Acylated Flavonol Glycosides From The Flowers Of *Aconitum Violaceum* staph.

Suman Yadav* and D. L. Verma

Department of Chemistry, Kumaun University, Soban Singh Jeena campus, Almora, Uttarakhand (India)-263 601.
E-mail : akyadava_09@rediffmail.com ; akyadava09@gmail.com
Mobile nos. +91-094129 66520; +91-094113 67240

Abstract: *Aconitum violaceum* staph (fam. Ranunculaceae), an alpine herbal constituent of Central Himalaya, has been used as a traditional medicine by tribal inhabitants of the region. Various ethnic groups, Bhotias, Darmese and Martolias use the plant for curing number of ailments, cough, asthma, neuro-disorders, inflammatory and heart related diseases. It is a small perennial herb with 1-1.5m in height, bearing dense spike of many dark or pale blue flowers. Aerial parts of *A. violaceum* was collected from alpine pastures of Sunderdhunga glaciers (4,000 m above msl) of Kumaun Himalaya in the month of July, 2008 and its authentification has been made from BSI, Dehradun and finally by Prof. Y. S. Pangati, Prof. Emeritus, Department of Botany at DSB campus, Nainital. A voucher specimen No. 46 of flower and leaves has been deposited in the Chemistry Department of Kumaun University at Almora campus. Aqueous-ethanolic extract of the aerial parts (500 gms) of *A. violaceum* was partitioned with CH_2Cl_2 and n-BuOH. The BuOH fraction gave antioxidative activity against the DPPH solution in UV-VS spectrophotometer at 715nm. It was evaporated to dryness under reduced pressure and residue was chromatographed on cellulose (Merck) CC and eluted with 40% HOAc. Two broad violet fluorescent bands were observed on CC and each was eluted and collected separately by monitoring with UV light. An eluate of faster moving band, representing FRAC-01 and a chromatographically identified catechol grouping flavonol glycosides, gave two flavonoids **(1)** and **(2)** after RPPC of the fraction on Whatman No. 3 PC using BAW as a developing solvent system, The compounds, **(1)** and **(2)**, a faster (Rf 42) and slower (Rf 39) moving components on PC in BAW solvent were identified as quercetin-3-O-β-D-glucosyl(1→2)(6″-O-transcaffeoyl)-β–D-gluco-pyranoside-7-O-α-L-rhamnopyranoside and quercetin-3-O-β-D-glucosyl(1→2)(6″-O-transcaffeoyl)-β–D-gluco-pyranoside-7-O-β-D-glucopyranoside, respectively. On comparing the antioxidative activity of compound, **(1)** and **(2)** with reference compound, quercetin, the activity profile was found in the order: quercetin > compound **(2)** > compound **(1)**.
[Suman Yadav and D. L. Verma. **Acylated Flavonol Glycosides From The Flowers Of *Aconitum Violaceum* staph.** Nature and Science 2010;8(12):239-243]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: *Aconitum violaceum,* flavonol, glycosides

Introduction:

Genus *Aconitum*, a pharmacologically important group of flowering plants of phanerogamic family, Ranunculaceae and a dominant species rich in the alpine pastures of Central Himalaya has been characterized by the presence of about 90 species and distributed widely throughout the temperate alpine regions of earth crust. About 10 species of *Aconitum* have been isolated from the alpine pastures and temperate regions of Kumaun Himalayas, a part of central Himalaya in Indian Himalayan Region (IHR). Various ethnic groups, Bhotias, Darmese, Martolia and Johares of Himalayan region use *Aconitum* species for curing number of ailments, cough, asthma, neuro-disorders, inflammatory and heart related diseases (Rawat, 1984). Various species of *Aconitum* native to Europian subcontinent have been used to cure gout, neuralgia, anticular, rheumatism and cardiac failure (Bisset, 1981).Genus *Aconitum*, rich source of secondary metabolites endowed with interesting biological activities, has been characterized by the presence of C_{19} and C_{20} diterpenoid alkaloids (Bisset, 1981; Colombo *et al.* 1988), flavonol glycosides and acylated flavonol glycosides of quercetin and kaempferol (Braca *et al*., 2003; Fico *et al*., 2000). In modern era of research in the field of medicines it has highly been recognized that various biological activities from the extracts of traditional medicinal plants have been attributed to the presence of polyphenolics, particularly flavonoids. Flavonoids, a group of prominent antioxidative compounds, have highly been reported to cure various diseases associated to oxidative stress produced in human body due to formation of excessive free radicals (Mora *et al*., 1990; Pietta, 2000; Halliwell, 1994). Catechol grouping in the flavonoid molecules is highly associated to enhance the antioxidative activity (Rice-Evans *et al*., 1997; Pietta, 2000). Ortho-dihydroxyl structure confirmed higher stability to the radical form and participated in the electron delocalization. Thus acetylation of flavonol glycosides with caffeic acid form higher antioxidative enhancement index compared to coumaroyl derivatives. Thus caffeoyl derivatives apparently had higher radical scavenging and antioxidative activity than the p-coumaroyl ester. For example, quercetin, a catechol group bearing

