Indian Journal of Chemistry
Vol. 46B, November 2007, pp. 1868-1872

Antifungal and phytochemical studies of *Eupatorium birmanicum* DC.

L Reena Devi[1], Th Shyamkeshor Singh[2] & Warjeet S Laitonjam[1]*

[1]Department of Chemistry, Manipur University, Canchipur 795 003, Manipur, India
[2]Department of Life Sciences, Manipur University, Canchipur 795 003, Manipur, India
Email: warjeet@yahoo.com

Received 18 April 2007; accepted (revised) 13 August 2007

The isolation of coumarin, β-sitosterol and β-sitosterol-D-glucoside from the chloroform extract; and *o*-coumaric acid, cerebroside **1**, ceramide **2**, and quercetin-3-*o*-rutinoside **3** from the methanol extract of the leaves of *Eupatorium birmanicum* DC. along with the antifungal study of the chloroform and methanol extracts from the plant are reported. The structures of the isolated compounds are characterized by different spectroscopic methods.

Keywords: Coumarin, β-sitosterol, β-sitosterol-D-glucoside, *Eupatorium birmanicum*

Eupatorium birmanicum DC. (*Aesteraceae*) belongs to the tribe *Eupatoriaea*. The juice extracted from the decoction of the leaves of the plant is used to control the burning sensation of chili[1]. The high intense of cough can be cured by applying or by ingesting the salad of leaves of *E. birmanicum*. One of the very interesting and common practice of this plant by the tribes of north-eastern region of India is the sprinkling of the bud's watery (just after freshly dipping into water) on epilepsy affected persons to cure the disease[1]. The chloroform extract of *E. birmanicum* exhibited potent anti-ulcer activity. The activity may be attributed to one or more of the bioactive constituents present in the plant. The results confirm the folklore claim for the leaves of *E. birmanicum* as anti-ulcer. No phytochemical work was done on the plant, *Eupatorium birmanicum* DC. Isolation of a number of flavonoid compounds from several *Eupatorium* species was reported[2,3]. The isolation of ceryl alcohol and β-sitosterol[4] and aromatic acids[5] from the leaves of *Eupatorium odoratum* (*Compositae*) was reported earlier. It was also reported the isolation of some sesquiterpenes, isosakuranetin, 2′-hydroxy-4,4′,5′,6′-tetramethoxy-chalcone, salvigenin, lupeol and β-amyrin[6]. The isolation of some chalcones and flavonoids from *E. odoratum* was also reported[7].

Herein, we report the isolation of coumarin, β-sitosterol and β-sitosterol-D-glucoside from the chloroform extract; and *o*-coumaric acid, cerebroside **1**, ceramide **2**, and quercetin-3-*o*-rutinoside **3** from the methanol extract of the leaves of *Eupatorium birmanicum* DC. The antifungal study of the isolated compounds from the plant is also reported. Four fungal species *viz*. *Fusarium oxysporum*, *Curvularia lunata*, *Sclerotiun rolfsii* and *Trichoderma viride* were selected for assessing the effect of different extracts of *E. birmanicum* DC. on their growth.

Results and Discussion

The cold light petroleum ether extract of the coarsely powdered, air dried leaves of *Eupatorium birmanicum* DC. were concentrated by distilling of the solvent using rotary evaporator and the residue was further extracted successively with chloroform and methanol. The extract was concentrated and evaporated to dryness. The chloroform extract was chromatographed over silica gel and on elution with petroleum ether-chloroform gave coumarin, β-sitosterol and β-sitosterol-β-D-glucoside.

