Chemical Examination Of Bergenia Stracheyi (Hk.) For Antioxidative Flavonoids

Sanyukta Chowdhary[!], Harish Kumar* and D.L. Verma[#]

[!] Department of Botany, Kumaun University, SSJ Campus Almora, Uttarakhand, India
[#] Department of Chemistry, Kumaun University, SSJ Campus Almora, Uttarakhand, India
*Department of Botany and Microbiology, Gurukul Kangri University, Haridwar- 249404, Uttarakhand, India
Phone: +91-1334-235003, 09410730060
E-mail: harish2129@gmail.com

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ABSTRACT： *Bergenia Stracheyi* (HK.), a perennial rhizometic herb of family Saxifragaceae, has frequently been reported from Afganistan to U.P. between 3300 – 4500m in alpine slopes. This is a common alpine species of Western Himalaya. It is found in the form of vast patches in sub-alpine and alpine areas. Its rootstock is thick, dark – brown with short stem, thick and fleshy. Leaves are stalked and sheathing at base, ovate or orbicular, cordate, margin fringed with short and stiff hairs. Flowers are white, pink or purplish in terminal corymbose cymes. Fruit is a conical capsule having minute seeds. It is a traditional medicinal plant and the local tribal inhabitants of the region use the rhizome of plant for curing kidney and gall bladder stone, cough and cold, heeling old wounds, cuts and burns, inflammation etc. Looking on the various biomedicinal uses of the plant in the various systems of medicines the objectives of the present chemical investigation of the plant are: Evaluation of antioxidative activity from flavonoidal positive fraction. Structural elucidation of active flavonol glycosides by chromatographic, hydrolytic and spectral methods. Three flavonol glycosides were isolated from the antioxidative activity guided fraction by PPC and these structures are identified as: Quercetin-3-O-α-L-rhamnoside, Kaempferol-3-O-α-L-rhamnoside and Kaempferol-3-O-rhamnosyl (1- 6) glucoside. Among these three flavonol glycosides, the compound (1) and (2) gave promising antioxidative activity while the compound (3) did not show effective activity. [Nature and Science. 2009;7(4):29-34]. (ISSN: 1545-0740).

Keywords: Chemical Examination; Bergenia Stracheyi; Antioxidative; Flavonoids

INTRODUCTION

Bergenia stracheyi (HK.) is a rhizometic herb species belonging to Saxifragaceae and is mainly found in Afghanistan to Uttarakhand, between 3300-4500 m in alpine slopes (Chauhan, 2000). The rhizome of *Bergenia stracheyi* is used as a folk medicine for its antiscorbutic, astringent, dirrretic, febrifuge and opthalmic properties (Pandey, 1995) and previous chemical and pharmacological studies on his species reported the occurrence of glycosides, gallic acid, tannic acid, mucilage, wax, albumens, starch etc. (Sharma, 2003). Flavonoids are also found in other species of *Bergenia* as glycosides (Farooq, 2005). Flavonoids have widely been associated with various biological activities such as antimicrobial, antioxidant, anti inflammation and anticancerogenic (Havsteen, 2002).

Antioxidants play a role in the maintenance of the pro/antioxidant balance by neutralizing the radicalar oxygen and nitrogen species which are responsible for deleterious processes in biological system (Ribeiro, 2002).

This paper deals with the detection of antioxidant compound from the aqueous- ethanolic extract of *Bergenia shracheyi* leaves. The fractionation of the extract through chromatography in order to isolate and elucidate the molecular structure of the compounds responsible for the antioxidant activity.

MATERIALS AND METHODS

Plant material and authentification:

Leaves of wild growing plants of *Bergenia stracheyi* (HK.) were collected in September 2006 from alpine slopes of Kumaun Himalaya. Voucher specimen of the collected plant (*Bergenia stracheyi* No. H.02) were confirmed and deposited at the herbarium of the Department of Botany, Kumaun University S.S.J.Campus, Almora, Uttarakhand.

