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Research Article

PHYTOCHEMICAL INVESTIGATION OF *ARNEBIA BENTHAMII* (WALL. EX G. DON) I.M. JOHN ST.

***M.A. Rather[1], D.P. Pandey[2], Sanjay Kumar[3], Tasir Sharief Per[4] and Younis Rashid[4]**
[1]Department of Chemistry, Govt. Degree College, Doda-182202, J&K, India.
[2]Department of Chemistry, Govt. P. G. College, Uttarkashi-249 193, Uttarakhand, India.
[3]Botanical Survey of India, CGO Complex, DF Block, Salt Lake City, Kolkata 700 064, West Bengal,India
[4]Department of Botany, Govt. Degree College, Doda-182202, J&K, India
[*]Author for Correspondence: aslam.aslam2006@rediffmail.com

ABSTRACT
Arnebia Forssk. (Boraginaceae) is a genus of hispid herbs, mostly confined to Asia, with a few species occurring in the drier parts of north Africa. Seven species of *Arnebia* are known to occur in India. *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst., is an erect, herbaceous perennial and major ingredient of the commercial drug available under the name "gaozaban", which has antibacterial, antifungal, anti-inflammatory and wound- healing properties. The roots yield a red pigment, shikonin (a dye), which has several medicinal properties and is marketed under the trade name "ratanjot". The chloroform extract of air dried aerial parts of *A. benthamii* on repeated coloumn chromatography over silica-gel afforded β-sitosterol, β-sitosterol-β-d-glucoside, kaempferol, kaempferol-7-o-methyl ether and aromadendrin. The identification of these compounds was made by concerted use of spectral and chemical methods.

Keywords: *Arnebia benthamii, β-Sitosterol, β-Sitosterol-β-D-glucoside, Kaempferol, Kaempferol-7-O-methyl ether, Aromadendrin*

INTRODUCTION
Arnebia genus was first established by Pher Forsskal in 1775 mostly confined to Asia with a few species occurring in the drier parts of North Africa. The genus is represented in India by 10 taxa including 8 species and 2 varieties viz., *Arnebia bhattacharyyae* K. Ambrish & S.K. Srivast., *A. benthamii* (Wall. ex G. Don) I.M. Johnst., *A. euchroma* (Royle) I.M. Johnst., *A. guttata* Bunge, *A. hispidissima* (Sieber ex Lehm.) A.DC., *A. linearifolia* A.DC., *A. griffithii* Boiss., *A. nandadeviensis* K. Chandra Sek. & R.S. Rawal , *A. euchroma* var. *grandis* (Bornm.) Kazmi and *A. guttata* var. *thomsonii* (C.B. Clarke) Kazmi, the genera mostly distributed in Jammu & Kashmir, Himachal Pradesh and Uttarakhand in North-West Himalaya to Uttar Pradesh, Punjab and Rajasthan in other parts of India (Kumar and Srivastava, 2014). The species is also assessed as endangered (E) in 2010 at state of Himachal Pradesh and critical endangered (CR) in 2003 at J&K and Uttarakhand (Ved, *et. al.* 2016).

A. benthamii is an erect, herbaceous perennial, 40-80 cm high with stout root stock, Stems simple, fistular, hispid, densely covered with white trichomes of tuberculated base. Flowers pink or purple to blue, calyx lobed, linear-lanceolate up to 4.5 cm long. The species can be identified with its allied species by having tubular corolla, tube up to 2.5 cm long (Kumar and Srivastava, 2014). It grows in alpine meadows at sandy-moist places from 2700-3300m altitude. It is a major ingredient of the commercial drug available under the name "Gaozaban" which has antibacterial, antifungal, anti-inflammatory and wound- healing properties (Manjkhola and Dhar, 2002). The roots yield a red pigment, Shikonin (a dye), which has several medicinal properties and is marketed under the trade name "Ratanjot" (Kirtikar and Basu,1984). Arnebin 1 and Arnebin 3 obtained from the other species of this genus are reported to possess anticancerous property (Harborne and Baxter, 1996). *A. euchroma* exhibits potent anti-HIV activity (Kashiwada, *et. al.*, 1995). *Arnebia* species constitute important herbal drugs of indigenous systems of medicine. Besides medicinal value this is so popular in Asia for imparting a pleasing red color to food stuffs, oils, fats and various galenicals. A variety of