The methanol extract on repeated chromatographic purification over a column of silica gel led to the isolation of four compounds. The methanol extract on elution with chloroform-methanol (95:5) gave *o*-coumaric acid or *o*-hydroxycinnamic acid. Its IR spectrum showed peaks at 3359 and 1668 cm^{-1} due to OH and CO groups, respectively. Its ^1H NMR showed signals at δ_H 12.22 (br s) due to –COOH proton, and the hydroxyl proton at δ_H 10.20. The olefinic protons showed doublets at δ_H 7.82 (J = 8.9 Hz) and at δ_H 6.51 (J = 8.9 Hz) due to H-3 and H-2, respectively. Aromatic protons showed signals at δ_H 7.57 (d, H-6′), 7.22 (t, H-4′), 6.90 (d, H-3′) and 6.82 (t, H-5′). The ^{13}C NMR spectrum showed signals at δ_C 168.93 (C-1),

157.44 (C-1′), 140.46 (C-3), 132.27 (C-5′), 129.53 (C-3′), 121.78 (C-2′), 120.26 (C-4′), 119.11 (C-6′) and 116.99 (C-2). Hydroxycinnamic acids usually occur in plants; and can exist as esters or as derivatives with sugars. *p*-Coumaric acid is very common in plants and they give blue/green spot in UV light. However, *o*-coumaric acid is very rare in plants. The TLC of *o*-coumaric acid gives yellow colour in UV light. The structure of *o*-coumaric acid was further confirmed when the PC of it was sprayed with 5% NaOH to give an intense yellow-green fluorescence under UV light. On elution with chloroform-methanol (93:7) gave the compound, **1**. It exhibited peaks at 3336 and 1637 cm^{-1} in the IR spectrum. Its ^1H NMR spectrum displayed singlet at δ_H 8.70 due to NH proton and triplets at δ_H 0.84 and at δ_H 1.22 due to CH_3 groups. The ^{13}C NMR spectrum of **1** showed signals at δ_C 175.81 due to CO group, and methyl groups showed signals at δ_C 14.43 and 23.09. Its mass spectrum showed peaks at *m*/*z* 843 (30%), 842 (100), 814 (70). Compound **1** was assigned as cerebroside. The structure of **2** was assigned as ceramide by comparing with the authentic compound. Its ^1H NMR and ^{13}C NMR spectral data were similar to those of compound **1**, except the absence of the sugar part.

A flavonoside, **3** was isolated from the methanol extract of *E. birmanicum* by elution with chloroform-methanol (85:15). Its ^1H NMR spectrum displayed singlet at δ_H 12.60 due to 5-OH proton and aromatic protons at δ_H 7.55 (s), 7.53 (s), 6.84 (d), 6.39 (s) and 6.19 (s). The ^{13}C NMR spectrum of **5** showed signals at δ_C 178.20 due to CO group, and methyl group showed signal at δ_C 18.57. Its mass spectrum showed peaks at *m*/*z* 634 (18%), 633 (100), 611 (5). From the above spectral data, the structure of **3** was assigned as quercetin-3-*o*-rutinoside.

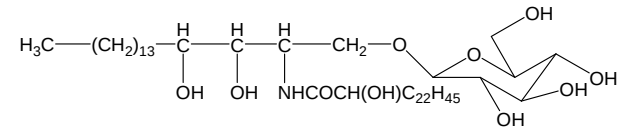
Four fungal species *viz*. *Fusarium oxysporum*, *Curvularia lunata*, *Sclerotiun rolfsii* and *Trichoderma viride* were selected for assessing the effect of chloroform and methanol extracts of *E. birmanicum* DC. on their growth. *E. birmanicum* DC. showed antifungal activities against one or more of the four fungal species (*F. oxysporum, C. lunata, T. viride* and *S. rolfsii*); different types of extracts of the plant obtained using different solvents (chloroform and methanol) showed differential inhibitory effect on the test fungal species, and the active principles produced by the plants seem to have narrow antifungal spectrum.

Experimental Section

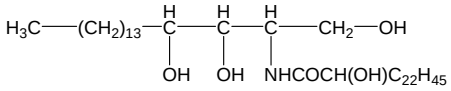
Melting points were determined in open capillary tubes and are uncorrected. Infrared (IR) spectra were recorded on an ATI Mattson Genesis FTIR spectrometer. Low resolution Chemical Ionization (CI) and Electron Impact (EI) mass spectra were recorded on a Fisons TRIO 2000 quadrupole mass spectrometer. Fast atom bombardment mass spectra (FAB-MS) were recorded on a VG AutoSpec 3000 mass spectrometer. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Varian Unity 500 (500 MHz), Bruker AC-300 and Varian XL (300 MHz) spectrometers. ^{13}C NMR spectra were recorded on Bruker AC-300 and Varian XL (75 MHz) spectrometers.