Method of extraction:

About 2.5 kg air dried and powdered leaves of *B. stracheyi* were extracted with 50% aqueous ethanol for five days by cold percolation method. The extract was decanted and concentrated under reduced pressure until only a small layer of H_2O (approx. 50 ml) remained. It was partitioned with CH_2Cl_2 and BuOH. The BuOH fraction was examined for antioxidative flavonoidal compounds.

Isolation of flavonoidal positive fraction from BuOH soluble:

The BuOH soluble was evaporated to dryness under reduced pressenre at 55^0 C in Rota evaporator. The residue of BuOH soluble was dissolve in 70% EtOH and it was banded in Whatman No. 3 PC using 20% HOAc as an eluent. A broad dark purple fluorescent band was observed on PC with UV light. It was cut and eluted with 70% aq - MeOH. The aqueous methanolic elute was evaporated to dryness and residue was chromatographed on cellulose (Merck grade) cc and eluted initially with H2O then increasing polarity with acetic acid. On eluting column with 10% HOAc two dark purple fluorescent bands were observed on column each band was eluted and collected separately by monitoring with UV light. The aqueous-acetic acid elute of two UV fluorescent bands, faster moving and slower moving, representing FRAC-01 and FRAC-02 respectively.

Evaluation of anti-oxidative activity of above CC eluted fraction:

Each fraction was examined in 2DPC for total flavonoidal constituents using BAW (n-BuOH-AcOH-H2O, 4: 1: 5, V\V, upper layer) and 30% HOAc as a developing solvents. 2DPC examination of FRAC-01 and FRAC-02 afforded flavonoidal compounds 5 and 3 respectively. Each flavonoidal positive fraction was screened for antioxidative activity by thin layer autochromatographic method.

The residue of FRAC-01 and FRAC-02 were dissolve in MeOH and methanolic soluble of each fraction was spotled on silica gel coated TLC. The TLC plate was developed with CHCl3 : MeOH (3 : 1) and after development the dried plate was sprayed with methanolic solution of DPPH (Aldrict chem.) free radical. FRAC- 01 and FRAC-02 gave positive antioxidative spots on TLC as they produced light yellow spots on purple back ground, while FRAC-01 did not give any antioxidative positive spots on TLC. Thus FRAC-01 was discarded and FRAC-02 was used for the isolation of antioxidative positive flavonoidal positive compounds.

Isolation of flavonoidal compounds from antoxidative positive fraction, FRAC-02:

The residue of FRAC-02 was chromatographed on Whatman No. 3 PC using BAW (4: 1: 5, V/V, upper layer) as an eluent. The dried and developed PC was inspected with UV light. Two major broad dark purple UV fluorescent bands were observed on PC were cut and eluted separately with 70% EtOH. The faster moving band, representing FRAC-02 (a) gave two dark purple UV fluorescent bands after its re-chromatography with 30% HOAc. The faster moving band, representing compounds (C) and slower moving compound, representing compound (B) were cut and eluted from PC by monitoring with UV light. Each compound was finally purified on Sephadex LH-20 cc using 50% aq. MeOH. The slower moving fraction, FRAC-02 (b) was further purified on Whatman No. 3 PC using BAW (4 : 1 : 5, V/V, upper layer) as an eluent, A broad dark purple UV fluorescent band was observed an PC was cut and eluted with 70% aq, EtOH separately. It was finally purified on sephadex LH-20 cc using 60% HOAc as an eluent. The isolated compound representing structure (A). (Structure 1).

