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**PHYTOCHEMICAL STUDIES ON *DESMOSTACHYA BIPINNATA* ROOTSTOCK****SHAKILA R¹, ARUL ANTONY S^{2*} AND GOPAKUMAR K³**

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ABSTRACT

The ethanolic extract of rootstock of *Desmostachya bipinnata* (Family: Poaceae) was subjected to column chromatography over silica gel and eluted with different solvents of increasing polarity. 5-Hydroxymethyl 2-furfural along with β -amyirin, β -sitosterol, β -sitosterol glucoside, stigmasterol glucoside and sucrose were isolated and reported for the first time from the plant. Their structures were elucidated by physical and spectroscopic data.

KEYWORDS : *Eragrostis cynosuroides*, Kusa, Tharuppai, asthma, diuretic.

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INTRODUCTION

Desmostachya bipinnata (Linn.) Stapf. syn. *Eragrostis cynosuroides* Beauv is a grass and belongs to the family Poaceae (Graminaea). It is known as Kusa in Ayurvedic system of medicine¹ and as Tharuppai in Siddha system of medicine. Root is used to treat diseases of urinary system, excessive vaginal discharge¹, irregular menses², asthma, jaundice³, abdominal and colic pain⁴. Root is used as gargle for gummosis and toothache⁵. It is consumed as a decoction (kudineer) for the above mentioned diseases^{6,7}. It is used as one of the ingredients in the ayurvedic formulations, viz., Karpuradyarka, Sukumara Ghrta, Asmari Ihara, Kasaya Curna, Trnapancamula Kvatha curna, Mutravirecaniya curna, Stanyajanana Kasaya curna. Coumarins, sugars, amino acids, carbohydrates⁸; flavonoids, flavonoid glycosides^{9,10}; a xanthene¹¹ were reported. Constituents of the essential oil from the leaves have also been reported and found to exhibit antimicrobial activity¹². Ethanolic extract and its constituents trycin and trycin-7-*O*-glucoside exhibited antiulcerogenic activity⁹, 4'-methoxy quercetin-7-*O*-glucoside showed antihelicobacter activity¹⁰. Isolation and identification of chemical constituents is important for finding out the active principles present in any medicinal plant¹³. In this communication, we report the isolation and identification of the chemical constituents from the ethanol extract of the rootstock of the plant. All the compounds are reported for the first time from the plant.

MATERIALS AND METHODS

Plant Material

The plant material was collected from Dharmapuri district in Tamil Nadu while in flowering during the month of August 2011. It was authenticated by Dr. R. Chelladurai, Botanist, Survey of Medicinal Plants Unit, Palayamkottai, Tamil Nadu. Voucher specimen of the plant (No. ACC. No. 7320) has been deposited in the Pharmacognosy department of Siddha Central Research Institute, Arumbakkam, Chennai-106.

Chemicals and Solvents

All solvents used were of analytical grade. For extraction of the plant material commercial grade solvents were used. They were purified by distillation. Chloroform and ethyl acetate were dried over anhydrous CaCl₂ and distilled. Commercial grade ethanol was dried over quick lime and distilled. Acme's silica gel (60-120 or 100-200 mesh) was used for column chromatography. Silica gel 60F₂₅₄ precoated aluminium plates (Merck, layer thickness 0.2 mm) were used for TLC. The spots were visualized by dipping in vanillin-sulphuric acid reagent and heating at 105°C till colour appearance.

Instruments

Melting points were determined by open capillary method on a heating block instrument and uncorrected. IR spectra were taken in KBr disc on a Perkin – Elmer grating FT-IR instrument. ¹H NMR and ¹³C NMR were taken in CDCl₃, CD₃OD or DMSO on a Bruker instrument (400 and 125 MHz respectively). Chemical shifts are given in δ scale with TMS as the internal standard.

Extraction, Isolation and Characterization

The rootstocks were shade dried and powdered in a hammer mill. Coarse powder (5 kg) was twice extracted with ethanol by cold percolation (48 h) method. The ethanol extract was filtered, concentrated in a rotary evaporator and finally dried in a vacuum. The total alcohol extract (48 g) was subjected to column chromatography over silica gel (100-200 mesh) and eluted with solvents of increasing polarity which afforded five compounds. The identities were further confirmed by comparison with authentic samples.

