

Isolation and characterization of the chemical constituents of *Sonchus wightianus* of Nepalese origin

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Abstract

Phytochemical screening of *Sonchus wightianus* of Nepalese origin were carried out. Phytochemical screening showed the presence of fatty acids, triterpenes, sterols, polyphenols, emodins, quinones, glycosides, polyose and anthracenosides. Compounds β -sitosterol, β -sitosterol glycoside, 1-Hexacosanol and Hexadecanoic methyl ester were isolated. 1-Hexacosanol and Hexadecanoic methyl ester are isolated for the first time from *Sonchus wightianus*.

Keywords: Phytochemical Screening, *Sonchus wightianus*, β -sitosterol, 1-Hexacosanol

Introduction

Sonchus is a perennial herb. The genus *sonchus* comprises 50 species distributed in Euroasia, around the Mediterranean and tropical Africa. The word “*Sonchus*” comes from the Greek word meaning “hollow” which refers to hollow stems. Some of the important species of the genus are *S. alpinus*, *S. arvensis*, *S. asper*, *S. macrocarpus*, *S. brachyotus*, *S. kirkii*, *S. wightianus*, *S. hymanii*, *S. oleraceus*, *S. crataegifolium*, *S. tuberifer svent* etc.¹ *Sonchus wightianus* is distributed throughout Nepal at an altitude of 600-2,500m, Afganistan, Pakistan, India and Sri Lanka.² It is a perennial herb with hollow stems and bitter milky juice. The alternate leaves are variable in size with prickly edges and pointed lobes. The flowers are bright yellow in color and blossom from June to September. It is commonly called as Sow Thistle and local name is Ban raayo or Dudhe. Numerous pharmacological activities on the genus *sonchus* have been reported. The leaves of the *S. wightianus* are used in earache by rural communities of India.³ The juice of *S. alpinus* is useful in deafness, gout, and old age. The roots of *S. arvensis* are used in cough, bronchitis, and asthma. The leaves are applied to swellings, while its latex is used for the treatment of eye diseases.⁴ The leaves and roots of the plant are used in indigestion and as a febrifuge, while its roots act as a vermifuge. Its stems are given as a tonic and sedative.⁴ In China, the extract of *Sonchus* species is used in appendicitis, tonsillitis and sore throat.

Despite the great significance of *Sonchus* species, not much work has been reported on the chemical and biological investigation of the *Sonchus wightianus* of Nepalese origin. Thus the present work is concentrated on the phytochemical screening of the aerial parts of *Sonchus wightianus*, isolation and identification of chemical constituents.

Experimental

General

Melting points were determined by using melting point apparatus from Griffin and George Company Limited (UK). Column chromatography was performed using silica gel of mesh no. 60-120. For thin

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layer chromatography "Silica gel-G" was used. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectra were recorded on UNITY plus-500 NMR spectrometer using TMS as internal standard and NMR spectra were recorded in chloroform (CDCl_3), DMSO, deuterated pyridine ($\text{C}_5\text{D}_5\text{N}$). The Chemical shifts values were expressed in δ (ppm) value. EI-MS measurements were carried out on BioTOF II triple quadruple mass spectrophotometer.

Collection of plant material

The plant material chosen for the study was the aerial part of the *Sonchus wightianus*. The material was collected from Kavreplanchowk district of Nepal. The aerial part of the plant was air dried in shade and made powder.

Phytochemical Screening

The method employed for phytochemical screening was based on the procedure put forward by Prof. I. Ciulei.⁵ This method involves selective and successive extraction of the plant material by using solvent of increasing polarity. By using different specific reagents the presence of main groups of natural products is analyzed.

Extraction and isolation of compounds

The extraction of chemical constituents of plant material powder i.e. aerial part of *Sonchus Wightianus* (500gm) was carried out by using Soxhlet apparatus successively with petroleum ether, ethyl acetate and methanol.

The petroleum ether fraction (10g) was subjected to column chromatography. The slurry was prepared by mixing petroleum ether fraction(10g) with silica (12.0g). The slurry was applied to the column and the column was eluted subsequently with the solvent of increasing polarity in the order of petroleum ether, chloroform, ethyl acetate and methanol. On eluting the column with petroleum ether- CHCl_3 (70:30), compound **SW-1(1-hexacosanol)** was obtained. On eluting the column with EtOAc- CHCl_3 (10:90), compound **SW-2 (β -Sitosterol)** was obtained.