compounds, such as naphthaquinones, benzoquinones, alkaloids, triterpenoids, steroids and flavonoids have been isolated and characterized from *Arnebia*. The naphthaquinones are the major constituents reported from *Arnebia*. *A. benthamii* is used for treatment of diseases of the tongue, throat, cardiac and febrifuge (Singh and Kachroo, 1976 and Chopra *et. al.* 1986).

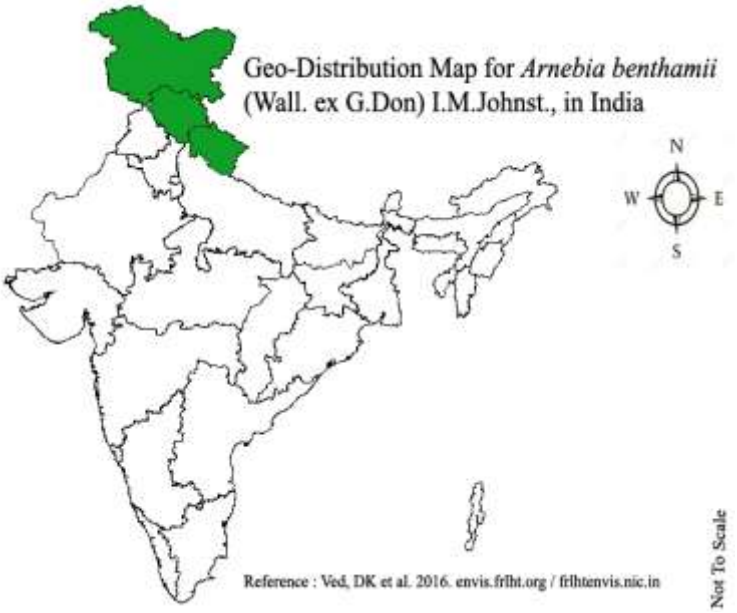


Figure 1: Geo-Distribution Map for *Arnebia benthamii* in India

MATERIALS AND METHODS

Plant Material:

Arnebia benthamii was collected from Daggan Dhar (Bhalessa) district Doda, Jammu & Kashmir, in July, 2010. The plant species were identified at Herbarium of Systematic Botany Division, FRI, (DD) Dehradun, Uttarakhand. The identity reconfirmed by one of the author (SK^3) at Central National Herbarium (CAL), Howrah. The voucher specimen (Hr. no. 60) was deposited in the herbarium of Department of Botany, Govt. P. G. College, Uttarkashi, Uttarakhand.

Figure 2: Collection site of *Arnebia benthamii*

Table 1: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR data (in ppm relative to TMS) of compound 4 in C_5D_5N.

C/H	$^{\delta}$C	Multiplicity (DEPT)	$^{\delta}$H (J in Hz)	HMBC Correlation (H→→C)
2	84.5	CH	5.45, *d* (11.5)	C-3, C-4, C-11, C-12, C-16
3	73.2	CH	5.02, *d* (11.5)	C-2, C-4, C-11
4	198.7	C	--	--
5	165.0	C	--	--
6	97.4	CH	6.49, *d* (2.0)	C-5, C-7, C-8, C-10
7	168.7	C	--	--
8	96.2	CH	6.35, *d* (2.0)	C-6, C-7, C-9, C-10
9	163.8	C	--	--
10	101.6	C	--	--
11	128.8	C	--	--
12	130.2	CH	7.73, *d* (8.5)	C-2, C-14, C-16
13	116.2	CH	7.23, *d* (8.5)	C-11, C-14, C-15
14	159.5	C	--	--
15	116.2	CH	7.23, *d* (8.5)	C-11, C-13, 14
16	130.2	CH	7.73, *d* (8.5)	C-2, C-12, C-14

