

Isolation and tentative characterization of stigmastane type steroids from the roots of *Senecio rufinervis* D.C.

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Abstract

Introduction: *Senecio rufinervis* D.C. locally known as Bhanwa contains sesquiterpene rich essential oil having analgesic and antimicrobial potential. To the best of our knowledge the plant has never been investigated for its secondary plant metabolites except its essential oil content. The objective of the present study was to isolate and characterize its phytoconstituents. **Material and Methods:** Petroleum ether extract from the roots of *S. rufinervis* D.C. was subjected to column chromatography yielded stigmastane type steroids. **Results:** The structure of the two compounds was assigned on the basis of chemical tests and spectral studies. **Conclusion:** The plant has never been investigated for the isolation of higher natural products other than essential oil, we have for the first time isolated steroids from the plant and tentatively characterized the isolated stigmastane type steroids, thus the plant has the potential for further isolation and absolute characterization of other natural products.

Key words: *Senecio rufinervis*, Petroleum ether extract, Steroids, MS, ¹H NMR, ¹³C NMR

INTRODUCTION

Senecio is one of the largest genera of the flowering plants and despite the separation of many species into other genera it still contains over 1000 species of varied form. Interest in the chemical study of *Senecio* species is stimulated by the observation that several species belonging to it are used in traditional medicine.^[1] A large variety of sesquiterpenoids,^[2] diterpenoids,^[3] triterpenoids,^[4] pyrrolizidines, and shikimic acid have been characterized from *Senecio* Species.^[5,6] Eremophilanes were isolated from *Senecio mairetianus*,^[7] *Senecio Nemorensis*, and *Senecio aegyptius*.^[8,9] Triterpenoids were isolated from the chloroform-methanol extract of *Senecio pseudotites*.^[10] Two diastereomeric triterpenes have been isolated from the aerial parts of *Senecio selloi*.^[11] Alkaloids were isolated from the roots of *Senecio macedonicus*.^[12] *Senecio rufinervis* is an aromatic plant containing an essential oil which is produced by many plants and confirms analgesic and anti-inflammatory activities.^[13-16] The plant has never been investigated for its

secondary plant metabolites except its essential oil content to the best of our knowledge.

MATERIALS AND METHODS

S. rufinervis D.C. was collected from Kilbury region Nainital (altitude 2600 m), Uttarakhand, India in September 2013 and authenticated by Botanical Survey of India, Dehradun, India. A voucher specimen (No. 112287) was deposited in the Applied Chemistry Department of Birla Institute of Applied Sciences Bhimtal, Nainital, Uttarakhand, India. The roots (750 g) were separated from the plants washed with water and dried under shade. The dried and coarsely powdered roots were successively extracted with petroleum ether

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(24 h), ethyl acetate (24 h), and 50% methanol in water (24 h) using soxhlet apparatus. Each fraction was concentrated under reduced pressure. Petroleum ether extract (16.6 g) was investigated using phytochemical tests and gave the Liebermann-Burchard test, Dragendorff's test, Shinoda test. Petroleum ether extract (11 g) was chromatographed over silica gel column and eluted with a solvent system of increasing polarity (petroleum ether:benzene, benzene:chloroform). A total of 77 fractions were collected. Fraction No. 44-48 (100% benzene) and fraction no. 60-62 (30% chloroform in benzene) were pooled together on the basis of TLC. Fraction 44-48 yielded 613 mg of the compound and fraction 60-62 yielded 125 mg of the compound. Both the compounds were further purified by repeated column chromatography to get the Compound A (60 mg) and Compound B (50 mg). Both the compounds gave the Liebermann-Burchard of steroids.^[17] The characterization of the isolated compounds was done by using spectroscopic techniques (MS, IR, ¹H and ¹³C NMR

RESULTS

Phytochemical analysis of the isolated compounds confirmed its steroidal skeleton. Compound A and B were isolated as brown colored wax and yellow colored liquid with a bitter almond like smell, respectively. Compound A [Figure 5] was isolated as brown color wax. The molecular formula of compound A was revealed as $C_{31}H_{48}O_4$ by the MS [Figure 1] data (m/z 485, $[M+H]^+$). The IR spectrum [Figure 2] showed the presence of hydroxyl (3435 cm^{-1}), carbonyl of ester (1736), C-O (1165 cm^{-1}) and CH stretch of $C=C$ (3019 cm^{-1}) group.^[18] ¹H NMR spectrum [Figure 3] showed the presence of two methyl singlet (δ 0.97 (s) and δ 1.25 (s) typical of steroidal nature.^[19] Two methyl doublets δ 1.00, signals at δ 5.51, δ 5.53 showed the presence of doubly bonded protons. The ¹³C NMR spectrum [Figure 4] indicated 31 carbon signals, including an ester carbonyl group at δ 173.5 and four double bonded carbons at δ 128.0, 128.2, 130.2, and 130.4.^[20,21]