Since the methanolic extract of the plant contained a mixture of a large number of chemical constituents of wide-range polarities, it is fractionated by solvents of different polarities like Hexane, Chloroform, EtOAc and MeOH respectively using vacuum liquid chromatography.

The methanol fraction (8g) was subjected to column chromatography. The slurry was prepared by mixing methanol fraction(8g) with silica (11g). The slurry was applied to the column and the column was eluted subsequently with the solvent of increasing polarity in the order of petroleum ether, chloroform, ethyl acetate and methanol. On eluting the column with 75% of EtOAc in CHCl_3 , compound **SW-3 (β -Sitosterol 3-O- β -D glycoside)** was obtained.

The chloroform fraction of methanol extract (2g) was subjected to column chromatography. The slurry was prepared by mixing petroleum ether fraction(2g) with silica (3g). The slurry was applied to the column and the column was eluted subsequently with the solvent of increasing polarity in the order of petroleum ether, chloroform, and methanol. On eluting column with 10% CHCl_3 in pet.ether, compound **SW-4 (Hexadecanoic methyl ester)** was obtained.

Result and Discussion

Phytochemical screening of S.wightianus

By the procedure given by Prof.I.Ciulei., the phytochemical screening of the plant *s. wightianus* was performed. The result are presented as below.

Photochemical screening of various extracts

S.N.	Group of the compounds	Pet. Ether Extract	EtOAc Extract	MeOH extract
1	Volatile oil	-	-	-
2	Basic alkaloids	-	-	-
3	Carotinoids	-	-	+
4	Terpenes/sterol	+	+	-
5	Fatty acid	+	+	-
6	Cumarins	-	-	-
7	Flavone aglycones	-	-	+
8	Emodins	+	+	+
9	Quinones	+	+	-
10	Polyphenols	-	+	+
11	Glycosides	-	+	-
12	Saponins	-	-	+
13	Anthrocenocides	-	-	+

+ = Presence, - = Absence

Analysis and Identification of Isolated chemical constituents

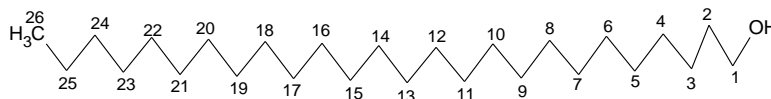
Compound: SW-1 (1-Hexacosanol)

Compound SW-1, white amorphous substance was found which was soluble in chloroform. The melting point of this compound was found to be 72⁰c. It was found to single spot on TLC with R_f value 0.46 in 75% CHCl₃/Pet.ether.

In ¹H-NMR spectrum triplet at δ 0.90 accounted for one methyl group. The big multiplet at δ 1.25 accounted for hydrogens of - CH₂ units. The triplet at δ 3.65 account for the protons of - CH₂ unit attached with electronegative oxygen atom.

¹³C-NMR showed the presence of 7 types of carbon atoms which have chemical shift at δ 63.01, 32.90, 32.04, 29.694, 25.83, 21.3 and 13.8 were accounted for carbon numbers 1, 2, 24, 4 to 23, 3, 25, 26 respectively.

On the basis of all these spectral analysis, the compound was identified as long chain fatty alcohol, 1-Hexacosanol⁶ From our best knowledge of literature survey, this compound is isolated for the first time from *Sonchus wightianus*.



1-Hexacosanol

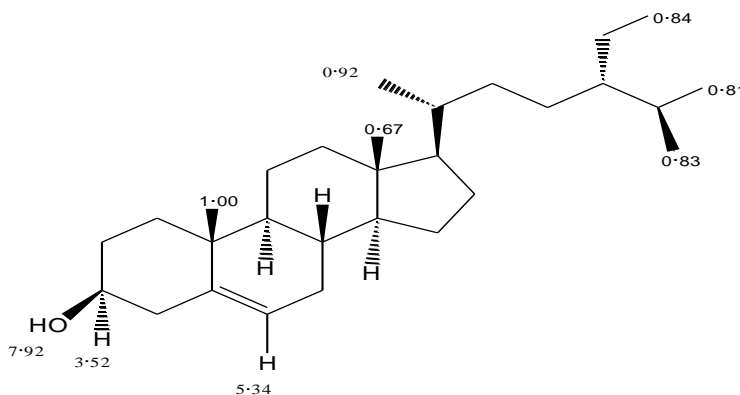
Compound: SW-2 (β -Sitosterol)