Isolation of Compound, I

Elution of the column with benzene (fractions 1 to 20) gave a gummy material which on crystallization from *n*-hexane gave colorless crystals (mp 196°C, lit. mp 197°C, yield 35 mg). It gave positive Noller's test for triterpenoid and a single spot (R_f = 0.45) on TLC over silica gel

with toluene: ethyl acetate (9:1) as the developing system. IR (KBr) ν_{\max} , cm^{-1} : 3293 (OH), 2946, 2870, 1647 (tri-substituted double bond), 1463, 1385 (gem dimethyl group), 1361, 1035 (C-O bending), 996 and 830 (tri-substituted double bond); ^1H NMR (δ , CDCl_3 , 500MHz) : 0.81 (3H, s, H-24), 0.85 (3H, s, H-23), 0.89 (6H, s, H-29 ad H-30), 0.96 (3H, s, H-25), 0.99 (3H, s, H-28), 1.02 (3H, s, H-26) 1.16 (3H, s, H-27), 3.24 (1H, t, J = 8.0 Hz, H-3), and 5.20 (1H, m, H-12); ^{13}C NMR (δ , CDCl_3 , 125MHz) : 38.61 (C-1), 25.99 (C-2), 79.05 (C-3), 38.78 (C-4), 55.20 (C-5), 18.39 (C-6), 32.68 (C-7), 39.82 (C-8), 47.66 (C-9), 36.97 (C-10), 23.54 (C-11), 121.71 (C-12), 145.20 (C-13), 41.74 (C-14), 28.10 (C-15), 26.17 (C-16), 32.50 (C-17), 47.26 (C-18), 46.85 (C-19), 31.08 (C-20), 34.75 (C-21), 37.16 (C-22), 27.25 (C-23), 15.50 (C-24), 15.58 (C-25), 16.82 (C-26), 26.96 (C-27), 28.40 (C-28), 33.33 (C-29) and 23.69 (C-30).

Isolation of Compound, II

Fractions 21 to 45 eluted with benzene a compound which on crystallization from hexane : chloroform mixture afforded colourless needles (mp 136°C, lit. mp 137°C, yield 125 mg). It answered Lieberman - Burchard test for sterols and gave single spot on TLC over silica gel (R_f 0.6) with toluene: ethyl acetate (4:1) as the developing system. IR (KBr) ν_{\max} , cm^{-1} : 3480 (hydroxyl); 2944, 2891, 1638 (trisubstituted double bond); 1463, 1382 (isopropyl); 1242, 1133, 1062, 969, 801 (trisubstituted double bond); ^1H NMR (δ , CDCl_3 , 500 MHz): 0.64 to 1.001, (18H, 6x CH_3), 3.55 (1H, brm, H-3), 5.35 (1H, brs, H-6); ^{13}C NMR (δ , CDCl_3 , 125 MHz) : 37.20 (C-1), 31.53 (C-2), 71.765 (C-3), 42.17 (C-4), 140.7 (C-5), 121.67 (C-6), 31.86 (C-7), 31.53 (C-8), 36.46 (C-10), 21.04 (C-11), 39.73 (C-12), 42.28 (C-13), 56.72 (C-14), 24.25 (C-15), 28.20 (C-16), 56.02 (C-17), 11.94 (C-18), 19.35 (C-19), 37.00 (C-20), 18.73 (C-21), 33.90 (C-22), 26.05 (C-23), 45.79 (C-24), 29.12 (C-25), 19.77 (C-26), 18.99 (C-27), 23.03 (C-28), 11.81 (C-29).

Isolation of Compound, III

Elution of the column with chloroform (fractions 61-75) a compound crystallized from hexane :

chloroform mixture as colourless needles (mp 34°C, lit. mp. 35°C, yield 20 mg). It gave a single spot (R_f = 0.50) on TLC with chloroform: methanol (8 : 0.5) as the solvent system. IR (KBr) ν_{\max} , cm^{-1} : 3398 (br, OH), 1672 (C=O), 1520; ^1H NMR (δ , CDCl_3 , 400 MHz): 9.585 (1H, s, CHO), 7.22 (1H, d, J=3.2 Hz, H-3), 6.52 (1H, d, J=2.4 Hz, H-4), 4.72 (2H, s, H-6); ^{13}C NMR (δ , CDCl_3 , 125 MHz): 177.736 (CHO), 160.753 (C-5), 152.329 (C-2), 123.998 (C-3), 110.01 (C-4), 57.553 (C-6).

