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Saussurea heteromalla (D. Don) Hand.-Mazz.: A new source of arctiin, arctigenin and chlorojanerin

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Saussurea heteromalla (D. Don) Hand.-Mazz. (Asteraceae), a Himalayan herb, has been investigated phytochemically for the first time in detail and the plant is being reported here as a new source of two lignans, *i.e.* arctigenin and its glycoside arctiin, and one sesquiterpene lactone, the chlorojanerin. The structures of these compounds, determined by UV spectra, LC-MS, ¹H NMR and ¹³C NMR spectroscopy are discussed. These compounds have been found pharmacologically important; arctigenin and arctiin are known to have anti-inflammatory activity and chlorojanerin has been investigated for the anti-ulcer and anti-viral properties.

Keywords: *Saussurea heteromalla*, arctiin, arctigenin, chlorojanerin, anti-inflammatory, anti-ulcer, anti-viral

The genus *Saussurea* DC. (Family – Asteraceae) is widely distributed in the higher altitudes of Indian Himalayas from Jammu and Kashmir eastwards to Arunachal Pradesh. *Saussurea heteromalla* is the only species seen profusely growing in the subtropical and also in the tropical Himalaya extending its distribution to the Shivalik hills¹. It is a 60-160 cm tall herb with lyrate-pinnatifid basal leaves in a rosette and upper leaves with white tomentose beneath. The heads have purple flowers with cottony or pubescent involucral bracts in 3-5 series. The tiny fruits (achenes) are 4-5 angled with muricated surface and possess white pappus hairs for dispersal². Some species of genus *Saussurea* like *S. costus* (Falc.) Lipschitz, are widely used in several indigenous systems of medicine for the treatment of various ailments, *viz.* asthma, inflammatory diseases, ulcer and stomach disorders³. The *S. heteromalla* also finds mention in Indian ethnomedicines. The seeds are used as carminative and for treating horse bite; the crushed leaves are applied for healing wounds^{4,5}.

This is the first report on detail chemical investigation on *S. heteromalla*. We have isolated biologically important arctigenin, arctiin and chlorojanerin from *S. heteromalla* and thus the species, which is easily cultivable at the lower altitudes of Himalaya, becomes a novel source of the compounds we are reporting. Arctigenin and arctiin (a derivative of arctigenin), reported for anti-inflammatory activity^{6,7}, have been isolated from species like *Arctium lappa*, *Wikstroemia viridiflora*, *Bupleurum fruticosum*, *Cnicus benedictus*, *Saussurea conica*, *Forsythia viridissima*, *Ipomoea cairica*, *Trachelospermum asiaticum*, and *Centaurea imperialis*. The chlorojanerin, isolated from the species like *Centaurea scoparia*, *Centaurea janeri* and *Jurinea derderioides*, has been investigated for anti-ulcer and anti-viral properties^{8,9}.

Results and Discussion

The ethyl acetate soluble fraction of the dichloromethane : methanol (1:1) extract of *Saussurea heteromalla* afforded three compounds (**1-3**). Compound **1** was obtained as white powder. LC-MS analysis of this gave the molecular weight as 534, corresponding to both positive and negative mode. Compound **2** showed molecular weight as 372. The general features of ¹H NMR spectra and mass fragmentation pattern of compound **1** resembles with those of compound **2**, but with a difference of one sugar unit between them. Mass spectra of compound **3** shows two peaks at *m/z* 421 [M+Na]⁺, 423 [M+Na+2]⁺ separated by 2 *m/z* unit and having intensity ratio 3:1; this pattern reveals presence of one chlorine atom. The spectral data, *viz.* UV-Vis, mass and NMR (¹H and ¹³C) of the compounds **1-3** were found to be in agreement with the published data¹⁰⁻¹³. The compounds **1** and **2** were established as the lignans, arctiin and arctigenin respectively and compound **3** was established as a sesquiterpene lactone, chlorojanerin (**Figure 1**).

This is the first report on the detail phytochemical investigation of the plant *S. heteromalla*. Interestingly, the compounds isolated and reported from *S. heteromalla* here, *i.e.*, arctigenin, arctiin and chlorojanerin are pharmacologically important. The arctiin and arctigenin have been reported for anti-

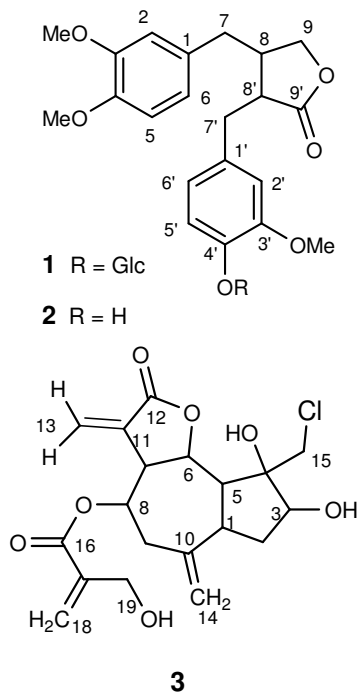


Figure 1 — Chemical structures of compounds 1-3

inflammatory activity and chlorojanerin has been investigated for its antiviral and anti-ulcer properties in literature. The plant is reported here as a new source of the above three useful compounds.