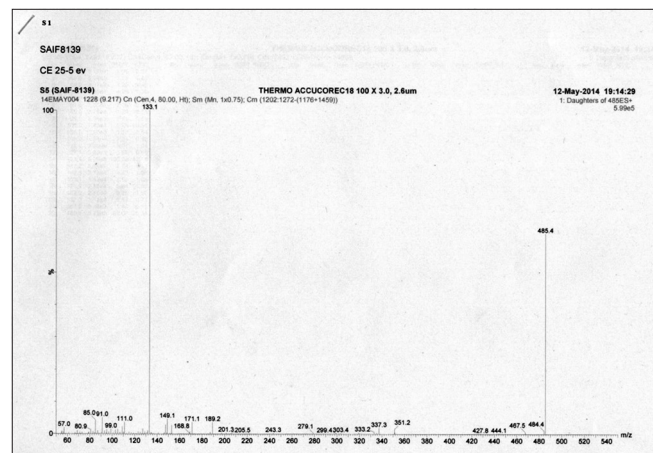


Figure 1: Mass spectrum Compound A

Compound B [Figure 5] was isolated as a yellow colored liquid. The molecular formula of compound B was revealed as $C_{31}H_{48}O_4$ by the MS data [m/z 485, $(M+H)^+$]. The spectral studies of the compound exactly same as we have discussed for compound A except the presence of a signal in ¹H NMR spectrum at δ 5.42 and 5.16 ppm revealed the presence of doubly bonded protons. ¹³C NMR spectrum also showed the presence doubly bonded carbons signaled at δ 123.2, 136.4, 142.4, and 124.0 ppm.

DISCUSSION

Steroids are widely distributed photochemical in the plants. In the present work, we have isolated steroids from the

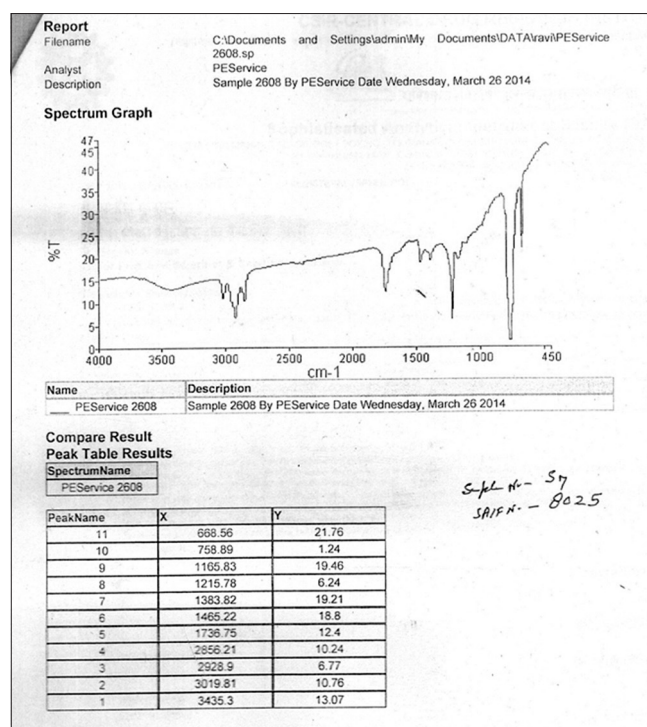


Figure 2: Infrared spectrum Compound A

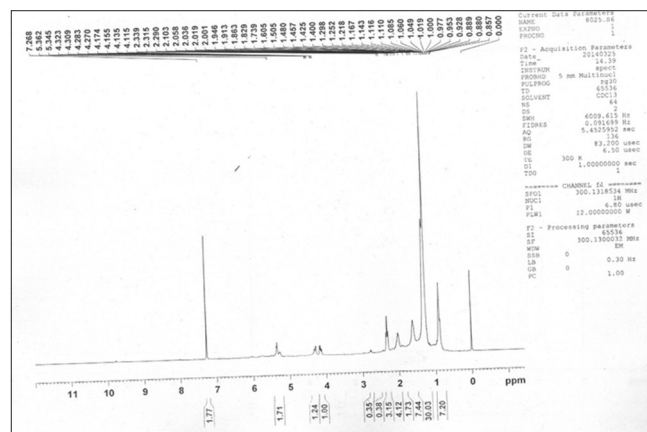


Figure 3: ¹H NMR spectrum Compound A

Table 2: Contd...

Position	Group	¹ H NMR (300 MHz, CDCl ₃) δ ppm	¹³ C NMR (300 MHz, CDCl ₃) δ ppm
6	CH	3.45	70.2
7	CH	5.42	123.2
8	C	-	136.4
9	C	-	142.4
10	C	-	35.2
11	CH	5.16	124.0
12	CH ₂	1.72	40.1
13	C	-	51.2
14	CH	2.10	60.4
15	CH ₂	1.62	28.0
16	CH ₂	2.33	27.2
17	CH	1.86	58.8
18	CH ₃	0.67	21.0
19	CH ₃	1.25	17.2
20	CH	1.60	39.2
21	CH ₃	1.06	19.5
22	CH	5.3	130.4
23	CH	5.3	130.4
24	CH	2.05	39.6
25	CH	1.94	29.9
26	CH ₃	1.29	39.9
27	CH ₃	1.86	29.3
24'1	CH ₂	1.00	22.9
24'2	CH ₃	1.00	22.9
3 Ac	C=O		173.5
	CH ₃		21.0

NMR: Nuclear magnetic resonance

our knowledge, the plant has never been investigated for the isolation of higher natural products other than essential oil, thus the plant has the potential for further investigations.

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