Healthy aerial parts of *Eupatorium birmanicum* DC. were selected and collected during the rainy season (June to August, 2001). After thorough washing, the plant materials were air dried under controlled condition (25°C) to avoid many changes occurring, followed by grinding of plant materials into small pieces by using hand grinder. The air-dried powdered plant material (2.5 kg) was soaked in petroleum ether for 3 days. The extract obtained was concentrated on a rotary vacuum evaporator at 40°C. This concentrated petroleum ether plant extract was semi-solid, sticky, yellow in colour and have characteristic odour. These processes of soaking, filtration and concentration were repeated for 3 times for exhaustive extraction of compounds present in the plant, which are soluble in petroleum ether. The residue plant material was air-dried at 25°C to vaporize petroleum ether completely. The air-dried plant materials which were devoid of trace of petroleum ether was soaked for 3 days in chloroform, followed by filtration and concentration as above. The processes of soaking, filtration and concentration were repeated for 3 times for exhaustive extraction of compounds present, which are soluble in chloroform. This concentrated chloroform plant extract was semi-solid, sticky, green in colour and have characteristic odour. Again, the residue plant material was air-dried at 25°C to vaporize chloroform completely. The exhaustive extraction procedures involving soaking for 3 days, filtration and concentration for 3 times were performed with methanol with the plant materials which were devoid of trace of chloroform. This concentrated methanol plant extract was also semi-solid, sticky, brown in colour and have characteristic odour. The chloroform extract was

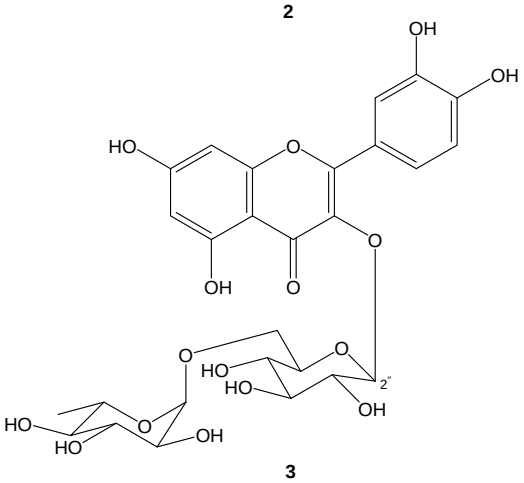
column chromatographed and eluted successively with petroleum ether, chloroform, and mixture of chloroform with increasing percentage of methanol. From the chloroform extract, three compounds – coumarin, β-sitosterol and β-sitosterol-β-D-glucoside were isolated. The methanol extract of *Eupatorium birmanicum* DC. was column chromatographed and eluted successively with petroleum ether, chloroform, and mixture of chloroform with increasing percentage of methanol. From the methanol extract of the plant, cerebroside **1**, ceramide **2**, quercetin-3-*o*-rutinoside **3** and *o*-coumaric acid were isolated **(Scheme I)**.



