# Isolation of Two Steroids and Two Flavonoids Having Antioxidant, Antibacterial and Cytotoxic Properties from Aerial Stems of *Equisetum Debile* Roxb.

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**Abstract:** Two steroids, namely, Stigmasterol (**1**) and Daucosterol (**2**) and two flavonoids, namely, Quercetin (**3**) and Quercetin-3-O-α-D-rhamnopyranoside (**4**) were isolated from n-hexane and ethyl acetate extract of the aerial stems of *Equisetum debile.* The structures of the compounds **1-4** were determined by the spectroscopic data analysis (IR, UV, $^{1}$H & $^{13}$C NMR). These compounds (**1, 3** and **4**) were isolated for the first time from this species so far. The cytotoxic potential of the pure compounds (**1-4**) were examined by using brine shrimp lethality bioassay. The isolated compounds **3** and **4** exhibited quite potent activity in brine shrimp lethality bioassay with $LC_{50}$ 1.30 and 1.96 µg/mL, respectively and they also demonstrated moderate to excellent antibacterial activity with a zone of inhibition of 12±1 mm against the tested pathogenic microorganisms. The two pure compounds **3** and **4** showed significant free radical scavenging activity with $IC_{50}$ 7.00 and 9.48 µg/mL, respectively.

**Keywords:** Bioactive phytoconstituents, Cytotoxicity, Free radical scavengers, Antibacterial screening, *Equisetum debile* Roxb.

## Introduction

*Equisetum debile* (Common name-Sumbak, Horsetails; Synonyms-*Equisetum diffusum*, *Equisetum telmateia*; Family-Equisetaceae) is a herb: native to tropical South Asia, but also found in the islands of the central Pacific, Indian, and South Atlantic islands [1]. The diversity of species increases from the equator to the temperate zone in the northern hemisphere, where as there are only four species in the Southern Hemisphere [2, 3]. In Bangladesh, *E. debile* wildly distributed in Chittagong and Jessore, mostly in shady hills and stream banks. This plant is administered as a cooling medicine; given for the treatment of gonorrhea and bone fractures. Decoction of plant is used for nasal polypus, various cancers of breast, liver, intestine, stomach, kidneys and tongue [4]. It is also used for the treatment of bone fracture, cystitis, arthritis, prostate disease, rheumatic and arthritic problems and it staunches wounds and stops nose bloods and reduces congaing up of blood [4].

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Previous phytochemical investigations resulted in the isolation of pinocembrin, chrysin, β-sitosterol, β-D-glycosylsitosterol, β-D-glucose, flavonoid glycosides and fatty acids [5, 6]. Flavonoid glycosides (kaempferol-3-O-sophoroside, kaempferol-3,7-O-β-D-diglucopyranoside, kaempferol-3-O-sophoroside-7-O- β-D-glucopyranoside, kaempferol-3-O-sophoroside and Caffeoyl-methylate-4-β-glucopyranoside), Megastigane diglucoside (3S,5R,6S,7E,9S) - megastigan - 7-ene-5,6-epoxy-3,9-diol-3,9-O-β-D-diglucopyranoside, (6R,9S)-3-oxo-a-ionol-9-O-β-D-glucopyranoside,(3S,5R,7E,9S)-9-[(β-D-glucopyranosyl) oxy] megastig-7-ene-3,5,6-triol), Phenylethyl - O - β - D - glucopyranoside, (Z) - 3 - hexenyl - O - β - D - glucopyranoside, L-tryptophan [7] and Debilosides A-C; Blumenol A, corchoinoside C, sammangaoside A [8].

*Equisetum debile* methanolic extract (100 & 1000 μg/mL) significantly inhibit the growth of shoots (hypocotyls) and roots (radicals) of rice when compared to control after three and seven days treatment. Furthermore, the *Equisetum debile* methanolic extract (50 to 1000 μg/mL) exhibit antioxidant properties and scavenge the free radical in dose dependent manner when compared with standard antioxidant (ascorbic acid). *Equisetum debile* methanolic extract also have antifungal property that inhibit 42.26% and 53.84% growth of *Aspergillus flavous* and *Aspergillus niger* respectively, while using the extract 200 μg/mL [9].

Antioxidant activity and phenolic composition of three different extracts (EtOAc, n-BuOH and $H_2O$) of field horsetail (*Equisetum arvense* L.) was reported [10]. The antioxidant activity of an aqueous extract (infusion) and respective ethyl acetate fraction of *Equisetum telmateia* Ehrh. (Equisetaceae) was studied and its anti-inflammatory and diuretic properties, had been evaluated by DPPH, TEAC and TBARS assays [11].