***Extraction and Isolation*:**
The air-dried and powdered aerial portion of *Arnebia benthamii* (2.0 kg) was extracted with light petroleum ether (60-80^0). The petroleum free mass was extracted with 60% ethanol. The ethanol extract was concentrated under reduced pressure and was partitioned with $CHCl_3$:H_2O (6:4) in a separatory funnel. The chloroform layer was separated & concentrated under reduced pressure to give chloroform extract. The chloroform extract (14.0 g) was column chromatographed over Si-gel using gradient elution with C_6-H_6-EtOAc (10:0→9:1) afforded compound 1 (145 mg), and compound 2 (105 mg), and various other fractions. The fractions with same component (monitored by TLC) were mixed, dried and on repeated column chromatography over Si-gel eluted with $CHCl_3$: MeOH (98:2) afforded compound 3 (180 mg), compound 4 (195 mg), and compound 5 (148 mg).

***Compound (1)*:** White needles (MeOH), M.P. 136-137^{0}C. $[\alpha]_D^{25}$: -37 (C=0.1, $CHCl_3$). Elemental Analysis: C=84.1%, H=12.08% (Calc. $C_{29}H_{50}O$) Molecular weight 414. IR (V_{max}^{KBr}) : cm^{-1} 3335, 2965, 2950, 2910, 1640 cm^{-1}.

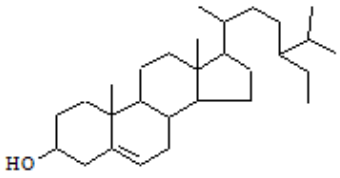
***Compound (2)*:** White crystals.(MeOH). M.P. 289-291. °C. $[\alpha]_D^{25}$: -41 (C=0.9, C_5H_5N). Elemental Analysis: C=72.68%, H=10.2% (Calc. $C_{35}H_{60}O_6$) Molecular weight 576. IR (V_{max}^{KBr}) : cm^{-1} 3410, 1640, 882 and 780.

***Compound (3)*:** Yellow needles (MeOH), M.P. 222-225°C Elemental Analysis: C=62.69%, H=3.17% (Calc. $C_{15}H_{10}O_6$) Molecular weight 286 EI-MS: m/z 286 [M]$^+$, 257, 229, 176, 165, 153, 133, 105, 77. IR (V_{max}^{KBr}) : cm^{-1} 3480, 3260, 1645, 1610, 1510, 1415, 1345, 1285, 1220, etc. ^{1}H-NMR: (300 MHz, CD_3OD): δ 6.13 (1H, *d*, *J*=2.5 Hz, H-6), 6.44 (1H, *d*, *J*=2.5 Hz, H-8), 6.81 (2H, *d*, *J*=8.7 Hz, H-13, 15), and 8.09 (2H, *d*, *J*=8.7 Hz, H-12, 16). ^{13}C-NMR (75 MHz, CD_3OD): δ 148.3 (C-2), 137.6 (C-3), 177.0 (C-4), 162.0 (C-5), 98.5 (C-6), 164.3 (C-7), 92.7 (C-8), 157.8 (C-9), 105.4 (C-10), 123.6 (C-11), 130.7 (C-12, 16), 116.3 (C-13, 15), 160.6 (C-14).