1



2



3

Scheme I

The extracts used for the determination of antifungal activity were chloroform and methanol extracts of *E. birmanicum* DC. Poisoned food technique described by Grover and Moore[8] was adopted for assessing the growth characteristic of the test fungal species. Czapek-Dox agar medium was used for maintenance and routine plating of fungal culture during the experiment. Requisite amounts of different plant extracts were separately dissolved in minimum amounts (5 mL) of respective solvents and the extract solutions were added separately to known amount of molten growth medium to give different extract concentrations. The concentrations selected for the study were 100, 500 and 1000 ppm for each type of plant extract. The extract treated media were poured in sterilized petri dishes (approx. 20 mL per plate) and left for solidification. Petri dishes containing Czapek-Dox agar medium and medium treated with the solvents were also prepared as controls. For each type of treatment, three replicated petri dishes were maintained. After solidification of the medium each plate was inoculated with one of the fungal species. The fungal innoculum was a mycelium block of 4 mm diameter of the particular fungus. The mycelium block was removed from the actively growing margins of 5 days old cultures of the four fungal species with the help of a sterilized cork borer. The mycelium block was aseptically placed upside down on the medium surface at the center of the Petri dish. The inoculated plates were incubated at 25 ± 1°C for different incubation periods. The length of incubation period was different for different fungal species. For *T. viride* the incubation period was 2-3 days, for *S. rolfsii* it was 4-5 days while for *F. oxysporum* and *C. lunata,* it was 5-6 days duration. After the incubation period, the diameters of fungal colony of treatment and control sets were measured in the mutually perpendicular directions. The results of the experiment were expressed directly as mean colony diameter (in mm) calculated from the three replicated plates and also as per cent relative growth. The percent relative growth was calculated from the following relationship:

$$\text{Per cent relative growth} = dt/dc \times 100$$

where, dc = mean colony diameter of control set

dt = mean colony diameter of treated set

The data thus obtained was statistically analyzed for significance using Duncan's Multiple Range Test.

The effect of chloroform extract of *E. birmanicum* DC. on the growth of the four fungal species is shown in **Table I**. Treatment with the plant extract showed inhibitory effect on growth of three fungal species (*F. oxysporum, C. lunata* and *T. viride*). However, the three fungi showed different degrees of sensitivity to the plant extracts. Significant growth inhibition of *F. oxysporum was* observed at 1000 ppm extract concentration. In case of *C. lunata* inhibitory effect was recorded in 500 ppm and higher concentrations. *T. viride* was found to be most sensitive to the plant

extract as growth inhibition was observed at all the three concentration (100, 500 and 1,000 ppm). The growth of *S. rolfsii* was not significantly affected by the extract at 100 and 500 ppm but at the highest concentration (1000 ppm), the plant extract showed slight stimulation on the growth of the fungus.

The effect of methanol extract of *E. birmanicum* DC. on the colony growth of the four fungal species is shown in the **Table II**. The methanol extract also showed more or less similar effect with that of chloroform extract. Treatment with the extract showed inhibitory effect against three fungal species *F.oxysporum, C. lunata* and *T. viride*, while the growth of *S. rolfsii* was stimulated by the extract.

As revealed by the **Tables I** and **II** it was observed that the test plant species, the type and concentration of the extracts influenced the growth of the four tested fungi. In some cases treatment with the plant extracts did not affect the growth rate of the fungi while in others extract treatment resulted in growth enhancement and still in other cases, fungal growth inhibition by the extracts was observed. Treatment with the plant extract showed inhibitory effect on growth of three fungal species (*F. oxysporum, C. lunata* and *T. viride*). However, the three fungi showed different degrees of sensitivity to the plant extracts. Significant growth inhibition of *F. oxysporum* was observed at 1000 ppm extract concentration. In case of *C. lunata* inhibitory effect was recorded in 500 ppm and higher concentrations. *T. viride* was found to be most sensitive to the plant extract as growth inhibition was observed at all the three concentration (100,500 and 1,000 ppm). The growth of *S. rolfsii* was not significantly affected by the extract at 100 and 500 ppm but at the highest concentration (1000 ppm) the plant extract showed slight stimulation on the growth of the fungus.

Table I — Antifungal study of the chloroform extract of *Eupatorium birmanicum*

Fungal sp.	Extract conc. (ppm)/Colony diameter (mm)				
	C	CS	100	500	1000
F. oxysporum	42.66a ± 1.52 (100.00)	41.66a ± 0.57 (97.65)	41.66a ± 1.52 (97.65)	40.66a ± 1.15 (95.31)	33.33b ± 0.57 (78.12)
C. lunata	37.33a ± 2.30 (100.00)	35.83a ± 2.40 (95.98)	37.33a ± 1.52 (100.00)	21.00b ± 1.00 (56.25)	0.66b ± 1.52 (55.34)
S. rolfsii	54.33a ± 1.15 (100.00)	53.33a ± 2.88 (98.15)	51.33a ± 3.05 (94.47)	55.00a ± 1.00 (101.23)	62.66b ± 2.08 (115.33)
T. viride	51.66a ± 1.52 (100.00)	48.33a ± 0.57 (93.55)	37.33b ± 1.15 (72.26)	32.66c ± 2.51(63.22)	15.33d ± 13.05 (29.67)