A high and significant antioxidant activity was detected in the ethyl acetate fraction. Analysis of the aqueous extract and the ethyl acetate fraction by HPLC-PAD-ESI/MS allowed the identification of the major phenolic compounds as flavan-3-ol, kaempferol and phenolic acid derivatives. Among the flavan-3-ols, A-type proanthocyanidins and afzelechin derivatives were detected as well as the more common B-type procyanidins, B2 and C1, whose identification was further confirmed by HPLC using detection involving chemical reaction with p-dimethylamino-cinnamaldehyde. The results suggested that the anti-inflammatory activity of E. telmateia could be due, at least in part, to the presence of compounds with antioxidant activity [12].

The cytotoxic potential of the different solvent extracts of Equisetum debile were examined by using brine shrimp lethality bioassay. However, n-hexane, ethyl acetate and n-butanol extract exhibited quite potent activity in brine shrimp lethality bioassay with LC50 4.25, 3.36 and 4.40 μg/mL, respectively. Further, ethyl acetate extract showed significant free radical scavenging activity with IC50 24.8 μg/mL. On the other hand, ethyl acetate and n-butanol extract demonstrated excellent antibacterial activity with a zone of 9±1 mm against the tested pathogenic microorganisms [13].

Therefore, the present study was undertaken to isolate and elucidate the structure of some pure compounds from the aerial stems of *Equisetum debile* by using extensive spectroscopic techniques and also to study the antioxidant, antibacterial and cytotoxic activity of the crude extracts as well as pure compounds (**1-4**).

## Materials and methods

### *General experimental procedures*

The NMR spectra were recorded using a Bruker DBX-400 MHz instrument (Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany), with chemical shift (δ) data reported in ppm relative to the solvent used. The spectras were taken by using $CDCl_3$ and $CD_3OD$ with tetra methyl silane (TMS) as standard reference. A Shimadzu FT-IR, 8400S, IR-prestige spectrometer (Shimadzu, Japan) was used for recording infrared spectrum. Major bands ($\nu_{max}$) were recorded in wave number ($cm^{-1}$) as potassium bromide (KBr) pellets. The UV absorbance was performed with a PerkinElmer Shelton, CT 06484 USA, Lambda 25 UV/VIS spectrometer. Vacuum rotary evaporator (BUCHI, Rotavapor R-210 Switzerland) was used for evaporating solvents. Melting points (m.p.) were determined by using an electro-thermal melting point apparatus SMP3 (Stuart Scientific, UK) and are uncorrected. All solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA).

### *Plant material*

Fresh aerial stems of *Equisetum debile* were collected from the shady hills of Kaptai, Chittagong, Bangladesh in October, 2009 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 34107) has been deposited to their museum.

### *Extraction and isolation of compounds*

Freshly collected aerial stems of *E. debile* were dried in an oven at 38˚C and crushed into powder. The crushed powder (516.2 g) was extracted with methanol (3.5L) for 5 days. The extract was concentrated to gummy mass (45.9 g) using Buchi Rotary Evaporator. The methanol extract (40g) was then partitioned in a separatory funnel by using successively with n-hexane, ethyl acetate, n-butanol and water. Then these extracts were concentrated by using rotary vacuum evaporator to provide n-hexane (11.0 g), ethyl acetate (9.0 g), n-butanol (8.5 g) and water (10.0 g) extracts.

The concentrated crude n-hexane extract (10.0g) was subjected to vacuum liquid chromatography (VLC) by using n-hexane and then eluted with mixtures of n-hexane and ethyl acetate with increasing the polarity of the solvents and finally with the mixtures of ethyl acetate and methanol to afford a total of 18 fractions (each 200 mL). VLC fractions 12-13 (90-100% ethyl acetate in n-hexane) (439.5mg) were then subjected to column chromatography (packed with silica gel-60) by using gradients of n-hexane-dichloromethane, then dichloromethane, followed by a gradient of dichloromethane-methanol, and finally with methanol to afford a total of 35 fractions (each 8 mL). Fractions 12-15 (showed violet colored single spot with tailing on TLC plate when visualized with anisaldehyde/sulphuric acid spray reagent) 23-25 (showed purple colored single spot with tailing on TLC plate when visualized with anisaldehyde/sulphurice acid spray reagent); these two fractions upon repeated washing with n-hexane and finally recrystallization, afforded pure compounds **1**(11mg, soluble in $CHCl_3$) and **2** (15mg, soluble in $CHCl_3$+ MeOH) respectively.