flavonol, has been shown to scavange various reactive oxygen species and has been implicated as inhibitors of lipid peroxidation (Mora *et al*., 1990). This compound possesses a wide range of therapeutic properties as cardiovascular protection, anti-cancer and anti-inflammatory (Narayana *et al*., 2001). Two caffeoyl derivatives of quercetin, quercetin-3-O-β-D-glucopyranoside-7-O-rhamnosyl (1→2) glucoside (1→6) caffeoyl and quercetin-3-O-β-D-glucosyl (1→2) caffeoyl (1→6)-glucopyranoside-7-O-α-L-rhamnoside were found to have more antioxidative potential than quercetin (Braca *et al*., 2003).

Aconitum violaceum Staph (Ranunculaceae) is widely distributed in the alpine pastures of central Himalaya. It is a small perennial herb with 1-1.5m in height bearing dense spike of many dark or pale blue flowers. This plant has been used as a prominent traditional medicine by various ethnic groups of alpine region of central Himalaya and it has been used to cure various ailments cough, asthma, inflammatory and heart related diseases (Rawat, 1984). Present communication reveals the isolation and characterization of caffeoyl grouping flavonol glycosides of quercetin from prominent antioxidative activity guided fractionation of n-BuOH soluble of aqueous-ethanolic extract of *Aconitum violaceum*.

Plant collection and authentification:

The plant *Aconitum violaceum* was collected from alpine pastures of Sunderdhunga glaciers (4000msl) of Kumaun Himalaya in the month of July, 2008. Its authentification has been carried out from BSI, Dehradun and finally by Prof. Y. S. Pangati, Professor Emeritus, Department of Botany, Kumaun University at DSB campus. Its voucher specimen No. 46 (flowers and leaves bearing twig) has been deposited in the Chemistry Department of Kumaun University at Almora campus.

Materials and Methods:

1. **Extraction**: 750 grams air dried and powdered sample of the flowers of botanically identified plant, *Aconitum violaceum* was extracted sequentially with 80% aq. MeOH and 50% aq.MeOH by cold percolation method. Two extracts were filtered and combined. The combined extract was evaporated to dryness under reduced pressure. The residue was partitioned between CH_2Cl_2: H_2O (1:1). After removal of CH_2Cl_2 soluble, the H_2O layer was further partitioned with n-BuOH.
2. **Screening of antioxidant activity of n-butanol soluble**: About 5 gms residue of n-butanol soluble was dissolve in MeOH. It was chromatographed on TLC of SiO_2 and developed with CH_2Cl_2: MeOH (3:1). The dried and developed TLC plate was inspected under UV light. Five violet fluorescent spots were discernible on TLC and their Rf values were measured. After locating UV active spots on TLC, it was sprayed with methanolic solution of DPPH. Two violet fluorescent spots turned to yellow in purple background, indicated the antioxidant activity of the n-BuOH fraction.
3. **Chromatographic fractionation of n-butanol soluble**: n-BuOH soluble, an antioxidative positive fraction, was chromatographed on cellulose (Merck) CC and eluted with 40% HOAc. Two broad violet fluorescent bands were observed on CC and each was eluted and collected separately by monitoring with UV light. An eluate derived from faster and slower moving bands are representing FRAC-01 and FRAC-02, respectively.
4. **Screening of antioxidant activity from FRAC-01 and FRAC-02**: The scavenging of DPPH free radical was observed in UV-VS spectrophotometer at 715nm against FRAC-01 but FRAC-02 did not show antioxidant activity.
5. **2DPC examination of FRAC-01 for total flavonoids positive compounds**: An eluate derived from FRAC-01 was evaporated to dryness and residue was dissolve in MeOH. It was chromatographed two-dimensionally on Whatman No. 1 PC using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) and 30% HOAc. The dried and developed PC was inspected under UV light. A total of four violet fluorescent flavonoids positive spots were observed on 2DPC. Their Rfs in BAW were measured at 52, 50, 43 and 39.
6. **Assignments of catechol-grouping flavonoids on 2DPC of FRAC-01**: The dried and developed 2DPC of FRAC-01 was sprayed with ammonical silver nitrate, of four flavonoids, two of which, at Rf 39 and 43 gave positive tests.
7. **Isolation of catechol-grouping compounds from FRAC-01**: Two compounds at Rf 39 and 43, a slower and faster moving components on PC in BAW solvent representing compounds **(2)** and **(1)**, respectively and a catechol group bearing compounds, were isolated from FRAC-01by RPPC using BAW as a developing solvent followed by Sephadex LH-20CC with 40% MeOH as an eluent for the final purification of each compound.
8. **Scavenging activity to DPPH radical**: An aliquot of 30ml of methanolic solution containing each pure compound was added to 3ml of 0.004% MeOH solution of DPPH determined after 30nm against a blank of methanol without DPPH (UV-

Perkin-Elmer-Lambda 11 spectrophotometer) and calculated (Cuendet *et al*., 1997). % decreasing intensity of DPPH at 715 (peak) was also calculated against reference compound, quercetin.

9. Structural elucidation of compounds **(1)** and **(2)** by chromatographic, hydrolytic and spectral methods, UV, ^{1}HNMR and MS:

Result and Discussion:

An antioxidative fraction, FRAC-01, isolated from 40% HOAc Cellulose CC fractionation of n-BuOH soluble of aqueous-ethanolic extract of the flowers of *A. violaceum* was chromatographed successively on Whatman No. 3 PC (RPPC with BAW) and Sephadax LH-20CC to give pure compounds **(1)** and **(2)**.

Compound **(1)**, a violet fluorescent on paper chromatogram under UV light (360nm), turned to orange with NA (Naturstoffreagenz-A) and dark brown with ammonical silver nitrate, indicating a flavonoid with ortho-dihydroxyl group in the B-ring. Methanolic solution of the compound gave positive tests with $FeCl_3$, Mg+HCl and α-naphthol, indicating a flavonoid glycoside. The UV shift on addition of diagnostic reagents confirmed the presence of free hydroxyl groups at positions C-3', C-4' and C-5 (Markham, 1982) (table no. 1).Complete acid hydrolysis of the compound gave quercetin (coPC), glucose (coPC), rhamnose (coPC) and caffeic acid (coPC). FAB-MS (-ve) of Compound **(1)**, exhibited a molecular ion peak at m/e 933 $[M-H]^-$ and other prominent peaks were observed at m/e 771(m/e933-glucose)$^-$, m/e 447(m/e 771-(glucose + caffeoyl)$^-$ and m/e 301(m/e 447- rham.)$^-$, supporting the release of two moieties of glucose, one molecule of rhamnose and one molecule of caffeic acid from quercetin. Enzymatic hydrolysis of compound with α-rhamnosidase gave a compound quercetin-3-di-O-glycoside, representing structure Compound **1(a)** and rhamnose(coPC).These data suggested that acyl group was located to the sugar moiety attached at 3-position and rhamnose was present at 7-position. Complete acid hydrolysis of compound **1(a)** gave quercetin (coPC), caffeic acid (coPC) and glucose (coPC). Deacetylation of compound **1(a)** with $NaHCO_3$ gave compound **1(b)** and caffeic acid (coPC). FAB-MS (-ve) of Compound **1(b)** gave a molecular ion at m/e 625$[M-H]^-$ and other ions observed at m/e 463 (m/e 625-glucose)$^-$ and m/e 301(m/e 463-glucose)$^-$, indicating successive elimination of two moieties of glucose from quercetin. The physico-chemical data of the compound **1(b)** were found similar to the compound, quercetin-3-O-sophoroside (Olsson *et al*., 1998).