C=control (0ppm conc.); CS = control with solvent; ± = standard deviation; Figures inside parentheses are the per cent growth relative to the control.
(The means in the same row followed by the same letter are not significantly different)

Table II — Antifungal study of the methanol extract of *Eupatorium birmanicum*

Fungal sp.	Extract conc.(ppm)/Colony diameter (mm)				
	C	CS	100	500	1000
F. oxysporum	70.33a ± 0.57 (100.00)	67.66b ± 0.57 (96.20)	67.33b ± 0.57 (95.73)	66.66b ± 1.15 (94.78)	66.66b ± 0.57 (94.78)
C. lunata	66.00a ± 1.00 (100.00)	65.33a ± 1.15 (98.98)	60.00b ± 12.00 (90.90)	60.33b ± 0.57 (91.40)	56.00c ± 2.00 (84.84)
S. rolfsii	37.33a ± 12.08 (100.00)	38.33a ± 1.15 (102.67)	37.33a ± 2.08 (100.00)	49.66b ± 1.52 (133.02)	49.66b ± 1.15 (133.02)
T. viride	49.33a ± 1.15 (100.00)	48.66ab ± 1.15 (98.64)	46.66bc ± 1.15 (94.58)	44.66cd ± 1.15 (90.58)	44.00d ± 1.00 (89.19)

C = control (0ppm conc.); CS = control with solvent; ± = standard deviation; Figures inside parentheses are the per cent growth relative to the control.
(The means in the same row followed by the same letter are not significantly different)

The methanol extract also showed more or less similar effect with that of chloroform extract. Treatment with the extract showed inhibitory effect against three fungal species, *F. oxysporum, C. lunata* and *T. viride*, while the growth of *S. rolfsii* was stimulated by the extract.

Cerebroside 1: White solid; m.p. 201-202°C. IR (KBr): 3336, 2920, 2851, 1637, 1537, 1465, 1081, 1038, 721 cm^{-1}; Mass (*m*/*z*,%): 844 (M-1, 5), 843 (35), 842(100), 823 (15), 814 (70); ^{1}H NMR ($CDCl_3$): δ 7.73 (1H, d, *J* = 8.93 Hz, H-4), 7.51 (2H, dd, *J* = 8.93, 4.9 Hz, H-5, H-7), 7.31 (1H, dd, *J* = 8.93, 4.9 Hz, H-6, H-8), and 6.43 (1H, d, *J* = 8.93 Hz, H-3); ^{13}C NMR (DEPT, $CDCl_3$): δ 175.81 (CO), 150.40 (CH), 136.20 (CH), 131.00 (CH), 130.81 (CH), 123.96 (CH), 105.73 (CH), 78.70 (CH), 78.59 (CH), 76.05 (CH), 75.31(CH), 72.58 (CH), 71.63 (CH), 70.62 (CH_2), 62.79 (CH_2), 51.89 (CH), 35.72 (CH_2), 34.02 (CH_2), 33.47 (CH_2), 33.46 (CH_2), 33.14 (CH_2), 32.27 (CH_2), 30.50 (CH_2), 30.18 (CH_2), 30.02 (CH_2), 29.98 (CH_2), 29.76 (CH_2), 29.68 (CH_2), 26.80 (CH_2), 26.00 (CH_2), 23.09 (CH_2), 14.43 (CH_3).