The concentrated crude ethyl acetate extract (8.0g) was subjected to vacuum liquid chromatography (VLC) by using n-hexane and then eluted with mixtures of n-hexane and ethyl acetate with increasing the polarity of the solvents and finally with the mixtures of ethyl acetate and methanol to afford a total of 18 fractions (each 200 mL). VLC fractions 16-17 (15-20% ethyl acetate in methanol ) (398.5mg) were then subjected to column chromatography (packed with sephadex LH-20) and eluted with 5% MeOH in $CHCl_3$ and the polarity of the mobile phase was increased gradually by adding more polar solvent such as MeOH. The eluted sample was collected in 35 test tubes (each 7mL). According to TLC pattern the test tubes (10-13) afforded on recrystallization and got yellow crystal **3** (20mg, soluble in MeOH) and test tubes (20-24) executed another yellow crystal **4** (15mg, soluble in MeOH).

## Bioassays

### *Cytotoxicity bioassays*

The cytotoxic activity was performed as described previously [14] by brine shrimp lethality bioassay method. The test samples for crude extracts as well as pure isolated compounds were dissolved in DMSO and serial dilution were made as 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.3095 µg/mL. On the other hand, vincristine sulphate (positive control) was dissolved in DMSO and serial dilution were made as 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.0781 µg/mL. Then each of these test solutions was added to test tubes containing 12 shrimps in simulated brine water (5 mL) and incubated at room temperature for 24 h. After 24 h, the median lethal concentration ($LC_{50}$) of the test samples was determined by a plot of percentage the shrimps against the logarithm of the sample concentrations (Finney method). Vincristine sulphate ($LC_{50}$= 0.57µg/mL) was used as positive control in this assay to compare the cytotoxicity of the test samples. Each treatment was replicated thrice.

### *Free radical scavenging activity test*

The free radical scavenging activity was assayed spectrophotometrically by DPPH method [15]. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical has a deep violet color due to its unpaired electron, and radical scavenging activity can be followed spectrophotometrically by a loss of absorbance at 525 nm. Sample stock solutions (1 mg/mL) were diluted to final concentrations of 100, 50, 10, 5 and 1 µg/mL in 70% ethanol or DMSO. DPPH ethanol solution (0.2 mM, 0.5 mL) was added to 1 mL of sample solutions of different concentrations, shaken well by vortex, and allowed to react at room temperature. The absorbance values were measured after 10 min at 525 nm by UV/Vis spectrophotometer. The free radical scavenging activity of samples was calculated according to the formula:

DPPH radical scavenging activity (%) = $[1-(Abs_{sample}-Abs_{blank})/Abs_{control}] \times 100$

Where $Abs_{sample}$ is the absorbance of the experimental sample, $Abs_{blank}$ is the absorbance of the blank, $Abs_{control}$ is the absorbance of the control.

As a blank, 70% EtOH or DMSO solvent (0.5 mL) and sample solution (1.0 mL) were used. DPPH solution (0.5 mL, 0.2 mM) and 70% EtOH or DMSO solvent (1.0 mL) was used as a negative control. The ascorbic acid (vitamin C) ($IC_{50}$=4.01μg/mL) was used as a positive control in this assay to compare the free radical scavenging activity of the test samples. Each treatment was replicated thrice.

### *Antibacterial screening*

The test samples were dissolved separately in specific volume of chloroform or methanol depending on their solubility. The antibacterial screening was then carried out by the disc diffusion method [16, 17]. The diluted samples were applied on to sterile blank discs (Oxoid, UK) at a concentration of 100 μg/disc for this test where Streptomycin (10 μg/disc, Oxoid, UK) used as a standard.

## Results and Discussion

Four pure compounds (**1-4**) (**Fig. 1**) were isolated from the aerial stems of *Equisetum debile* with the help of different chromatographic techniques and elucidated their structure by various spectroscopic analyses as well as by comparison of their spectral data with previously available in the literature.