RESULTS and DISCUSSION

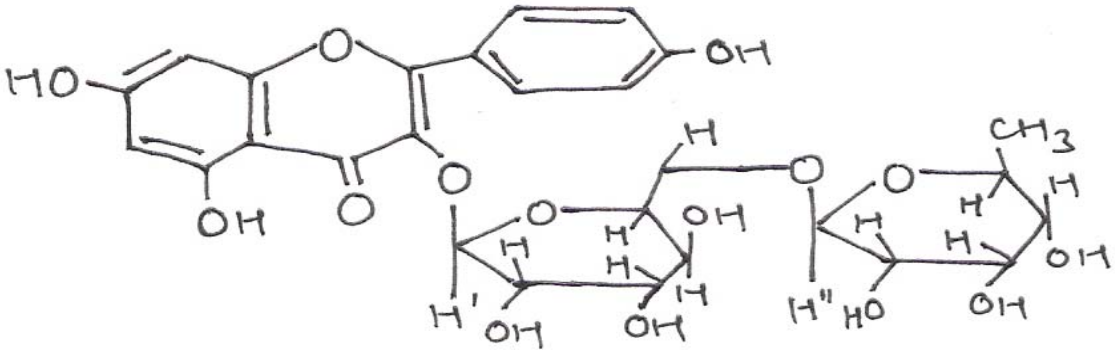
Compound (A) appeared dark purple under UV light changing to fluorescent yellow with NH3, indicating a flavone with free hydroxyls at C-4' and C-5 (Mabry et al., 1970). When cellulose TLC of Compound (A) was sprayed with NA reagent, it turned to yellow, indicating presence of p-hydroxy substituted B-ring and absence of orthodehydroxyl group in the B-ring (Homberg and Geiger, 1980; Geiger and Homberg, 1983). Compund (A) gave positive Feigl spot test for suger. Total acid hydrolysis of (A) with 2N HCL yielded rhamnose and glucose sugar and kaempferol, all identified by chromatographic comparison and spectral method. The aglycone, kaempferol was identified by 'HNMR (DMSO - d6, 400 MHz). `HNMR spectrum of aglycone showed the expected signals in the aromatic region, i.e. two meta coupled doublets each with J=2.0 H2 at δ 6.20 and 6.44, for the A-ring H-6 and H-8 protons, and two ortho coupled symmetrical doublets each with J=8.8 H2, at δ 6.94 and 8.02. Which correspond to the protons of H-3', H-5' and H-2', H-6' of B-ring. A low field broad singlet of δ 12.5, represent chelated 5-OH group of flavonoid nucleus.

Compound (A) appeared dark purple under UV light while its acid hydrolysed aglycone, kaempferol exhibited dull yellow colour on PC with UV light, indicating a release of sugar moieties from the 3-position (Sayed et.al., 1999). The `HNMR (DMSO - d6, 400 MHz) of (A) showed two ortho coupled symmetrical doublets each with J=8.5 H2 at δ 6.82 and 8.03 which correspond to the H-3', H-5' and H-2' and H-6' of the B-ring and the two meta coupled doublets each with J=2.0 H2 at 6.21 and 6.45, for H-6 and H-8, of the A-ring. Two anomeric protons at δ 5.28 (d, J=7.5 H2) and δ 4.38 (d, J=2H2) were attributed to glucose moiety (B- configuration) directly linked to the aromatic ring at the 3-position and a rhamnose (δ-configuration) linked to the 3-O-glucosyl moiety. The rhamnosyl methyl appeared as doublet at δ 110 (J=5.0 H2). The remaining sugar protons were observed in the range δ 2.98 - 4.02. The appearance of anomeric proton of rhamnose terminal sugar at δ 4.38, indicated it is attached with C-6 of primary glucose sugar moiety.