Ceramide 2: White solid; m.p. 130-32°C. IR (KBr): 3332, 3221, 2919, 2851, 1622, 1067 cm^{-1}; Mass (*m*/*z*,%): 684 (M+1, 15%), 683 (M^+, 90), 668(20), 654 (15), 413 (100); ^{1}H NMR ($CDCl_3$): δ 7.73 (1H, d, *J* = 8.93 Hz, H-4), 7.51 (2H, dd, *J* = 8.93, 4.9 Hz, H-5, H-7), 7.31 (1H, dd, *J* = 8.93, 4.9 Hz, H-6, H-8), and 6.43 (1H, d, *J* = 8.93 Hz, H-3); ^{13}C NMR (DEPT, $CDCl_3$): δ 175.38 (CO), 150.38 (CH), 135.96 (CH), 130.98 (CH), 130.85 (CH), 123.95 (CH), 77.02 (CH), 73.18 (CH_2), 73.07 (CH), 72.62 (CH), 62.18 (CH_2), 53.13 (CH), 35.87 (CH_2), 34.32 (CH_2), 34.00 (CH_2), 33.46 (CH_2), 33.14 (CH_2), 32.28 (CH_2), 30.50 (CH_2), 30.18 (CH_2), 30.03 (CH_2), 29.98 (CH_2), 29.77 (CH_2), 29.67 (CH_2), 26.89 (CH_2), 25.98 (CH_2), 23.10 (CH_2), 14.43 (CH_3).

Quercetin-3-O-rutinoside 3: White solid; m.p. 162-64°C. IR (KBr): 3423, 2933, 1655, 1602, 1362, 1295, 1064 cm^{-1}; FAB MS (*m*/*z*,%): 633 (M+Na, 100%), 611 (M+1, 5), 303 (20); ^{1}H NMR (DMSO-d_6): δ 12.60 (1H, s, 5-OH), 7.55 (1H, s, H-6′), 7.53 (1H, s, H-2′), 6.84 (1H, d, *J* = 4.63 Hz, H-5′), 6.39 (1H, s, H-8), 6.19 (1H, s, H-6), 2.51 (2H, s, CH_2), 0.99 (3H, d, *J* = 4.55 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ 157.29 (C–2), 134.15 (C–3), 178.20 (C–4), 162.06 (C–5), 99.59 (C–6), 165.11 (C–7), 94.47 (C–8), 157.44 (C–9), 104.76 (C–10), 122.45 (C–1′), 116.09 (C–2′), 145.62 (C–3′), 149.29 (C–4′), 117.11 (C–5′), 122.02 (C–6′), 102.07 (3–glc–C–1″), 74.94 (3–glc–C–2″), 76.76 (3–glc–C–3″), 70.87 (3–glc–C–4″), 77.32 (3–glc–C–5″), 67.85 (3–glc–C–6″), 101.59 (2″–rha–C–1‴), 71.43 (2″–rha–C–2‴), 71.22 (2″–rha–C–3‴), 72.72(2″–rha–C–4‴), 69.09 (2″–rha–C–5‴), 18.57 (2″–rha–C–6‴).

Acknowledgements

The authors are thankful to the RSIC, NEHU, Shillong, IICB, Kolkata and RSIC, CDRI, Lucknow for the spectral data, and the DST, New Delhi for the financial support.

References

1 Sinha S C, *Medicinal Plants of Manipur* (Mass and Sinha, Manipur Cultural Integration Conference), Imphal, Manipur, India, **1996**.

2 Kupchan S M, Sigel C W, Hemingway R J, Knox J R & Udayamurthy M S, *Tetrahedron*, 25, **1969**, 1603.

3 Wagner H, Iyenger M A, Horhammer L & Herz W, *Phytochemistry*, 10, **1972**, 1504.

4 Ahmad M & Nabi M N, *Sci Res Dacca Pak*, 4, **1967**, 154.

5 Ahmad M & Nabi M N, *Sci Res Dacca Pak*, 6, **1969**, 37.

6 Bose P K, Chakrabarti P, Chakrabarti S, Dutta S P & Barua A K, *Phytochemistry*, 12, **1973**, 667.

7 Barua R N, Sharma R P, Thyagarajan G & Hertz W, *Phytochemistry*, 17, **1978**, 1807.

8 Grover R K & Moore J D, *Phytopathology*, 52, **1962**, 876.