Isolation of Compound, IV&V

Elution of the column with chloroform: ethanol (9:1) which on crystallization in acetone gave colourless crystals (mp 270°C, yield 50 mg). It gave positive Liebermann Burchard test for steroids and also answered anthrone sulphuric acid test for sugar. It gave a single spot (R_f = 0.58) on TLC over silica gel with chloroform : methanol (8.5 : 1.5) as the developing system. The ^1H NMR and ^{13}C NMR data however showed it to be a mixture of compound IV and V. IR (KBr) ν_{\max} , cm^{-1} : 3412 (OH), 2933 & 2800 (aliphatic C-H stretching), 1645, 962 & 800 (disubstituted double bond), 1373 (isopropyl); ^1H (δ , CDCl_3 , 400 MHz): 0.66-1.00 (6 methyl groups), 3.65 (H-3), 5.34 (H-6), 5.15 (d, J=14.8, H-22) and 5.03 (d, J=8.4 Hz, H-23); ^{13}C NMR (δ , CDCl_3 , 125 MHz): 29.23 (C-1), 28.64 (C-2), 76.70 (C-3), 41.82 (C-4), 140.41 (C-5), 121.25(C-6), 31.38 (C-7,8), 49.57 (C-9), 36.18 (C-10), 20.56 (C-11), 38.26 (C-12), 41.70 (C-13), 56.14 (C-14), 23.83 (C-15), 28.66 (C-16), 55.38 (C-17), 11.62 (C-18), 19.10 (C-19), 35.46, 41.82 (C-20) 19.06, 20.56 (C-21) 35.46, 138.03 (C-22), 25.35, 128.79 (C-23), 45.09, 50.57 (C-24), 28.78, 31.38 (C-25), 18.86, 19.10 (C-26), 18.56, 19.06 (C-27), 23.83, 25.56 (C-28), 12.11, 11.67 (C-29).

Isolation of Compound, VI

Elution of the column with chloroform: ethanol (1:1) gave colourless compound which on recrystallization with aqueous ethanol afforded colourless crystals (m.p.185-187° (decomp); lit.mp.186°C, yield 270 mg). It answered for sugar. It gave a single spot (R_f = 0.55) on TLC over silica gel with Ethyl acetate : Methanol: Acetic acid (4:5:0.5) as the system. ^1H NMR (δ

ppm, DMSO, 400 MHz): 4.20 (1H, d, J=7.8 Hz, H-1), 3.65 (1H, dd, J=1.0 Hz, 3.0 Hz, H-4), 3.55 (1H, dd, J = 8.0 Hz, 9.0 Hz, H-2), 3.52 (1H, dd, J=7.0 Hz, 11.0 Hz, H-6), 3.30 (1H, dd, J = 5.0 Hz, 11.0 Hz, H-6), 3.15 (1H, dd, J=3.0 Hz, 9.0 Hz, H-3) and 3.03 (1H, dd, J = 1.0 Hz, 5.0 Hz, H-5); ^{13}C NMR (δ ppm, DMSO, 125 MHz): 64.90 (C-1'), 106.20 (C-2'), 83.90 (C-3'), 79.15 (C-4'), 76.65 (C-5'), 64.06 (C-6'), 94.68 (C-1), 73.62 (C-2), 74.94 (C-3), 71.85 (C-4), 75.10 (C-5) & 62.80 (C-6).

