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Chemical constituents from *Sarcostemma acidum* (Roxb.) Voigt

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Abstract

Sarcostemma acidum (Roxb.) Voigt is a valuable medicinal plant to treat a number of diseases in India. Four compounds were isolated from the whole plant of *S. acidum*. The structures were characterized as Friedelin (**1**), *β*-amyrin (**2**), *β*-sitosterol (**3**) and 4*α*-Carboxy-4*β*-methyl-5*α*-cholesta-8,24-dien-3*β*-ol (**4**) by extensive spectroscopic (NMR, FT-IR and mass) analysis and compared their spectral data with literature. The *in vitro* anti-inflammatory activity of isolated compounds (**1-4**) and extract of *S. acidum* was carried out against the denaturation of protein using egg albumin method compared with standard dichlofenac sodium. The compounds showed the inhibition of protein denaturation in a concentration dependent manner.

Keywords: *S. acidum*; triterpenoid; isolation; anti-inflammatory.

1. Introduction

Natural products have been utilized as a source of medicine throughout history. Many modern pharmaceuticals are prepared from natural products [1]. There is an increase in trend of utilizing naturally occurring products in recent years and the active isolates from natural source are often screened for new drug discoveries. Natural product chemists are impressed by the fact that active biomolecules display an unbelievable range of diversity in terms of their structure as well as their biological and physical characteristics [2-3].

Triterpenoids and steroids have become a topic of intense interest due to their significant biological activities. They are extensively distributed throughout the plant and marine animal kingdoms [4].Their chemical structures are constructed by a carbon skeleton of C_5 isoprene units and can be distinguished from each other by the number of constituent isoprene units [5].

Sarcostemma acidum (Asclepiadeae) is a medicinal plant found in sub-Himalayan region of Northeast India, Bihar, West Bengal and many places of South India in dry rocky places at an altitude of 1350 m [6]. It has been used as traditional medicine for the treatment of rheumatism, ear ache, dog bite, chronic ulcer, snakebite, mental diseases, wounds and sinusitis etc. [7]. The plant exhibited numerous biological activities including anti-ulcer [8], antimicrobial [9], hepatoprotective [10], anti-inflammatory [11] and several other activities [12, 13]. The genus of the plant has reported rich source of triterpenoids [14, 15].

In our study, we have shown the isolation of compounds from *S. acidum* by chromatographic techniques and structure elucidation by various spectroscopic methods. Further, inhibition of protein (albumin) denaturation of isolated compounds of *S. acidum* was also studied.

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2. Experimental

2.1. Materials and methods

^{1}H- and ^{13}C-NMR were recorded with a Bruker AVANCE DPX 500 MHz NMR spectrometer, Switzerland with internal reference as tetramethyl silane (TMS). Chemical shifts (δ) value are reported in ppm and coupling constant (*J*) in Hz. Melting point was recorded using BUCHI M-560 capillary melting point apparatus and is uncorrected. Electron impact ionization mass spectrums (EIMS) were recorded with Trace DSQ GC-MS instrument of Thermo Fisher Scientific, USA. A high resolution mass spectrum (HRMS) was recorded in a Waters XEVO G2-Xs QTOF instrument. A FT-IR spectrum in Elmer FT-IR 2000 spectrometer on a thin film using chloroform was recorded. Silica gel 60-120 mesh was used for Column chromatography. Thin layer chromatography (TLC) experiments were performed using pre-coated Silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany). Fractionated solvents from column chromatography were evaporated to dryness under reduced pressure by Rotavapor; BUCHI, Labortechnik AG, Flawil, Switzerland.

2.2. Plant material

The plant *S. acidum* was collected from Jorhat district of Assam, India in February, 2018. The plant material was identified by Dr. M. Bordoloi and a voucher specimen was deposited in the herbarium at the CSIR-North East Institute of Science and Technology, Jorhat. The collected plant materials were further crushed, powdered and used for extraction.