Deacetylation of compound **(1)** with $NaHCO_3$ followed by enzymatic hydrolysis with β-glucosidase gave compound **1(c)**. It was identified as quercetin-3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside. On basis of enzymatic hydrolysis and deacetylation of compound **(1)**, it was established that the caffeoyl group is attached to primary sugar, sophoroside at 6" position. Finally the attachment of caffeoyl group to primary sugar, sophorose was identified by ^{1}HNMR studies (table 1):

Table 1. ^{1}HNMR of compound (1) in DMSO-d_6 (400MHz):

Shift (δ)	Multiplicity (J=Hz)	Identification (H)
Aglycone:		
6.42	1H, d, 2.0	H-6
6.76	1H, d, 2.0	H-8
6.89	1H, d, 8.5	H-5'
7.16	1H, dd, 8.5, 2.0	H-6'
7.30	1H, d, 2.0	H-2'
Sugar region:		
5.61	1H, d, 7.5	H-1"
5.56	1H, d, 1.20	H-1""
5.16	1H, d, 7.2	H-1'"
5.54	1H, dd, 2.0, 12.0	H-6(a)"
5.52	1H, dd, 2.0, 12.0	H-6(b)"
3.10-4.10	m	Remaining protons of glucose
Caffeoyl group:		
6.20	1H, d, 16.0	α-H
6.40	1H, d, 16.0	β-H
6.70	1H, d, 8.5	H-5"'"
6.88	1H, dd, 8.5, 2.0	H-6""
7.10	1H, d, 2.0	H-2""

^{1}HNMR: ^{1}HNMR spectrum of **(1)** showed a typical pattern of a flavonol with a quercetin aglycone together with signals attributed to sugar moieties and acyl residue. Three anomeric protons arising from the sugar moieties appeared at δ5.61 (1H, d, J=7.5), δ 5.16 (1H, d, J=7.2) and δ 5.56 (1H, d, J=1.20) were attributed to H-1" of glucose (primary), H-1'" of glucose attached to 2" of primary sugar and H-1"" primary rhamnose directly attached to 7-position, respectively. An ABX system of the anomeric protons of caffeoyl group was observed at δ 6.70 (1H, d, J=8.5), δ 6.88 (1H, dd. 8.5, 2.0) and δ 7.12 (1H, d, J= 2.0Hz) and α, β- protons with trans-configuration were also observed at 6.22 and 7.40 each with J=16.0Hz. On the basis of ^{1}HNMR studies, the downfield shift of H-6" (a) and H-6" (b) of 3-O-glucose moiety, indicate the attachment of caffeoyl moiety to C-6 position. On the basis of ^{1}HNMR studies, the compound **(1)** was

identified as quercetin-3-O-β-D-glucopyranosyl (1→2) (6"-O-transcaffeoyl)-glucopyranoside-7-O-α-L-rhamnopyranoside.

UV spectrum of Compound **(1)** (table 2) showed two absorption maxima at 333nm and 265nm, indicating the presence of substituted aromatic rings and α, β unsaturated ketone in the molecule (Agarwal, 1989).

Table 2. UV spectra of compound (1):

Shift reagents	λ_{max}/nm
MeOH	265, 300sh, 333
NaOMe	279, 386
NaOAc	268, 380
NaOAc/H_3BO_3	268, 359
$AlCl_3$	279, 300sh, 430
$AlCl_3$/HCl	280, 339, 400

Compound **(2)**, appeared as a violet fluorescent spot on paper chromatogram under UV light, indicating the substitution of C-3 hydroxyl group (Mabry *et al*., 1970). This spot changed to orange with NA (Naturstoffreagenz-A) reagent and dull brown with ammonical silver nitrate, indicating the presence of ortho-dihydroxyl group or catechol group bearing B-ring. Methanolic solution of the compound gave positive colour reactions with Mg+HCl, $FeCl_3$ and α-naphthol. UV spectra of Compound **(2)** gave two bands at (λ_{max}, nm) 255 and 339 and shifts obtained with diagnostic reagents, NaOMe (271, 387), NaOAc (260, 384), NaOAc/H_3BO_3 (258, 360) and $AlCl_3$ (274, 300sh, 438), indicating an acylated flavonol-3, 7-diO-glycoside (Mabry *et al*., 1970). Complete acid hydrolysis of the compound gave quercetin (coPC), Caffeic acid (coPC) and glucose (coPC).