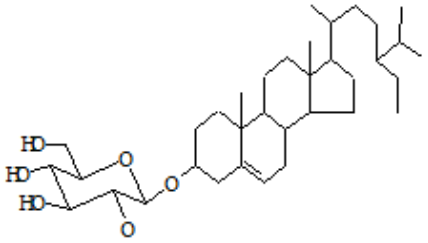
***Compound (4)*:** Yellow needles (MeOH) , M.P. 226-227°C Elemental Analysis: C=63.98%, H=3.97% (Calc. $C_{16}H_{12}O_6$) Molecular weight 300 EI-MS: m/z 300 [M]$^+$, 285, 281, 269, 167, 153, 133, 107, 77. IR (V_{max}^{KBr}) : cm^{-1} 3480, 3260, 1650, 1600, 1500, 1420, 1350, 1280, 1220, etc. ^{1}H-NMR: (300 MHz, CD_3OD): δ 3.83 (3H, *s*, -OMe), 6.23 (1H, *d*, *J*=2.5 Hz, H-6), 6.49 (1H, *d*, *J*=2.5 Hz, H-8), 6.87 (2H, *d*, *J*=8.7 Hz, H-13, 15), and 8.06 (2H, *d*, *J*=8.7 Hz, H-12, 16). ^{13}C-NMR (75 MHz, CD_3OD): δ 148.3 (C-2),

137.4 (C-3), 177.4 (C-4), 162.1 (C-5), 98.5 (C-6), 167.0 (C-7), 92.7 (C-8), 158.1 (C-9), 105.4 (C-10), 123.6 (C-11), 130.7 (C-12, 16), 116.3 (C-13, 15), 160.6 (C-14), 56.4 (-OMe).

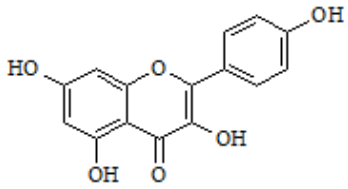
***Compound (5)*:** Yellow crystalline solid (MeOH), M.P. 165^{0}C. Elemental Analysis: C=52. 19%, H=5.13%, (Calc. $C_{15}H_{12}O_6$) Molecular weight 288 EI-MS: m/z 288 $[M]^+$, 259, 165, 153, 134, 107,77. IR (V_{max}^{KBr}) : cm^{-1} 3480, 3260, 1650, 1600, 1500, 1420, 1350, 1280, 1220, etc. ^{1}H-NMR: (400 MHz, CD_3OD; Table 1); ^{13}C-NMR (100 MHz, CD_3OD Table-1).



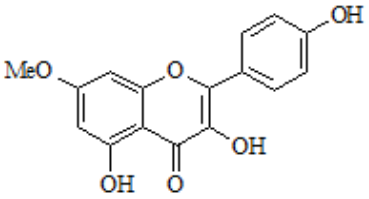
1. β-sitosterol



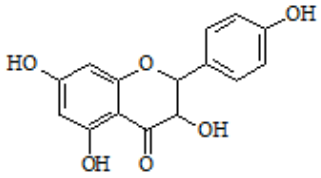
2. β-sitosterol-β-D-glycoside



3. Kaempferol



4. Rhamnocitrin (kaempferol-7-O-methyl ether).



5. Aromadendrin

Figure 3: Compounds in the plant

RESULT AND DISCUSSION

Compound **1** and **2** were identified as β-sitosterol and β-sitosterol-β-D-glycoside by CO-TLC, CO-IR and MMP with authentic sample (Baker *et. al.* 1954 and Porter *et. al.* 1982). Elemental analysis of **3** was corresponded to the molecular formula $C_{15}H_{10}O_6$ that was confirmed by the presence of molecular ion peak at m/z 286 in its EI-mass spectrum. It gave olive green colour with $FeCl_3$ and positive Shinoda test which indicate the presence of flavonoidal skeleton (Shinoda, 1928). Its IR spectrum displayed two absorption band at 3480 and 3260 cm^{-1} for chelated and non-chelated OH groups respectively. Other IR absorption bands were observed at 1645 and 1610 cm^{-1} (α,β-unsaturated C=O) and 1510, 1415 cm^{-1} (stretching of ether function).