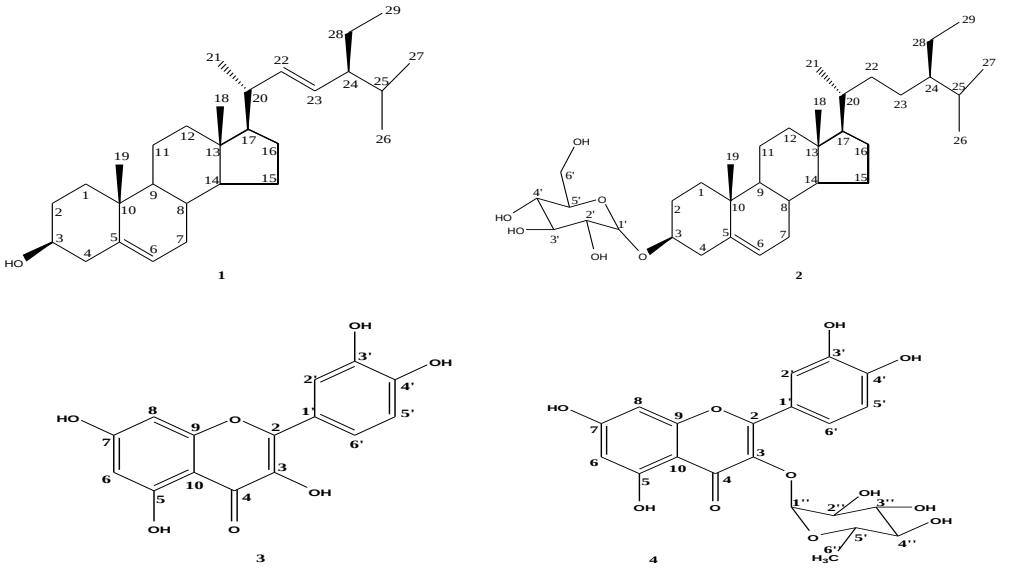

***Fig. 1:*** *The structures of compounds* ***1-4****.*

**Stigmasterol (1)**: Colorless needles; m.p. 137-139˚C (Uncorr.) (Lit. m.p. 135-137˚C) [18, 19]; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3290 (m, H-bonded O-H str.), 2910 (s, C-H str. $sp^3$), 1673 (m-w, C=C str. $sp^2$), 1565 (m, C-H bend. $CH_2$ gr.), 1445 (m, C-H bend. $CH_3$ gr.), 1275 (s, C-O str. due to alcohol) and 870 (alicyclic gr.).

***Table 1**: Comparative $^{1}H$ & $^{13}C$-NMR data of Compound **1** (400 MHz, $CDCl_3$).*

| C No. | $^{1}H$ (Observed) δ ppm | $^{1}H$ (Reported [20, 21]) δ ppm | $^{13}C$ (Observed) δ ppm | $^{13}C$ (Reported [20, 21]) δ ppm |
|---|---|---|---|---|
| 1 | | | 37.3 | 37.3 |
| 2 | | | 31.7 | 31.7 |
| 3 | 3.51(1H, m, H-3) | 3.58(1H, m, H-3) | 71.8 | 71.8 |
| 4 | | | 42.3 | 42.2 |
| 5 | | | 140.8 | 140.8 |
| 6 | 5.35 (1H, m, H-6) | 5.40 (1H, m, H-6) | 121.7 | 121.7 |
| 7 | | | 31.9 | 31.9 |
| 8 | | | 31.9 | 31.9 |
| 9 | | | 51.2 | 50.2 |
| 10 | 1.0 (3H, s, $CH_3$-10) | 1.0 (3H, s, $CH_3$-10) | 36.5 | 36.4 |
| 11 | | | 21.1 | 21.1 |
| 12 | | | 39.7 | 39.7 |
| 13 | 0.69 (3H, d, J=7.2 Hz, $CH_3$-13) | 0.69 (3H, d, J=7.2 Hz, $CH_3$-13) | 42.2 | 42.2 |
| 14 | | | 56.8 | 56.9 |
| 15 | | | 24.3 | 24.4 |
| 16 | | | 28.2 | 28.9 |
| 17 | | | 56.1 | 56.0 |
| 18 | 0.72 (3H, s, $CH_3$-18) | 0.70 (3H, s, $CH_3$-18) | 11.8 | 12.0 |
| 19 | | | 19.8 | 19.4 |
| 20 | 1.02 (3H, d, J=8.0 Hz, $CH_3$-20) | 1.02 (3H, d, J=8.0 Hz, $CH_3$-20) | 40.4 | 40.5 |
| 21 | | | 21.2 | 21.2 |
| 22 | 5.18 (1H, dd, J=15.2, 8.8 Hz,) | 5.18 (1H, dd, J=15.2,8.8 Hz) | 138.3 | 138.3 |
| 23 | 5.04 (1H, dd, J=15.2, 8.4 Hz) | 5.07(1H, dd,J=15.2,8.4 Hz) | 129.3 | 129.3 |
| 24 | | | 50.2 | 51.6 |
| 25 | | | 31.7 | 31.9 |
| 26 | 0.83 (3H, d, J=8.0 Hz, $CH_3$-26) | 0.94 (3H, d, J=8.0 Hz, $CH_3$-26) | 21.0 | 19.0 |
| 27 | 0.79 (3H, d, J=8.0 Hz, $CH_3$-27) | 0.83 (3H, d, J=8.0 Hz, $CH_3$-27) | 19.0 | 21.1 |
| 28 | | | 25.4 | 25.4 |
| 29 | 0.92 (3H, t, J=6.4 Hz, $CH_3$-29) | 0.85 (3H, t, J=6.4 Hz, $CH_3$-29) | 12.0 | 12.2 |