Enzymatic hydrolysis of Compound **(2)** with β-glucosidase yielded a 3-monoglycoside of quercetin, representing compound **2(a)** and glucose (coPC). The data suggested that the acyl group was located at 3-position and glucose was present at the 7-position. To assign the nature of sugar moiety present at the 3-position and position attachment of caffeoyl group, the compound **(2)** was deacetylated with $NaHCO_3$. On deacetylation it gave a violet fluorescent compound on PC under UV light, representing compound **2(b)**. Its chromatographic behaviour, UV, ^{1}HNMR and MS data were found similar to those reported in the literature to the compound quercetin-3-O-sophoroside-7-O-β-D-glucoside. These data suggesting that the β-glucosidase hydrolysis of Compound **(2)** resulted release of two glucose moieties, one from terminal position of sophorose sugar and another glucose directly from 7-position of quercetin. Deacetylation of Compound **2(a)** with $NaHCO_3$ gave caffeic acid (coPC) and quercetin-3-O-β-D-glucopyranoside (coPC). Thus, it has been established that caffeic acid is attached to the primary glucose moiety of sophorose sugar. The position attachment of caffeic acid to primary glucose moiety of sophorose sugar was confirmed by ^{1}HNMR of Compound **2(a)** (in DMSO-d_6, 400MHz) (table 3).

Table 3. ^{1}HNMR of compound 2(a):

Shift (δ)	Multiplicity(J=Hz)	Identified protons
6.18	1H, d, 2.0	H-6
6.38	1H, d, 2.0	H-8
6.88	1H, d, 8.5	H-5'
7.86	1H, dd, 8.5, 2.0	H-6'
7.40	1H, d, 2.0	H-2'
5.65	1H, d, 7.5	H-1"
4.54	1H, dd, 2.0, 12.0	H-6"(a)
4.52	1H, dd, 2.0, 12.0	H-6"(b)
3.10-4.10	(m)	Remaining protons of glucose
Caffeic acid region		
6.23	1H, d, 16.0	α-H
7.41	1H, d, 16.0	β-H
6.70	1H, d, 8.5	H-5"'
6.88	1H, dd, 8.5, 2.0	H-6"'
7.00	1H, d, 2.0	H-2"'

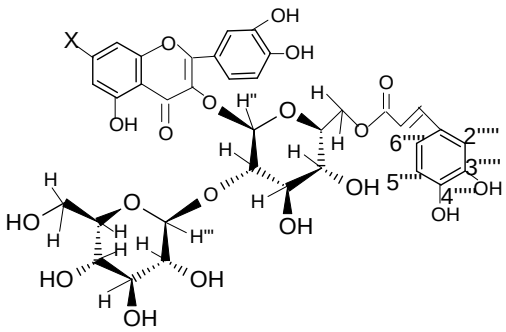
The downfield shift of H-6" (a) and H-6" (b) protons, indicating the caffeoyl group is attached to 6"-position of primary glucose of sophorose moiety. The (-)ve FAB-MS of Compound **(2)** was in agreement with the assigned structure as it showed the molecular ion at m/e 949[M-H]$^-$ and other prominent ions observed at m/e 787 [m/e 949-gluc.]$^-$, m/e 463 [m/e 785-(gluc.+caffeoyl)]$^-$ and m/e 301[m/e463-gluc.]$^-$ and its ^{1}HNMR spectra (DMSO-d_6) 400MHz .

On the basis of chromatographic behaviour, hydrolytic methods, UV, ^{1}HNMR (table 4) and MS studies, the compound **(2)** was identified as quercetin-3-O-β-D-glucosyl (1→2) (6"-O-transcaffeoyl)-β-D-glucopyranoside-7-O-β-D-glucopyranoside (Figure 1).