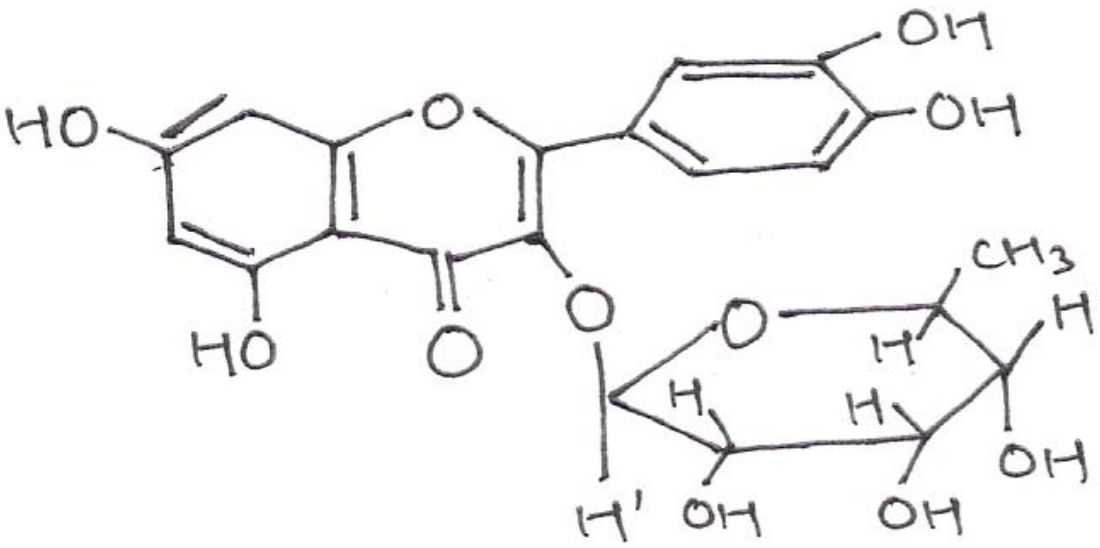
H_2O_2 oxidation of compound (A) with H_2O_2 and reacting mixture was evaporated to dryness and residue was dissolved in isopropanol. Isopropanol soluble was chromatographed on Whatman No. 1 PC using BAW (n-BuOH-AcOH-H2O, 4 : 1 : 5, V/V, upper layer) as a developing solvent. The PC was inspected with UV light. A dull yellow UV fluorescent spot on PC appeared at Rf 60 under UV light was identified as kaempferol. The chromatographic properties of it were found similar to Kaempferol, which was isolated as an aglycone from the complete acid hydrolyzed mixture of compound (A). After identification of UV fluorescent spot as kaempferol, on PC of isopopanol soluble from H2O2 oxidised mixture, the chromatogram was sprayed with benzidine reagent. The PC was dried in oven at 110^{o}C for 10 minutes. A brown spot was visualized at Rf, 13 in BAW and it was identified as rutinose sugar by CoPC with its standard. Thus, compound (A) was identified as Kaempferol-3-O-rhamnosyl (1-6) glucoside or kaempferol-3-O-rutinoside. (Structure 1).

Compound (B) a dark purple UV fluorescent on PC with UV light, was isolated from FRAC-02, a fraction derived from 10% HOAc cellulose cc fractionation of BuOH soluble. On the basis of chromatographic properties (Rf values and colour reactions with UV/NH3, UV/NA and UV/ZrOCl2 it was identified as a Quercetion-3-O-mono glycoside (Mabry *et al*., 1970; Mabry and Markham, 1975). Complete acid hydrolysis of the compound with 2NHCl gave Quercetin (COPC) and rhamnose (COPC). Thus the compound (B) was identified as Quecetein-3-O-α-L-rhamnoside. The `HNMR (DMSO-d6; 400 MH2), of compound (B), gave two meta coupled doublets each with J=2.0 H2, at 6.20 and 6.40, representing H-6 and H-8 of the A-ring. Three proton signals at 7.32 (1H,d, J=2.0 H2), 6.88 (1H,d,J=8.5) and 7.20 (1H, d,d, J=2.0 H2 and 8.5 H2), correspond to the H-2', H-5' and H-6' protons of B-ring. The anomeric proton signal appeared as doublet at 5.56 (J= 1.15) assigned to a α- rhamnose moiety directly linked to aromatic ring at 3 position. Thus, the compound (B) was identified as Quercetin-3-O-α-L rhamnoside. (Structure 2).