These spectral features (**Table 1**) are in close agreement to those observed for Stigmasterol [20, 21]. On the above evidences, compound **1** was identified as Stigmasterol.

**Daucosterol (2)**: White amorphous solids; m.p. 268-272˚C (Uncorr.) (lit. m.p. 276-279˚C) [19]; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3490-3340 (m, H-bonded O-H str.), 2910 (s, C-H str. $sp^3$), 1640 (m-w, C=C str. $sp^2$), 1560 (m, C-H bend. $CH_2$ gr.), 1515 (m, C-H bend. $CH_3$ gr.), 1360 (s, C-O str. due to alcohol) and 870 (alicyclic gr.).

***Table 2:*** *Comparative $^{1}H$ & $^{13}C$-NMR data of Compound **2** (400 MHz, $CDCl_3$ + $CD_3OD$).*

| C No. | $^{1}H$ (Observed) δ ppm | $^{1}H$ (Reported [22, 23]) δ ppm | $^{13}C$ (Observed) δ ppm | $^{13}C$ (δ ppm) (Reported [22,23]) |
|---|---|---|---|---|
| 1 | | | 36.8 | 37.6 |
| 2 | | | 31.5 | 30.2 |
| 3 | 3.51(1H, m, H-3) | 3.58(1H, m, H-3) | 78.7 | 78.5 |
| 4 | | | 39.3 | 39.2 |
| 5 | | | 139.9 | 140.9 |
| 6 | 5.35 (1H, m, H-6) | 5.37 (1H, m, H-6) | 121.6 | 121.9 |
| 7 | | | 31.5 | 32.0 |
| 8 | | | 31.9 | 31.9 |
| 9 | | | 49.8 | 50.2 |
| 10 | 1.0 (3H, s, $CH_3$-10) | 1.0 (3H, s, $CH_3$-10) | 36.2 | 36.8 |
| 11 | | | 20.6 | 21.1 |
| 12 | | | 38.1 | 39.8 |
| 13 | 0.69 (3H, d, J=7.2 Hz, $CH_3$-13) | 0.69 (3H, d, J=7.2 Hz, $CH_3$-13) | 41.9 | 42.4 |
| 14 | | | 56.3 | 56.7 |
| 15 | | | 23.9 | 24.4 |
| 16 | | | 28.7 | 28.4 |
| 17 | | | 55.6 | 56.1 |
| 18 | 0.72 (3H, s, $CH_3$-18) | 0.70 (3H, s, $CH_3$-18) | 11.2 | 11.8 |
| 19 | | | 19.1 | 19.3 |
| 20 | 1.02 (3H, d, J=8.0 Hz, $CH_3$-20) | 1.02 (3H, d, J=8.0 Hz, $CH_3$-20) | 36.2 | 36.3 |
| 21 | | | 18.4 | 18.9 |
| 22 | 5.18 (1H, dd, J=15.2, 8.8 Hz,) | 5.18 (1H, dd, J=15.2,8.8 Hz) | 35.5 | 34.1 |
| 23 | 5.04 (1H, dd, J=15.2, 8.4 Hz) | 5.07(1H, dd,J=15.2,8.4 Hz) | 27.7 | 26.2 |
| 24 | | | 45.5 | 45.9 |
| 25 | | | 28.7 | 29.3 |
| 26 | 0.83 (3H, d, J=8.0 Hz, $CH_3$-26) | 0.85 (3H, d, J=8.0 Hz, $CH_3$-26) | 19.4 | 19.1 |
| 27 | 0.79 (3H, d, J=8.0 Hz, $CH_3$-27) | 0.83 (3H, d, J=8.0 Hz, $CH_3$-27) | 18.7 | 19.8 |
| 28 | | | 22.6 | 23.2 |
| 29 | 0.92 (3H, t, J=6.4 Hz, $CH_3$-29) | 0.94 (3H, t, J=6.4 Hz, $CH_3$-29) | 11.2 | 12.0 |
| C-1′ | 4.13 (1H, d, J=8.0Hz, H-1′) | 4.41(1H, d, J=8.0Hz, H-1′) | 100.7 | 101.5 |
| C-2′ | 2.94 (1H, m, H-2′) | 3.23 (1H, m, H-2′) | 73.1 | 74.0 |
| C-3′ | 3.05 (1H, m, H-3′) | 3.42 (1H, m, H-3′) | 76.1 | 76.9 |
| C-4′ | 3.13 (1H, m, H-4′) | 3.42 (1H, m, H-4′) | 69.6 | 70.7 |
| C-5′ | 3.00(1H, m, H-5′) | 3.29 (1H, m, H-5′) | 75.6 | 76.3 |
| C-6′ | 3.47(1H, dd,J=12.0, 4.4Hz $H_a$-6′) 3.57(1H, dd,J=12.0, 3.3Hz,$H_b$-6′) | 3.84(1H, dd,J=12.0, 4.4Hz $H_a$-6′) 3.75(1H, dd, J=12.0, 3.3Hz, $H_b$-6′) | 61.7 | 62.2 |