The ^{1}H-NMR spectrum of **3** displayed two *meta*-coupled doublet (*J*=2.0 Hz) each for 1H at δ 6.13 (H-6) and 6.44 (H-8) and two *ortho*-coupled A_2B_2-type doublet (*J*=8.7 Hz) at δ 6.81 (H-13, 15) and 8.09 (H-12, 16) in aromatic region suggested the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring in the molecule. The presence 1,4-di-substituted phenyl was further confirmed to be *p*-hydroxyphenyl system by the ^{13}C-chemical shift of the carbon signals at δ 130.7 (C-12, 16) and 6 116.3 (C-13, 15) which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) (Pouchart and Compbell,1974). The ^{13}C-NMR spectrum also showed the presence of C=O group at δ 177.0, a benzylic carbon (C-2) at δ 148.3 and an oxygen bonded ethylenic carbon (C-3) at δ 137.6. The downfield ^{13}C-chemical shifts of aromatic carbon atoms at δ 162.0 (C-5), 164.3 (C-7), and 160.6 (C-14) as compared to ^{13}C-chemical shifts of other aromatic carbon atoms confirmed the presence of a flavonoidal skeleton (Markham, 1982 and Seikel, 1962). These ^{13}C-chemical shifts suggested that C-5, C-7 and C-14 carbon atoms are substituted with phenolic functions.

On the basis of above spectral data compound 3 was identified as kaempferol. It was further confirmed by comparison of spectral data with reported data (Markham, 1982) and co-TLC and MMP with an authentic sample.

The ^{1}H-NMR and ^{13}C-NMR data of **4** were similar to those of **3**. In aromatic region of ^{1}H-NMR spectrum, two *meta*-coupled doublet (*J*=2.0 Hz) each for 1H at δ 6.23 (H-6) and 6.49 (H-8) and two *ortho*-coupled A_2B_2-type doublet (*J*=8.7 Hz) at δ 6.87 (H-13, 15) and 8.06 (H-12, 16) suggested the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring. The later ring was further confirmed to be *p*-hydroxyphenyl system from the ^{13}C-chemical shift of the carbon signals at δ 130.7 (C-12, 16) and δ 116.3 (C-13,15) which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) (Pouchart and Compbell,1974). In aliphatic region the ^{1}H-NMR spectrum displayed an integrated singlet for 3H at δ 3.83 was assigned for -OMe group attached at C-7 position which is confirmed by downfield ^{13}C-chemical shift of C-7 at δ 167.0. The ^{13}C-NMR spectrum also showed the presence of C=O group at δ 177.4, a benzylic carbon (C-2) at δ 148.3 and an oxygen bonded ethylenic carbon (C-3) at δ 137.4.

On the basis of these spectral data compound **4** was identified as rhamnocitrin (kaempferol-7-O-methyl ether). It was further confirmed by comparison of spectral data with reported values [Markham *et. al.* 1972] and co-TLC and MMP with an authentic sample.

Compound **5** was obtained as yellow crystalline solid analyzed for $C_{15}H_{12}O_6$ which was substantiated by the presence of molecular ion peak at m/z 288 in the El^{+}-mass spectrum. It gave olive green colour with $FeCl_3$ and responded positive to Shinoda's test which indicate the presence of flavonoidal skeleton (Shinoda, 1928). The IR spectrum of compound **5** displayed two absorption band at 3485 and 3260 cm^{-1} for chelated and non-chelated OH groups respectively. The absorption bands at 1650 and 1600 cm^{-1} in IR spectrum indicated presence of an α, β-unsaturated Carbonyl group in the molecule. The IR spectrum also showed stretching of ether function at 1500, 1420 cm^{-1}.