2.3. Extraction and isolation

The whole plant of *S. acidum* (500g) was macerated thrice exhaustively with ethanol (EtOH) (3 x 4.5 L each) for 72 h at room temperature and it was concentrated under reduced pressure at 45°C using rotary evaporator. The resulting crude extract (20 g) was stored at (-) 20°C in a Deep freezer.

The extract (4 g) was chromatographed over a column of silica gel using solvent system started with 100% *n*-hexane followed by gradually increased polarity using EtOAc (*n*-Hexane/EtOAc ratios 10:1, 9:1, 7:3, 1:1, 1:2, 1:7 and 1:9) to give 50 eluents. Then, the eluents were analyzed by TLC and the eluents having similar R_f values were combined together get 6 different combined fractions. The solvent was evaporated under reduced pressure by rotary evaporator at 48°C. Fraction 1 was found prominent in TLC and obtained as a pure compound **1** (800 mg). Fraction 2 was purified by preparative TLC (*n*-Hexane/EtOAc, 10:1) to obtain compound **2** (65 mg). The purification of the fraction 3 by column chromatography over silica gel using solvent system started with 100% *n*-hexane followed by *n*-Hexane/EtOAc ratios (10:1), (9:1), (7:1), (5:1) to get total of 40 subfractions. From these, subfraction 1 was purified by preparative TLC (*n*-Hexane/EtOAc, 9:1) to obtain compound **3** (72 mg). Further, subfraction 3 was purified by preparative TLC (*n*-Hexane/EtOAc, 7:3) to obtain compound **4** (44 mg). The remaining fractions have not shown prominent TLC spot and they were not considered for further study. The physicochemical and spectrometric data of compound (**1-4**) as follows:

Friedelin (**1**): white solid; melting point: 250–255°C. FT-IR (KBr, $CHCl_3$, cm^{-1}): 2924, 2854, 1715, 1461, 1377 and 665. ^{1}H-NMR (500 MHz; $CDCl_3$; ppm) δ_H 2.40 (m, 1H), 2.38 (m, 2H), 2.25 (q, *J* =6.5 Hz, 1H), 1.96 (m, 1H), 1.77–1.33 (superposed signals, 21H), 1.29 (s, 3H), 1.18 (s, 3H), 1.05 (s, 3H), 1.00 (d, *J* = 3 Hz, 3H), 0.95 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.72 (s, 3H). ^{13}C-NMR (125 MHz; $CDCl_3$; ppm) δ_C 213.39 (C-3), 59.44 (C-10), 58.21 (C-4), 53.08 (C-8), 42.76 (C-18), 42.15 (C-5), 41.52 (C-2), 41.26 (C-6), 39.68 (C-14), 39.24 (C-22), 38.28 (C-13), 37.42 (C-9), 35.99 (C-16), 35.61 (C-11), 35.33 (C-19), 35.03 (C-29), 32.76 (C-21), 32.40 (C-15), 32.08 (C-28), 31.78 (C-30), 30.49 (C-12), 29.98 (C-17), 28.16 (C-20), 22.28 (C-1), 20.26 (C-26), 18.67 (C-27), 18.22 (C-7), 17.94 (C-25), 14.65 (C-24), 6.83 (C-23). EIMS (426.34, calc. 426.39). Elemental analysis: Found C, 83.17%; H, 11.55%; O, 2.98%; $C_{30}H_{50}O$ requires C, 84.44%; H, 11.81%; O, 3.75%.