Materials and Methods

The whole plant materials of *S. heteromalla* were collected from the foothills of Himalayas in April 2001 and May 2008 and characterized by Arvind Saklani. The first collection was done from Kangra, Himachal Pradesh and the extracts prepared from this collections were stored at a low temperature in in-house plant extract library. The second collection was made from Nainital district in Uttarakhand. The voucher specimen (Collection No.- AS00255) has been deposited with the herbarium of Natural Products Botany Department, Piramal Life Sciences, Mumbai for future reference.

General Procedure

HPLC: Shimadzu LC-2010CHT Liquid Chromatograph machine (Lichrosphere RP-18 column, 125 × 4 mm, 5 μ); NMR spectra: Bruker 300 MHz in CDCl₃ with TMS as internal reference; mass spectrometer: Bruker Daltonics ESI-Q-TOF Micro TOFQ; Preparative HPLC: Waters Prep LC 4000 with Waters™ 486 tunable absorbance detector, C-18 seppak cartridge (1 g) from Phenomenax: CombiFlash®

Sq 16X Teledyne Technologies Company ISCO attached with UV/VIS detector, RediSep® Flash Column silica 12 g Teledyne Isco: all the solvents used for prep-HPLC purification were of HPLC grade and solvents used for normal phase chromatography was distilled from commercial solvents of Rankem.

Preparation of extracts and isolation

The air-dried and powdered material of the whole plant *S. heteromalla* (400 g) was extracted with 2 L dichloromethane : methanol (1:1) at room temperature for 12 hr and filtered. The process was repeated with the mass 2 more times. All 3 extracts were then combined and dried on a rotary evaporator under reduced pressure to give a dark brown residue (15 g). This extract was suspended in water : methanol mixture (8.5:1.5, 300 mL) and partitioned with petroleum ether (300 mL × 5) followed by EtOAc (300 mL × 5). The ethyl acetate soluble portion was combined and dried on a rotary evaporator under reduced pressure to give a brown residue (5.5 g). This was further purified by RediSep® silica flash-column using CombiFlash® instrument. The column was eluted with chloroform and methanol gradient (100% chloroform to 20% methanol in chloroform in 60 min, detection 254 nm) to obtain three fractions (F1–F3).

Fraction 1 (250 mg) was further purified by C-18 seppak cartridge with water : acetonitrile gradient system. All the fractions were then pooled on the basis of TLC and lyophilized to obtain 25 mg of white coloured powder of compound **1**. Fraction-2 (15 mg) was purified on semi preparative HPLC (mobile system: water : acetonitrile (98:2), to 100% acetonitrile in 30 min; detection 220 nm; flow rate 5 mL/min) to yield white colored 2 mg of compound **2**. Fraction-3 (210 mg) was purified by C-18 seppak cartridge using mixture of water and acetonitrile in step gradient mode to obtain compound of white crystals. This was further subjected to semi preparative HPLC (mobile system: water : acetonitrile (49:1), to 100% acetonitrile in 30 min; detection 220 nm; flow rate 5 mL/min) to give 69 mg of white crystalline powder, Compound **3**.

Compound **1** White amorphous powder, UV λ_{max} (MeOH): 278, 230 nm; LC-MS (MW): *m/z* 535.2 [M+H]⁺, 569 [M+Cl]⁺, 1113.4 [2M+HCOO]⁺, 552 [M+NH₄]⁺, 557 [M+Na]⁺, 573 [M+K]⁺; ¹H NMR (300 MHz, CDCl₃): δ 6.4 (1H, brs, 2H), 6.75 (1H, d, *J* = 8.2 Hz, H-5'), 6.56 (1H, dd, *J* = 8.2 Hz, 1.8, H-6'), 2.54 (2H, m, H-7'), 2.51 (1H, m, H-8), 3.57 (1H, dd, *J* = 8.1