Table 4. ^{1}HNMR spectra of compound (2):

Shift (δ)	Multiplicity (J=Hz)	Identified protons [H]
Aglycone:		
6.40	1H, d, 2.0	H-6
6.78	1H, d, 2.0	H-8
6.89	1H, d, 8.5	H-5'
7.18	1H, dd, 8.5, 2.0	H-6'
7.30	1H, d, 2.0	H-2'
Sugar region:		

5.61	1H, d, 7.5	H-1"
4.54	1H, dd, 2.0, 12.0	H-6"(a)
4.52	1H, dd, 2.0, 12.0	H-6"(b)
5.16	1H, d, 7.2	H-1""
4.80	1H, d, 7.5	H-1"'
3.10-4.10	(m)	Remaining protons of three glucose molecules
Caffeoyl group:		
6.20	1H, d, 16.0	α-H
7.40	1H, d, 16.0	β-H
6.70	1H, d, 8.5	H-5""
6.88	1H, dd, 8.5, 2.0	H-6""
7.10	1H, d, 2.0	H-2""



Compound **X (Sugar)**
(1) rhamnose; (2) glucose

Figure 1. Compound and **X (Sugar)**

Corresponding author*
Dr. (Mrs.) Suman Yadav
¾, Block 4, New Professor's Colony,
Lower Mall Road, Almora,
Uttarakhand – 263 601 (India)
Phone: +91-5962- 231977; 09211367240
E-mail: akyadava_09@rediffmail.com;
akyadava09@gmail.com

References:

1. Agarwal, PK. Carbon-13 NMR of flavonoids, Elsevier, Amserdum. 1989.
2. Bisset, NG. Arrow poisons in China, Part II, Aconitum-Botany, Chemistry, Pharmacology. J. of Ethnopharmacology, 1981. **4**: 247-336.
3. Braca, A; Fico, G; Morelli,I; Simon, FD.; Tome, F and Tommasi, ND. Antioxidant free radical scavenging activity of flavonol glycosides from different species. J. of Ethnopharmacology, 2003. **86:** 63-67.
4. Cuendet, M; Hostettmann, K and Potterat, O. Iridoid glucosides with free radical scavenging properties from *Fragraea glumei*. Helvertica Chimica Acta, 1997. **80**: 1144-1152.
5. Fico, G; Braca, A; Bilia, AR; Tome, F and Morelli, I. Journal of Natural Products, 2000. **63**: 1563-1565.
6. Halliwell, B. Free radicals, antioxidants and human disease. Curiosity, cause of consequence, Lancet, 1994. **344**: 721-724.
7. Lim, CE.; Park, JH. and Park, CW. Flavonoids variation in the *Aconitum jaluense*, complex (Ranunculaceae) in Korea, Plant Systemic Evolution, 1999. **218**: 125-131.
8. Mabry, TJ; Markham, KR and Thomas, MB . The systematic identification of flavonoids. Springer Verlag, 1970. 1-310.
9. Markham, K.R. Techniques of Flavonoids Identification. A.P., London. 1982
10. Mora, A. Paya, M. Rios, IL. and Alcaraz, MJ. Structural activity relationships of polymethoxy flavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation, Biochemical Pharmacology, 1990. **40**: 793-797.
11. Narayana, KR. Reddy, MS. Chaluvedi, MR. and Krishna, DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential, Ind. J. Pharm, 2001. **33**: 2-16.
12. Olssom, LC; Veit, M; Weissenbock, G and Bornman, JF. Differential flavonoids response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry, 1998. **49(4):** 1021-1028
13. Pelletier, SW. and Keith, LH. In alkaloids, Edited (RHE. Manske) XII, 1969. 01-202.
14. Pietta, PG. Flavonoids as antioxidants, journal of Natural Products, 2000. **63**: 1035-1042.
15. Rawat, GS. Study on the high altitude flowering plants of Kumaun Himalaya. Ph.D. thesis, Kumaun University, Nainital, India, 1984.
16. Rice-Evans, C. Miller, NJ. and Pagana, G. Structural antioxidant activity relationship of flavonoids and phenolic acid. Free radical Biology and Medicine, 1996. **20:** 933-56.
17. Rice-Evans, C. Miller, NJ. and Pagana, G. Antioxidant properties of Phenolic compounds, Trends in Plant Science, 1997. **2:** 152-159.

11/18/2010