The ^{1}H-NMR spectrum of compound **5** displayed presence of two *meta*-coupled doublets (*J* = 2.0 Hz, each for 1H, at δ 5.45 and 5.02), and two A_2B_2-type doublets (*J* = 8.5 Hz, each for 2H, at δ 7.73 and 7.23), in the aromatic regions suggesting the presence of a *tetra*-substituted and a 1,4-disubstituted phenyl ring. In the heterocyclic region of ^{1}H-NMR spectrum appearance of two doublets (*J* = 11.5 Hz) (at δ 5.45 and 5.02) corroborated with the H-2 and H-3 protons of dihydroflavonols. The 1,4-disubstituted phenyl ring as deduced by ^{1}H-NMR was determined to be *p*-hydroxyphenyl system from the ^{13}C-NMR chemical shifts of carbon signals at δ 130.2 (C-12, 16) and 116.2 (C-13, 15) which fairly corresponded with those of hydrogen carrying carbons of *p*-cresol (δ 115.3, 130.2) (Pouchart and Compbell,1974). The ^{13}C -NMR spectrum also showed presence of a carbonyl carbon δ 198.7 (C-4), a benzylic carbon at δ 84.5 (C-2) and oxygen bonded heterocyclic carbon at δ 73.2 (C-3). In agreement with above discussed 1H- and ^{13}C-NMR data the DEPT spectrum showed presence eight methane carbon (two of double intensity) and seven quaternary carbon atoms. The assignment of protonated carbon atoms and proton attached with them were

made by HMQC experiment. The crucial data for the structure determination were provided by HMBC spectrum which displayed ^{1}H-^{13}C long-range correlation and thus the entire structure was mapped out.
On the basis of above discussed spectral data **5** was characterized as aromadendrin which was confirmed by comparison of its physical data with the reported data (Markham, 1972).

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REFRENCES

Baker SA, Bourna BJ and Whiffen HD, (1954). Methods of Biochemical Analysis, 3rd Edn. (ed. D. Glick). *Inter Sciences* 213.
Chopra RN, Nayar, SL and Chopra IC (1986). *Glossary of Indian Medicinal Plants CSIR*, New Delhi.
Harborne JB and Baxter H, (1996). Dictionary of Plant Toxins, *John Wiley*, New York.
Kashiwada Y, Nishizawa M, Yamagishi T, et al. (1995). Anti-AIDS agents. 18. Sodium and potassium salts of caffeic acid tetramers from Arnebia euchroma as anti-HIV agents. *Journal of Natural Products* **58, 392-400**.
Kirtikar KR and Basu BD (1984). *Indian Medicinal Plants* , **3,** 697, Bishen Singh Mahendra Pal Singh, Dehradun.
Kumar Ambrish and Srivastava SK (2014). Taxonomic Studies on the Genus Arnebia Forssk. (Boraginaceae) in India, *Taiwania,* **59**(4) 315.
Manjkhola S and Dhar U (2002). Conservation and utilization of *Arnebia benthamii* (Wall. ex G. Don) Johnston - a high value Himalayan medicinal plant. *Current Science* **83**(4) 484.
Markham K R (1982). *Techniques in Flavonoid Identification*, Academic press, Inc. New York, New York.
Markham KR, Ternai B, Stanley R, Geiger H and Mabry TJ (1978). 13C NMR studies of flavonoids – III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* 34 1389.
Porter LJ, Newmann RH, Foo LY, Weng H and Hemingway RW (1982). *Journal of Chemical Society Perkin Trans*, **1**, 1217).
Pouchart CJ and Compbell JR (1974). 'The Library of NMR Spectra', **Vol-IV**, *Aldrich Chemical* Co., Milwaukee, pp. 144.
Seikel MK (1962). The Chemistry of Flavonoid Compounds (ed. T. A. Giessmann), Pergamon press, New York, 34.
Shinoda J (1928). A new biologically active flavones glycoside from the roots of Cassia fistula Linn. *Jou rnal of Pharmaceutical Society of Japan* **48 214-220**
Singh, G and Kachroo P (1976). Forest Flora of Srinagar. Bishen Singh Mahendra Pal Singh, Dehradun.
Ved D.K and G. S Goraya (2008). Non-Timber Forest Produce as livelihood Option for Rural Communities of Mid-Himalays in Himachal Pradesh (2008) - Foundation for Revitalisation of Local Health Traditions, Bangalore.