β-amyrin (**2**): white solid; melting point: 189-191°C. FT-IR (KBr, $CHCl_3$, cm^{-1}): 3360, 1650. ^{1}H-NMR (500 MHz; $CDCl_3$; ppm) δ_H 4.49 (ddt, *J* = 16.5, 11.5, 4.5 Hz), 1.16 (s, 3H), 1.14 (s, 3H), 1.09 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.85 (s, 3H). ^{13}C-NMR (125 MHz; $CDCl_3$; ppm) δ_C 141.60 (C-13), 122.01 (C-12), 76.63 (C-3), 49.67 (C-5), 47.40 (C-9), 43.04 (C-18), 40.79 (C-19), 39.27 (C-14), 38.94 (C-8), 37.82 (C-4), 36.00 (C-1), 35.06 (C-22), 34.82 (C-10), 34.59 (C-21), 34.52 (C-29), 33.11 (C-17), 32.41 (C-7), 32.05 (C-20), 30.34 (C-28), 30.07 (C-2), 29.70 (C-23), 28.95 (C-15), 28.23 (C-16), 27.80 (C-27), 25.45 (C-30), 23.63 (C-11), 19.61 (C-6), 18.42 (C-26), 18.21 (C-25), 16.20 (C-24). EIMS (426.27, calcd. 426.38). Elemental

analysis: Found C, 84.11%; H, 10.42%; O, 3.29%; $C_{30}H_{50}O$ requires C, 84.44%; H, 11.81%; O, 3.75%.

β-sitosterol (**3**): White powder; melting point: 136-137°C. FT-IR V_{max} (KBr, $CHCl_3$, cm^{-1}): 3421, 2957, 2936, 2891, 2867, 1708, 1666, 1463, 1381, 1134, 1051, 970, 772, 665. ^{1}H-NMR (500 MHz; $CDCl_3$; ppm) δ_H 5.35 (t, *J* = 3 Hz, 1H), 5.15 (dd, *J* = 8.5, 15 Hz, 1H), 5.01 (dd, *J* = 8.5, 15 Hz, 1H), 3.53 (m, 1H), 1.01 (s, 3H), 0.68 (s, 3H). ^{13}C-NMR (125 MHz; $CDCl_3$; ppm) δ_C 140.73 (C-5), 121.74 (C-6), 71.82 (C-3), 56.85 (C-17), 56.75 (C-14), 51.23 (C-9), 45.80 (C-24), 42.27 (C-13), 42.20 (C-4), 39.66 (C-12), 37.23 (C-1), 36.49 (C-10), 36.14 (C-20), 33.91 (C-22), 31.88 (C-8), 31.63 (C-2), 31.61 (C-7), 29.10 (C-25), 28.42 (C-16), 25.42 (C-23), 24.36 (C-15), 23.03 (C-28), 22.67 (C-11), 19.83 (C-26), 19.40 (C-19), 19.01 (C-27), 18.69 (C-21), 12.27 (C-29), 11.98 (C-18). EIMS (414.31, calcd. 414.38). Elemental analysis: Found C, 82.22%; H, 12.02%; O, 3.77%; $C_{29}H_{50}O$ requires C, 83.99%; H, 12.15%; O, 3.86%.

4α-Carboxy-4β-methyl-5α-cholesta-8,24-dien-3β-ol **(4):** White powder; melting point: 125-127°C. FT-IR V_{max} (KBr, $CHCl_3$, cm^{-1}): 3404, 2961, 2952, 1706, 1668, 1440, 1024, 970, 772, 665. ^{1}H-NMR (500 MHz; $CDCl_3$; ppm) δ_H 5.11 (t, *J* = 3.6 Hz, 5H), 1.18 (s, 3H), 1.06 (s, 3H). ^{13}C-NMR (125 MHz; $CDCl_3$; ppm) δ_C 170.96 (-COOH), 145.10 (C-9), 139.50 (C-25), 124.19 (C-8), 121.51 (C-24), 80.83 (C-3), 58.91 (C-4), 55.24 (C-17), 55.11 (C-14), 46.65 (C-5), 41.57 (C-13), 39.67 (C-12), 37.59 (C-20), 37.01 (C-1), 36.71 (C-10), 33.23 (C-22), 32.44 (C-7), 30.97 (C-2), 28.29 (C-16), 25.84 (C-23),23.58 (C-15), 23.41 (C-6), 21.23 (C-11), 18.13 (C-19), 17.88 (C-21), 14.03 (C-18). HR-MS (-ve mode) for $C_{29}H_{46}O_3$ at m/z 441.3043 (Calcd. 442.3447). Elemental analysis: Found C, 76.87%; H, 9.28%; O, 10.92%; $C_{29}H_{46}O_3$ requires C, 78.68%; H, 10.47%; O, 10.84%.