White amorphous solid was found which was soluble in chloroform, ethylacetate and methanol. The melting point of this compound was found to be 130⁰C. The compound had R_f value of 0.43 in 20% ethyl acetate in hexane. By Co-TLC with authentic sample, it was found that the compound have the same R_f value of that β -Sitosterol. The mass spectrum showed M⁺ at m/z at 414 corresponding to the molecular formula (C₂₉H₅₀O).

The IR spectrum showed presence of –OH group (3434 cm⁻¹). The absorption bands at 2961 cm⁻¹ and 1383 cm⁻¹ were due to the C-H stretching and bending respectively. A peak at 1063 cm⁻¹ showed the presence of C-O stretching.

The ¹H-NMR spectrum showed two singlets at δ 1.00 and at 0.67 are methyl group of C-19 and C-18 respectively. The doublets at δ 0.92 (d, J = 6.1 Hz); 0.81 (d, J = 6.9 Hz); 0.83 (d, J = 6.9 Hz) and 0.84 (t, J = 7.3Hz) were accounted for methyl group at C-21, C-25, C-27 and C-29. A doublet at δ 5.35 (d, J = 20.5 Hz) in ¹H-NMR can be accounted for an olefinic proton at C-6. Other multiplet at δ 3.52 (ddd, J = 13.2, 16.0, 16.0 Hz) equivalent to a singlet proton was assigned for the proton of C-3. The low field signal may be due to the attachment of β -OH group at C-3 carbon. Thus, the assignment of hydroxyl group at C-3 and the double bond at C-5 were assigned accordingly. Three multiplets equivalent to two protons each appeared at δ 1.83, 2.00 and 2.27 were assigned for 3-CH₂ groups. The remaining protons have appeared as multiplets at δ 1.05-1.65.

The respective fragments were assigned by the mass spectrum with m/z 399, 396, 381, 329, 273, and 255. From all these spectral analysis, the compound was β -Sitosterol, previously isolated from large number of Nepalese plant species.⁷

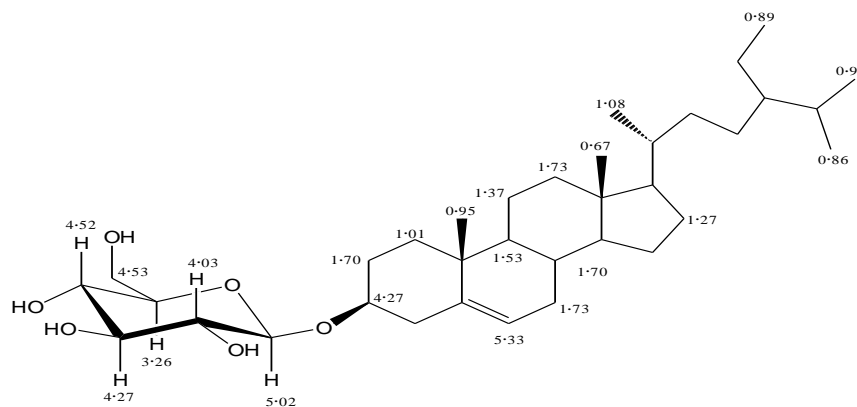


β -Sitosterol With Chemical Shift Value Of ¹H-NMR

Compound: SW-3 (β -Sitosterol 3-O- β -D glycoside)

Compound SW-3 melting point above 284°C with R_f value 0.66 (1:9, methanol: chloroform) was white crystalline compound. ¹H- NMR spectrum pattern was similar to that of β -sitosterol with some additional peaks relating to a carbohydrate moiety. The multiplet at δ 4.27 was assigned for the proton of C-3. Its deshielding may be due to attachment of β -O-glucosyl moiety at C-3 carbon. The proton signals at δ 3.96, 4.03, 4.27, 4.52, 4.53, and 5.02 in the deshielded region were assigned for respective Gly- 5, Gly-2, G-3, Gly-4, Gly-6, and Gly-1 protons of glucosyl moiety. ¹³C-NMR was also similar to β -sitosterol with additional six peaks confirming the glucose ring. It showed the presence of 35 carbons of which chemical shift at δ 102.60, 75.33, 78.4, 71.74, 78.16, and 62.88 were assigned for carbon atoms of glucosyl moiety.