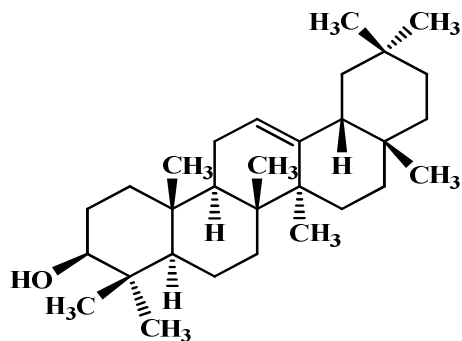
RESULTS AND DISCUSSIONS

All the isolated compounds I-VI were identified by their physical and spectroscopic data. The identified compounds were β -amyrin (I)¹⁴, β -sitosterol (II)¹⁵, 5-hydroxy methyl-2-furfural

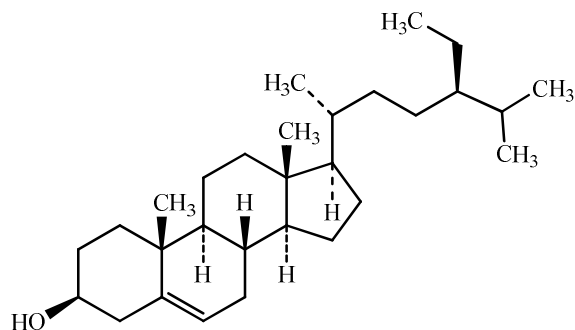
(III)^{16,17}, β -sitosterol-3-O- β -D-glucopyranoside and stigmasterol-3-O- β -D-glucopyranoside (IV & V)¹⁸ and sucrose (VI)¹⁹. In the ^1H NMR of compound IV and V, the methyl groups appeared in the region δ 0.66-1.01 of which H-18 appeared most upfield at δ 0.66 as three proton singlet. The most downfield methyl was H-19 of stigmasterol glucoside at δ 1.00 while that of β -sitosterol glucoside appeared at δ 0.97. H-3 appeared as one proton multiplet at δ 3.65. H-6 appeared as one proton broad singlet at δ 5.34. The presence of stigmasterol glucoside was also confirmed by the side chain olefinic proton H-22 and H-23. The ^{13}C NMR signals (Table 1) also confirmed the mixture of IV and V.

Table 1
 ^{13}C assignment of Compound (IV and V)

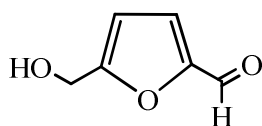
Carbon No.	δ_c - β -Sitosterol glucoside (ppm) (Compound IV)	δ_c - Stigmasterol glucoside (ppm) (Compound V)
C-1	29.23	
C-2	28.64	
C-3	76.70	
C-4	41.82	
C-5	140.41	
C-6	121.25	
C-7	31.38	
C-8	31.38	
C-9	49.57	
C-10	36.18	
C-11	20.56	
C-12	38.26	
C-13	41.70	
C-14	56.14	
C-15	23.83	
C-16	28.66	
C-17	55.38	56.14
C-18	11.62	
C-19	19.10	19.06
C-20	35.46	41.82
C-21	19.06	20.56
C-22	35.46	138.04
C-23	25.35	128.78
C-24	45.09	50.57
C-25	28.78	31.38
C-26	18.86	19.10
C-27	18.56	19.06
C-28	23.83	25.56
C-29	12.11	11.67



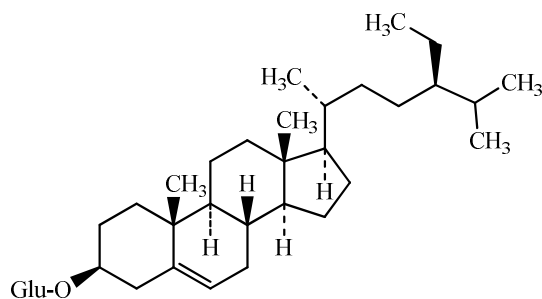
I



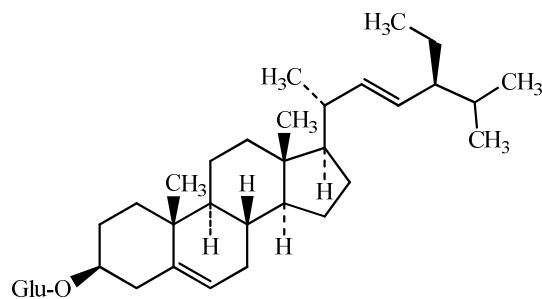
II



III



IV



V

CONCLUSION

5-Hydroxymethyl 2-furfural, along with β -amyrin, β -sitosterol, β -stisterol glucoside, stigmasterol glucoside and sucrose were isolated and reported first time from the ethanolic extract of the rootstock of *D. bipinnata*.

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