It is evident from the spectrum (**Table 2**) that $^{1}H$ NMR and $^{13}C$ NMR spectral data of compound **2** are almost identical to that reported data for Stigma-5-en-O-β glucoside or Daucosterol [22, 23]. Therefore, the structure of the compound **2** was established as Stigma-5-en-3-O- β glucoside or Daucosterol.

**Quercetin (3):** Yellow crystals; m.p. 305-307˚C (Uncorr.) (Lit. m.p. 310-312˚C) [24, 25, 19] ; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3431, 3385, 1650, 1600, 1550, 1505, 1200.

***Table 3**: Comparative $^{1}H$ & $^{13}C$-NMR data of Compound **3** (400 MHz, $CD_3OD$).*

| C No. | $^{1}H$ (Observed) δ ppm | $^{1}H$ (Reported [24, 25, 26,19]) δ ppm | $^{13}C$ (Observed) δ ppm | $^{13}C$(Reported [24, 25,26,19]) δ ppm |
|---|---|---|---|---|
| 2 | | | 148.0 | 148.2 |
| 3 | | | 137.2 | 137.2 |
| 4 | | | 177.3 | 177.5 |
| 5 | | | 162.4 | 162.6 |
| 6 | 6.18 (1H, d, J=1.8 Hz, H-6) | 6.22 (1H, d, J=1.8 Hz, H-6) | 99.3 | 99.4 |
| 7 | | | 165.6 | 165.7 |
| 8 | 6.39 (1H, d, J=1.8Hz, H-8) | 6.44 (1H, d, J=1.8Hz, H-8) | 94.4 | 94.6 |
| 9 | | | 158.2 | 158.4 |
| 10 | | | 104.5 | 104.7 |
| 1′ | | | 124.1 | 124.3 |
| 2′ | 7.73 (1H, d, J=1.9Hz, H-2′) | 7.71 (1H, d, J=1.9Hz, H-2′) | 116.0 | 116.1 |
| 3′ | | | 146.2 | 146.3 |
| 4′ | | | 148.8 | 150.3 |
| 5′ | 6.89 (1H, d, J=8.4Hz, H-5′) | 6.92 (1H, d, J=8.4Hz, H-5′) | 116.2 | 116.1 |
| 6′ | 7.64 (1H, dd, J=9.2, 2.0Hz, H-6′) | 7.57 (1H, dd, J=9.2, 2.0Hz, H-6′) | 121.7 | 121.8 |

**Quercetin-3-O-α-D-rhamnopyranoside (4)**: Yellow crystals; m.p. 188.5˚C (Uncorr.) (Lit. m.p. 183-185˚C) [19, 24, 25]; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3332, 3089, 1658, 1600, 1550, 1508, 1369, 1161.