Both ¹H-NMR and ¹³C-NMR values of glucosyl moiety were confirmed by comparing with authentic values of glucose. Further the above structural arrangement was confirmed by two-dimensional spectroscopy (¹H-¹H COSY, HMBC and HMQC). They gave correlations of protons and carbon atoms. They correlated the presence of glycosyl moiety at 3 position of steroid framework All the spectral values obtained from ¹³C-NMR, ¹H-NMR, HMBC and HMQC identified the compound as β - Sitosterol glycoside.⁸



β -Sitosterol 3-O- β -D glycoside With Chemical Shift Value of ¹H-NMR

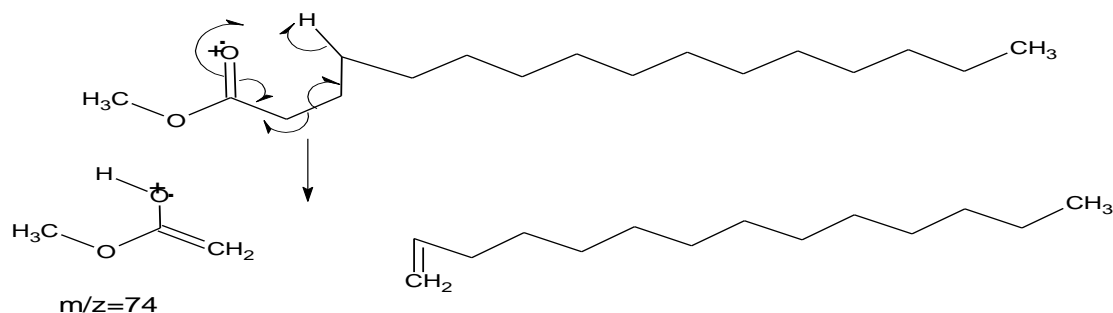
Compound: SW-4 (Hexadecanoic methyl ester)

Compound SW-4, white amorphous compound was found to single spot on TLC with R_f value 0.43 in 15% CHCl₃/Pet.ether. The compound had melting point 74°C. The mass spectrum showed M⁺ at 270.4 corresponding to the molecular formula C₁₇H₃₄O₂.

In ¹H-NMR spectrum triplet at δ 0.90 (J=13.1, 3H) accounted for one methyl group. The singlet at δ 3.64 (J=3.52, 3H) accounted for methyl group of (-OCH₃) unit. The multiplet at δ 2.29 (J= 17, 2H) were due to the hydrogens of -CH₂ attached to C=O. The broad multiplet at δ value 1.19 to 1.33 was due to the 13 - CH₂ groups (26 H).

Further the structure was confirmed by mass spectrum analysis. The molecular ion at m/z 270 is clearly seen. The most characteristic and base peak is m/z 74 resulting the McLafferty rearrangement. There is no prominent peak at [M-OH] and [M-COOH]. These represent there is no short chain. Beside the McLafferty rearrangement peak there is series of hydrocarbon clusters at interval of 14 mass units.

On the basis of all these spectral analysis, the compound was identified as Hexa decanoic methyl ester.⁹ From our best knowledge, this compound is isolated for the first time from *Sonchus wightianus*.



The mass fragmentation patterns of SW-4

Conclusions

Phytochemical screening of different extracts of *Sonchus wightianus* showed the presence of fatty acids, triterpenes, sterols, polyphenols, emodins, quinones, glycosides, polyose and anthracenosides. Compounds β -sitosterol and 1-Hexacosanol were isolated from petroleum ether extract of the plant and identified. Similarly, compounds β -sitosterol glycoside and Hexadecanoic methyl ester were isolated from methanol extract and identified.

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