenin showed strong antibacterial activities against *B. megaterium* and *E. coli*. Recently it has been reported that an aqueous fraction of stems of *D. scandens* showed anti-bacterial activity against *E. coli* (Sittiwet and Puangpronpitag, 2009). The findings are of significance especially noting that *E. coli* causes serious nosocomial infection such as urinary tract infections, cardiovascular, and surgical-site infections (Sittiwet and Puangpronpitag, 2009). *B. megaterium* causes a plant disease viz., white to very light tan blotching and streaking of wheat leaves and considered soil saprophyte (Abdel-Monaim et al., 2012). Moreover compounds can be used as pesticide in agriculture which show activity toward *C. fusca* (Kotrikla et al., 1999).

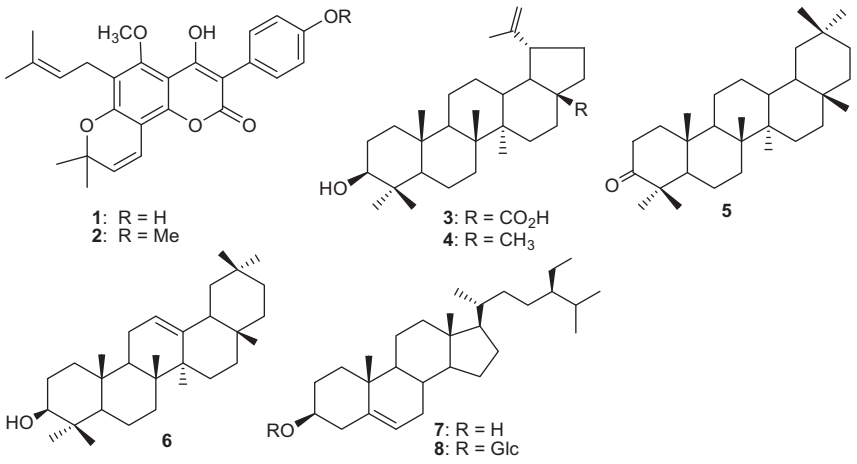

Figure 1 Structure of different compounds isolated from *D. scandens*.

Table 1 Inhibition zones (mm) caused by pure metabolites (scandenin and scandenin A), and different fractions from root (DSR) and stem (DSS) against different microbes in agar diffusion bio-assay.[a]

Compound/Fractions	Antibacterial Ec	Antibacterial Bm	Antialgal Bm	Antifungal Mv
Scandenin (**1**)	8 + PI 10	14 + PI 16	8.5	PI 9
Scandenin A (**2**)	9 + PI 11	PI 8	7	PI 8
DSR-1	6	PI 6	6	6
DSR-2	5.5	7	6.5	6
DSR-3	6	6	6	6
DSR-4	6	7	6	6
DSR-5	6	8 + PI 9	6.5	6
DSR-6	6	8	6	PI 7.5
DSR-7	6	6	6	5 + PI 7
DSR-8	6	10 + PI 11	6	5 + PI 6
DSR-9	6	6	6	PI 4.5
DSR-10	7	PI 7	7	7
DSR-11	8	8	5.5	6
DSR-12	6	PI 6	7	0
DSS-1	7	PI 6	5.5	PI 6
DSS-2	6	PI 6	6	PI 6
DSS-3	6	PI 7	6	6
DSS-4	6	PI 6.6	6	PI 5.6
DSS-5	6	PI 6	6	PI 6
DSS-6	6	6	6	6
DSS-7	6	7	6.5	6 + PI 6.5
DSS-8	6	8	6.5	PI 6
DSS-9	8	7	7	5 + PI 6
DSS-10	7	PI 6	6.5	PI 6.5
‘	14	18	0	0
Tetracycline	18	18	PI 10	0
Nystatin	0	0	0	20
Actidione	0	0	35	50
Acetone	0	0	0	0

[a] *Chlorella fusca* (Chl), *Microbotryum violaceum* (Mv), *Escherichia coli* (Ec), *and Bacillus megaterium* (Bm). Application of pure substances at a concentration of 0.05 mg (50 μL of 1 mg/mL). PI = partial inhibition, i.e. there was some growth within the zone of inhibition.

Similarly, all tested fractions and compounds viz., scandenin (**1**) and scandenin A (**2**) showed noticeable algicidal activity against *C. fusca*. Interestingly, fractions and compounds **1** and **2** showed appreciable antifungal behavior toward *M. violaceum*.

4. Conclusion

The overall result of the present study leads to the conclusion that different extracts of *D. scandens* exhibit appreciable antibacterial, antifungal and antialgal activities. Preliminary study

showed that scandenin (**1**) showed strong antibacterial activity and convincing antifungal and antialgal properties. The results suggest further quantitative and elaborate investigation.

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