***Table 4**: Comparative $^{1}H$ & $^{13}C$-NMR data of Compound **4** (400 MHz, $CD_3OD$).*

| C No. | $^{1}H$ (Observed) δ ppm | $^{1}H$ (Reported [24, 25, 26,19]) δ ppm | $^{13}C$ (Observed) δ ppm | $^{13}C$ (Reported [24, 25,26,19]) δ ppm |
|---|---|---|---|---|
| 2 | | | 158.4 | 158.4 |
| 3 | | | 136.2 | 134.3 |
| 4 | | | 179.6 | 177.9 |
| 5 | | | 163.1 | 161.4 |
| 6 | 6.18 (1H, d, J=1.8 Hz, H-6) | 6.22 (1H, d, J=1.8 Hz, H-6) | 99.7 | 98.8 |
| 7 | | | 165.8 | 164.2 |
| 8 | 6.39 (1H, d, J=1.8Hz, H-8) | 6.44 (1H, d, J=1.8Hz, H-8) | 94.6 | 93.7 |
| 9 | | | 159.4 | 157.3 |
| 10 | | | 105.8 | 105.5 |
| 1′ | | | 121.9 | 121.2 |
| 2′ | 7.74 (1H, d, J=1.9Hz, H-2′) | 7.71 (1H, d, J=1.9Hz, H-2′) | 109.6 | 109.1 |
| 3′ | | | 146.8 | 145.3 |
| 4′ | | | 150.8 | 148.4 |
| 5′ | 6.89 (1H, d, J=8.4Hz, H-5′) | 6.92 (1H, d, J=8.4Hz, H-5′) | 116.2 | 115.5 |
| 6′ | 7.65 (1H, dd, J=9.2, 2.0Hz, H-6′) | 7.57 (1H, dd, J=9.2, 2.0Hz, H-6′) | 122.7 | 121.9 |
| 1′′ | 4.53(1H, s, H-1′′, α-anomaric H) | 4.55(1H, s, H-1′′, α-anomaric H) | 103.5 | 102.4 |
| 2′′ | 3.88 (1H, m, H-2′′) | 3.89 (1H, m, H-2′′) | 71.8 | 70.7 |
| 3′′ | 3.48(1H, m, H-3′′) | 3.45(1H, m, H-3′′) | 73.3 | 70.8 |
| 4′′ | 3.34(1H, m, H-4′′) | 3.38(1H, m, H-4′′) | 72.1 | 71.9 |
| 5′′ | 3.81(1H, m, H-5′′) | 3.85(1H, m, H-5′′) | 72.0 | 71.2 |
| 6′′ | 0.97 (3H, d, J=6.0 Hz, $CH_3$-6′′) | 0.98 (3H, d, J=6.0 Hz, $CH_3$-6′′) | 17.6 | 17.1 |

From UV, IR, $^{1}H$, $^{13}C$, DEPT-135 NMR data (**Table 3-6**) and other related data which are available in the literatures [24, 25, 26, 19] led us to conclude that compound **3** and **4** are characterized as Quercetin and Quercetin-3-O-α-D-rhamnopyranoside, respectively.

***Table 5:*** *UV spectral shifts for compound 3*

| Ethanol solution | Spectral maxima ($\lambda_{max}$ nm) | Spectral effect | Structural diagnosis |
|---|---|---|---|
| Alone | 257, 292, 375 | Bathochromic shift | 3-OH free |
| (Com.**3** + 2M NaOH) | 271, 318, 415 | Bathochromic shift | 4`-OH free |
| (Com.**3** + 5% $AlCl_3$) | 265, 386, 436 | Bathochromic shift | 5-OH free |
| (Com.**3** + Powdered NaOAc) | 269, 298, 378 | 12 nm shift in band-I | 7-OH free |
| (Com.**3** + NaOAc- $H_3BO_3$) | 259, 263, 391 | 17 nm bathochromic shift in band-III | 3', 4'-di OH free |

***Table 6:*** *UV spectral shifts for compound 4*

| Ethanol solution | Spectral maxima ($\lambda_{max}$ nm) | Spectral effect | Structural diagnosis |
|---|---|---|---|
| Alone | 251, 268, 363 | 12 nm hypschromic shift compared to Quercetin (band-III 375 nm) | 3-OH substituted |
| (Com.**4** + 2M NaOH) | 254, 266, 415 | Bathochromic shift | 4'-OH free |
| (Com.**4** + 5% $AlCl_3$) | 258, 383, 436 | Bathochromic shift | 5-OH free |
| (Com.**4** + Powd. NaOAc) | 263, 288, 375 | 12 nm shift in band-I | 7-OH free |
| (Com.**4** + NaOAc- $H_3BO_3$) | 259, 268, 389 | 16 nm bathochromic shift in band-III | 3', 4'-di OH free |

*Equisetum debile* methanolic extract (100 & 1000 µg/mL) significantly inhibit the growth of shoots (hypocotyls) and roots (radicals) of rice when compared to control after three and seven days treatment. Furthermore, the *Equisetum debile* methanolic extract (50 to 1000 µg/mL) exhibit antioxidant properties and scavenge the free radical in dose dependent manner when compared with standard antioxidant (ascorbic acid). *Equisetum debile* methanolic extract also have antifungal property that inhibit 42.26% and 53.84% growth of *Aspergillus flavous* and *Aspergillus niger* respectively, while using the extract 200 µg/mL [9].