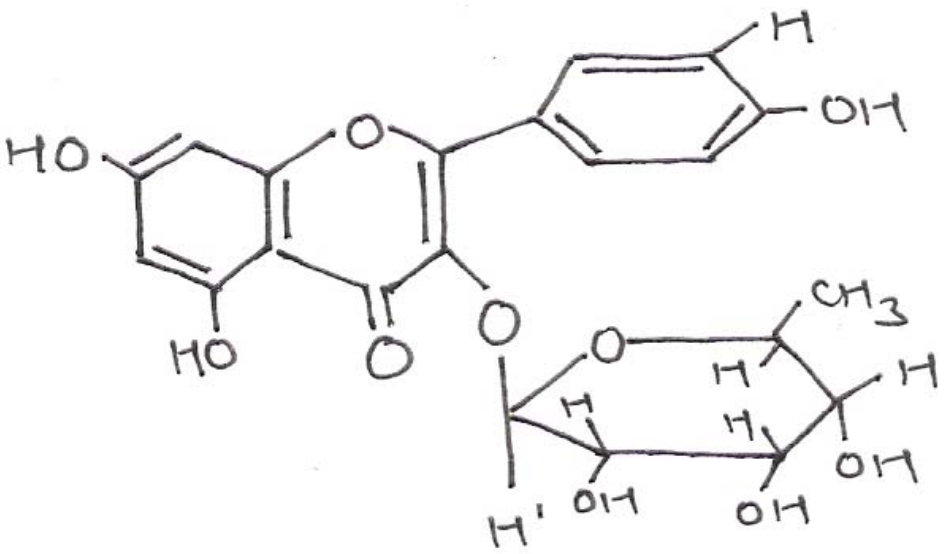
Compound (C), a dark purple UV fluorescent compound, was isolated from FRAC-02, a fraction derived from 10% HOAc fractionation of BuOH soluble on cellulose cc. On the basis of chromatographic properties (RF values and colour reations, UV, UV/NH3, UV/NA, UV/ZrOCl2), it was identified as a flavonol-monoglycoside (Mabry *et al*., 1970; Markham, 1982; Yahara *et.al*., 2000). Complete hydrolysis of compound with 2NHCl, gave a flavonol aglycone, kaempferol. It was identified by `HNMR spectra. In `HNMR spectra four doublets were observed in aromatic region at δ 6.20, 6.40, 6.94 and 8.02, which were assignable to H-6, H-8, H-3', 5' and H-2', 6' respectively. The sugar released after the acid hydrolysis of the compound was identified as rhamnose (CoPC). The compound (C) gave violet spot on PC with UV light while its aglycone, gave dull yellow UV fluorescence, indicating release of sugar moiety from 3-position (Mabry *et al*., 1970). The `HNMR of the compound (C) was found similar to the compound (B) in sugar region. Thus, the compound (C) was identified as Kaempferol-3-O-α-L- rhamnoside. (Structure 3).



Structure 1. Kaempferol-3-O-rutinoside



Structure 2. Quercetin-3-O-α-L-rhamnoside



Structure 3. Kaempferol-3-O-α-L-rhamnoside

CONCLUSIONS

The pursue for antioxidant compounds from the ethanol extract of *Bergenia stracheyi* (HK.) leaves led to the isolation and identification of two flavonol glycosides. Flavonoids are secondary metabolities widespread in plant kingdom and their occurrence in leaves has been related to the need of protection from the oxidative processes (Ribeiro *et al*., 2002). Thus flavonol glycosides isolated in this work might play a role in the protecting system in various oxidative processes system in various oxidation processes occurs in human beings.

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Corresponding author:
Harish Kumar
Department of Botany and Microbiology
Gurukul Kangri University
Haridwar- 249404
Uttarakhand, India
Phone: +91-1334-235003, 09410730060
E-mail: harish2129@gmail.com

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