Hz, H_a-9), 4.11 (1H, dd, $J = 8.1$ Hz, H_b-9), 6.61 (1H, d, $J = 1.6$ Hz, 2 -H), 6.9 (1H, d, $J = 8.2$ Hz, H-5), 6.58 (1H, dd, $J = 8.2$ Hz, 1.6, H-6), 2.83 (2H, m, 7 -H), 2.4 (1H, m, 8 -H), 3.81 (3H, COMe), 3.77 (3H, OMe), 3.67 (3H, COMe); glucose: 4.81 (1H, d, $J = 7.2$ Hz) for hemiacetal proton of sugar unit, multiplet in the range of 3.41 to 4.96 corresponds to the other protons of sugar; ¹³C NMR (75 MHz, CDCl₃): δ 131.01 (C- 1), 112.09 (C-2), 149.12 (C-3), 56.16 (C-3 OMe), 56.14 (C4-OMe), 148.01 (C-4), 111.59 (C- 5), 120.80 (C-6), 38.0 (C-7), 41.4 (C-8), 70.75 (C-9), 130.0 (C-1'), 113.32 (C-2'), 149.0 (C-3), 56.13 (C-3', COMe), 145.0 (C-4'), 117.0 (C-5'), 122.0 (C-6'), 34.42 (C-7'), 46.80 (C-8'), 178.9 (C-9', C=O); glucose: 103.0 (C-1''), 73.3 (C-2''), 72.1 (C-3''), 69.6 (C-4''), 76.1 (C-5''), 61.2 (C-6''); Mol. Formula: C₂₇H₃₄O₁₁.

Compound 2. White amorphous powder, UV λ_{\max} (MeOH): 280, 222 nm; LC-MS (MW): m/z 373 [M+H]⁺, 389 [M+NH₄]⁺, 411 [M+K]⁺, 355 [M-OH]⁺, 137 (fragment-guiacyl), 151 (fragment-veratryl); ¹H NMR (500 MHz, CDCl₃): δ 6.48 (1H,d, $J = 1.9$ Hz, H-2), 6.77 (1H, d, $J = 7.5$ Hz, H-5), 6.57 (1H, dd, $J = 7.5$ Hz, 1.5 Hz, H-6), 2.57 (2H, m, H-7), 2.51(1H, m, H-8), 6.65 (1H, d, $J = 1.7$ Hz, H-2'), 6.85 (1H, d, $J = 8$ Hz, H-5'), 6.63 (1H, dd, $J = 8.5$ Hz, 1.8 Hz, H-6'), 2.98 (2H, m, H-7'), 2.49 (1H, m, H-8'), 3.92 (1H, dd, $J = 8.5$ Hz, 7.1 Hz, H-9_a), 4.16 (1H, dd, $J = 9.3$ Hz, 7.5 Hz, H_b -9), 3.84 ((C-3 OMe), 3.83 (C4-OMe), 3.87 (C-3, COMe); Mol. Formula: C₂₁H₂₄O₆.

Compound 3. White crystalline powder, UV λ_{\max} (MeOH): 190, 214 nm; LC-MS (MW): m/z 399 [M+H]⁺, 421 [M+Na]⁺, 423 [M+Na+2]⁺, 437 [M+K]⁺; ¹H NMR (300 MHz, CDCl₃): δ 3.64 (1H, ddd, $J = 9$ Hz, $J = 8.5$ Hz, H-1), 2.5 (1H, m, H_a-2), 1.6 (1H, dd, $J = 15$ Hz, 8 Hz, H_b-2), 4.18 (1H, brd, $J = 6.8$ Hz, H-3), 2.35 (1H, d, $J = 10$ Hz, H-5), 4.75 (1H, dd, $J = 11$ Hz, 9 Hz, H-6), 3.18 (1H, m, H-7), 5. 16 (1H, m, H-8), 2.68 (1H, dd, $J = 15$ Hz, 4.5 Hz, H_a-9), 2.4 (1H, d, $J = 15$ Hz, H_b-9), 6.2 (1H, d, $J = 3.6$ Hz, H_a-13), 5.6 (1H, d, H_b-13), 5.17 (1H, brs, H_a-14), 4.8 (1H, brs, H_b-14), 4.33 (1H, d, 11.6 Hz, H_a-15), 3.95 (1H, d, $J = 12.2$

Hz, H_b-15), 6.3 (1H, s, H_a-18), 5.9 (1H, s, H_b-18), 4.1 (2H, brs, H-19); ¹³C NMR (75 MHz, CDCl₃): δ 49.39 (C-1), 37.2 (C- 2), 77.2 (C-3), 84 (C-4), 57 (C-5), 77.2 (C-6), 46.6 (C-7), 76.12 (C-8), 37.26 (C-9), 141.6 (C-10), 136.2 (C-11), 169.0 (C-12), 122.8 (C-13), 117.51 (C-14), 45.98 (C-15), 164.8 (C-16), 136.2 (C-17), 126.3 (C-18), 61.8 (C-19); Mol. Formula: C₁₉H₂₃ClO₇.

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