The free radical scavenging activity of the four pure compounds (**1-4**) were assayed by using DPPH method. The $IC_{50}$ value for Vitamin C and two pure compounds **3** and **4** were found to be 4.01, 7.00 and 9.48 µg/mL, respectively (Table 7). In comparison with the positive control (ascorbic acid), it can be mentioned that compound **3** and **4** showed significant antioxidant activity. Thus, compound **3** and **4** may be used as scavengers due to the presence of phenolic character.

The cytotoxic activity of the pure isolated compounds (**1-4**) were determined by using brine shrimp lethality bioassay. The $LC_{50}$ for crude vincristine sulphate (positive control) and four pure compounds (**1-4**) obtained from Finney method were found to be 0.57, 2.15, 2.21, 1.30 and 1.96 µg/mL, respectively (Table 8). In comparison with the positive control (Vincristine sulphate), it can be mentioned that all the test samples were lethal to brine shrimp nauplii. All pure compounds demonstrated quite potent activity in brine shrimp lethality bioassay. These positive results suggested that they may contain antitumor or pesticidal activity.

Further, the antibacterial activity of all four pure compounds (**1-4**) were subjected to screening at 100µg/disc by using disc diffusion method. The two pure compounds **3** and **4** showed significant antibacterial activity against almost all tested pathogenic microorganisms having the zone of inhibition of 9 ± 1 mm each (Table 9).

***Table 7:*** *$IC_{50}$ values of pure compounds from E. debile (Free radical scavenging activity).*

| Samples | $IC_{50}$ (µg/mL) | Samples | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| VC | 4.01 | B-3 | 7.00 |
| B-1 | - | B-4 | 9.48 |
| B-2 | - | | |

VC: Vitamin C (Ascorbic acid, Std.); B-1, B-2, B-3 and B-4 for pure compounds **1**, **2, 3** and **4,** respectively.

***Table 8:*** *$LC_{50}$ values of pure compounds from E. debile (Cytotoxic activity).*

| Samples | $LC_{50}$ (µg/mL) | Samples | $LC_{50}$ (µg/mL) |
|---|---|---|---|
| VS | 0.57 | B-3 | 1.30 |
| B-1 | 2.15 | B-4 | 1.96 |
| B-2 | 2.21 | | |

VS: Vincristine sulphate (Std.); B-1, B-2, B-3 and B-4 for pure compounds **1**, **2, 3** and **4**, respectively.

***Table 9:*** *Antibacterial screening of pure compounds (100 µg/disc) from E. debile (Antibacterial activity).*

| Sample | Diameter of zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *B.cereus* | *B.mega-tyerium* | *B. subtilis* | *S.aureus* | *E.coli* | *S.sonnei* | *S.dysen-teriae* |
| ME | NA | 7 | NA | NA | NA | NA | 7 |
| B-1 | NA | 8 | 9 | NA | NA | 7 | 9 |
| B-2 | NA | NA | 9 | 7 | NA | NA | 10 |
| B-3 | 12 | 13 | 10 | 11 | 13 | NA | 10 |
| B-4 | 10 | 12 | NA | 13 | 11 | 9 | NA |
| Strepto-mycin | 22 | 23 | 17 | 17 | 17 | 15 | 27 |

ME: Methanol extract; B-1, B-2, B-3 and B-4 for pure compounds **1**, **2, 3** and **4,** respectively; NA: no activity observed; Streptomycin (Std.) (10.0 µg/disc).

## Conclusions

It is concluded that four compounds (**1-4**) were isolated from the n-hexane and ethyl acetate extracts of the aerial stems of *E. debile* and characterized by an extensive spectroscopic evidences among them three compounds namely, Stigmasterol (**1**), Quercetin (**3**) and Quercetin-3-O-α-D-rhamnopyranoside (**4**) were isolated for the first time from this species so far. The antioxidant, antibacterial and cytotoxicity screening of the different solvent extracts as well as four pure isolated compounds (**1-4**) were found to be consistent with the folk uses of *E. debile* by local people.

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