2.4. Protein denaturation using egg albumin[16]

A reaction mixture (3mL) comprises 0.45 mL of egg albumin (hen's egg), 2.5 mL phosphate buffer (pH: 6.8) and 0.05 mL of different concentration of test samples (10-100 µg/mL). The samples were incubated at 37ºC for 20 min and then the temperature was increased to 57ºC for 3 min. After this, the samples were cooled for some time and the absorbance was determined by spectrophotometer at 660 nm. Distilled water was served as control and Diclofenac sodium was used as reference drug. The percentage inhibition was determined by the formula:

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

3. Results and Discussion

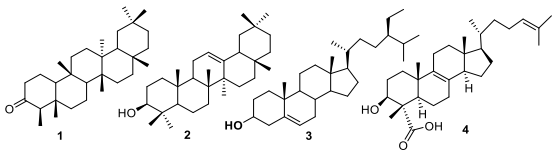

Fig. 1: Structure of compounds (**1**-**4**)

Compound **1** was obtained as a white amorphous solid (800 mg) with molecular formula $C_{30}H_{50}O$, by elemental analysis and mass spectrometry, EIMS (m/z 426.34 $[M]^+$). In FT-IR spectrum, the absorption band at 1715 cm^{-1} was assigned for the carbonyl group in the molecule. The presence of carbonyl group was confirmed by the characteristic peak displayed by ^{13}C NMR at δ_C 213.39. The ^{13}C-NMR displayed two groups of thirty carbon signals, each with significant differences in chemical shift. The ^{1}H NMR signals at δ_H 1.18, 1.05, 1.00, 0.95, 0.88, 0.87 and 0.72 were assigned for methyl groups. A quartet signal at δ_H 2.25 with *J* = 6.5 Hz was assigned for the methane group. There was observed no vinylic proton signal. There was observed 21 superposed signals at δ_H 1.77–1.33. These spectroscopic data revealed that the compound **1** seemed to be friedelin (Figure 1) and it was confirmed by comparison of their spectral data with the literature [17].

Compound **2** was obtained as a white amorphous solid (65 mg) with molecular formula $C_{30}H_{50}O$, by elemental analysis and mass spectrometry, EIMS (m/z 426.27 $[M]^+$). In FT-IR spectrum, the absorption band at 3372 cm^{-1} was assigned for the presence of hydroxyl group. The ^{1}H NMR signals at δ_H 1.16, 1.14, 1.09, 1.04, 1.03, 0.99, 0.95, 0.85 were assigned for methyl groups. In ^{13}C NMR spectrum displayed recognizable signals at δ_C141.60 and 122.01 due to alkene double bonds at C-13 and C-12 respectively. The presence of olefinic proton was confirmed by the ^{1}H NMR signal at δ_H 4.49. Based on these evidences, the structure of the molecule was assigned as *β-amyrin* (**2**) (Figure 1) and confirmed by comparison of their spectral data with the literature [18].

Compound **3** was obtained as a white amorphous solid with molecular formula $C_{29}H_{50}O$, by elemental analysis and mass spectrometry, EIMS (m/z 414.31).

The FT-IR spectrum indicated the presence of hydroxyl group (3421 cm^{-1}) and C=C (1666 cm^{-1}) moiety in the molecule. In the ^{1}H NMR spectrum, singlets at δ_H 0.68 and 1.01 each integrated to three protons were assigned to methyls at quaternary carbons of steroids. Two doublets at δ_H0.81 and 0.83 integrated to three protons were assigned to side chain of methyls at C-27 and C-26. The triplet signal at δ_H 5.35 integrated to one proton was assigned to olefinic proton. The ^{13}C NMR signals displayed at δ_C 140.73 and 121.74 were assigned for the double bond present in between C-5 and C-6. The muliplet signal at δ_H 3.53 for one proton was assignable to a proton under hydroxyl group. The ^{13}C NMR revealed signals for methyls at 11.98, 19.40, 18.69, 19.83, 19.01 and 12.27. All these evidence led to characterize the compound as *β*-sitosterol (**3**) (Figure 1). The structure of the compound was further confirmed by comparing with literature [19].

Compound **4** was obtained as a white amorphous solid (44 mg) with molecular formula $C_{29}H_{46}O_3$, by elemental analysis and mass spectrometry, HRMS (m/z 441.3043). In FT-IR spectrum, the absorption band appears at 3404 cm^{-1} due to the presence of hydroxyl group in the molecule. The ^{13}C NMR spectrum at δ_C 170.96 was assigned for the COOH group. The ^{13}C NMR signals at 11.98, 19.40, 18.69, 19.83, 19.01 and 12.27 were assigned for the methyl groups. The structure of the molecule was assigned as 4*α*-Carboxy-4*β*-methyl-5*α*-cholesta-8,24-dien-3*β*-ol (**4**) (Figure 1) and confirmed by comparing with literature [20].

The anti-inflammatory activity of *S. acidum* extract and isolated compounds were studied towards the denaturation of egg albumin which are summarized in table 1 and depicted in figure 2. The compounds (**1-4**) exhibited significant anti-inflammatory activity at 100–500 µg/mL by protein denaturation inhibition compared with the standard Dichlofenac Sodium.

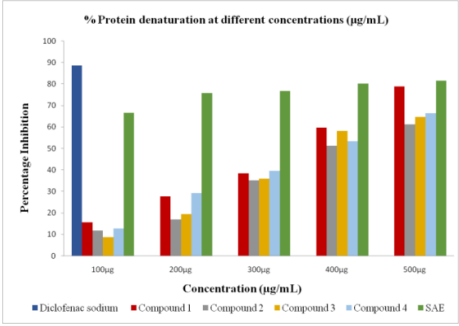

Fig. 2: Effect of samples on protein denaturation

In our study, two triterpenoids (**1-2**) and two steroids (**3-4**) were isolated from this *Sarcostemma* species. Compounds **1** and **4** were isolated for the first time from this species.

Table 1 *In vitro* protein denaturation of crude extract and isolated compounds of *S. acidum*

	Conc. (µg/mL)	Treatment(s)					
		Compound **1**	Compound **2**	Compound **3**	Compound **4**	EtOH Ext.	Standard
Absorbance	100µg	0.481±0.002	0.503±0.001	0.520±0.003	0.498±0.001	0.190±0.002	0.054±0.001
	200µg	0.414±0.003	0.474±0.001	0.460±0.001	0.404±0.003	0.138±0.003	
	300µg	0.351±0.005	0.370±0.002	0.365±0.001	0.345±0.001	0.132±0.001	
	400µg	0.230±0.001	0.278±0.003	0.239±0.002	0.266±0.003	0.113±0.001	
	500µg	0.124±0.001	0.221±0.002	0.201±0.001	0.191±0.002	0.105±0.001	
% Protein denaturation	100µg	15.61	11.75	8.77	12.63	66.66	90.52
	200µg	27.36	16.84	19.29	29.12	75.78	
	300µg	38.42	35.08	35.96	39.47	76.84	
	400µg	59.64	51.22	58.07	53.33	80.18	
	500µg	78.94	61.22	64.73	66.49	81.57	
Absorbance are expressed in mean ± SE of 3 replicates; Absorbance of Control = 0.570±0.002							

4. Conclusions

From the plant *S. acidum*, four compounds namely, Friedelin (**1**) and β-amyrin (**2**), β-sitosterol (**3**) and 4α-Carboxy-4β-methyl-5α-cholesta-8,24-dien-3β-ol (**4**) were isolated and structures were determined by extensive use of spectral analysis. Further, compound (**1-4**) along with extract of *S. acidum* exhibited anti-inflammatory activity comparing with standard Dichlofenac Sodium.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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