Flavonol glycosides from antioxidative activity guided fractionation of aqueous-ethanolic extract of *Bauhania retusa* Roxb.

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ABSRACT: *Bauhania retusa* Roxb., a traditional medicinal and leguminous fodder plant of Kumaun hills, is widely distributed in sub-tropical and terai regions of Himalaya ranging altitude from 3000ft. to 4,500ft. Leaves of the plant have widely been used for curing cough, bronchitis, diuretic, diabetes, dysentery, sores, liver-dysfunction, inflammation, ulcers, piles and skin diseases by various tribal folks of Kumaun Himalaya. Leaf flavonoids derived from various food and fodder plants have widely been recommended for curing diseases related to oxidative stress. The n-BuOH fraction from aqueous-ethanolic extract (1:1) of the leaves of *Bauhania retusa* was fractionated on cellulose cc using 30 to 50% HOAc. All the dark purple fluorescing bands observed on cc were eluted and collected separately by monitoring with UV light. The combined fraction derived from the elute of dark purple fluorescing bands gave antioxidative activity against the methanolic solution of DPPH free radical in UV-VIS spectrophotometer at 515 nm. The flavonol glycoside, Quercetin-3-O-rutinoside, Kaempferol-3-O-α-δ-rhamno-pyranosyl-(1→2)-β-D-glucopyranoside-7-O-α-δ-rhamnopyranoside, Quercetin-3-O-α-δ-rhamnopyranosyl (1→2)-β-D-glucopyranoside-7-O-α-δ-rhamnopyranoside and Quercetin-3-O-β-D-glucosyl(1→2)glucoside-7-O-β-D-glucoside were identified from antioxidative active fraction of aqueous-ethanolic of aqueous-ethanolic extract of *Bauhania retusa*. [Nature and Science. 2009;7(4):63-65]. (ISSN: 1545-0740).

Key Words: *Bauhania retusa* Roxb., antioxidative activity, flavonol.

INTRODUCTION

Bauhania retusa Roxb., a member of Caesalpionioideae group of leguminous phanerogamic plant, is a tree bearing broad entire leaves, white-pinkish flowers and brown-yellow pods. Genus *Bauhania* comprises 300 species with cosmopolitan in distribution. Most of the *Bauhania* species of Kumaun Himalaya have been reported as a traditional medicines for curing number of ailments like diabetes, inflammation, snake bite, dysentery, sores, liver disorders, ulcers, piles and skin diseases by various ethnic groups (Chopra *et al*., 2002; Pande and Jain, 2002; Singh, 2002). Flavonoidal compounds, a diversified group of polyphenolic heterocyclic compounds and integral constituents of food and fodder plants, have widely been used for diseases related to oxidative stress like diabetes, strokes, arthritis, cancer, inflammation and antibiotic. *Bauhania* extracts have widely been identified as antioxidants and are rich source of flavonoidal compounds (Rahman and Begum, 1966; Gupta *et al*., 1980; Kumar *et al*., 1990). Many flavonol glycosides have been obtained from some fodder species of *Bauhania* (Salatino *et al*., 1998; Yahara *et al*., 1994).

MATERIAL AND METHODS

1. **Authentification of plant species**: *Bauhania retusa* was identified by Prof. P.C. Pande, Department of Botany, Kumaun University S.S.J. Campus Almora Uttarakhand, India. The leaves, pods and twigs bearing flowers have been deposited (Vouchar speciman No. 40) in the Chemistry Department of Kumaun University SSJ Campus, Almora Uttrakhand.
2. **Extraction and Separation of Flavonoids**: The leaves of *Bauhania retusa* was collected from Lohaghat (District Champawat of Uttrakhand) sites of Kumaun. About 3 kg air dried powered leaves of *B. retusa* was extracted sequentially with 70% EtOH and 50% EtOH by cold percolation for many days. The two extracts were filtered and combined and evaporated to dryness under reduced pressure at 70^0 in Rota evaporator until H_2O layer remained. It was partitioned with CH_2Cl_2 and BuOH successively. The BuOH soluble was fractionated on cellulose cc (Merck) using H_2O-AcOH as an eluent. On eluting cc with 30% HOAc and 40% HOAc, a number of dark

purple fluorescing bands were observed and were eluted and collected separately by monitoring cc with UV light. All elutes derived from dark purple fluorescing bands were combined and concentrated under reduced pressure. The residue was chromatographed on Whatman No.3 PC using BAW (n-BuOH-AcOH-H2O, 4:1:5, V/V, upper layer) as a developing solvent. Three dark purple fluorescing broad bands observed on PC under UV light at Rf 47, 40 and 36, representing FRAC-01, FRAC-02 and FRAC-03 respectively.

3. **Antioxidative active screening**: Each fraction was examined for antioxidative activity by the standard thin layer autobiography methods (Cuendet *et al*., 1997 and 2000).
   TLC thin layer plate of FRAC-01, 02 and 03 were spotted and developed with CHCl3: MeOH (3:1). The dried and developed plates were sprayed with methanolic solution of DPPH free radical. Three antioxidative active yellow spots were observed on TLC of FRAC-01 while no antioxidative active spots were observed with FRAC-02 and FRAC-01.
4. **Isolation of flavonoids from antioxidative active FRAC-01**: The eluate of FRAC-01, an antioxidative active fraction, was chromatographic on Whatman No.1 PC using repeated development with BAW (4:1:5, upper layer). Two major dark purple fluoresent bands were observed on PC at Rf 56 and 48 and were cut and eluted separately with 70% MeOH. The elute of faster moving band gave compound (1) and three compound (2-4) were isolated from slower moving band by sephadex LH-20 cc using 40% MeOH as an eluent.

RESULT AND DISCUSSION

Compound **(1),** gave a molecular ion at m/z 609 $[M - H]^-$ in FAB-MS (-ve) and other prominent ions identified at 463 $(m/z\ 609\text{- rha})^-$ and 301 (m/z 463-gluco), indicating successive elimination of terminal rhamnose and glucose from Quercetin. It was further supported by complete acid hydrolysis of (1) with 2NHCl and gave Quercetin (CoPC), rhemnose (CoPC) and glucose (CoPC). The compound (1) was identified as Quercetin-3-O-rutinoside by comparison of spectroscopic and physical data with authentic sample or the reported values in literature.

Compound **(2)** gave a molecular ion at m/z 739 (FAB-MS -ve) calculated for $C_{33}H_{40}O_{19}$. It gave positive tests with Mg + HCl, $FeCl_3$ and α-naphthol. Acid hydrolysis of 2 with 2NHCl gave Kaempferol (CoPC), galactose (CoPC) and rhamnose (CoPC). H_2O_2 oxidation of 2 affored a flavonol glycoside 2(a) and robinose sugar (CoPC), indicating the robinose sugar is attached at C-3 of Kaempferol. The compound 2(a) was identified as Kaempferol-7-O-α-δ-rhamnoside (CoPC). Hydrolysis of 2 with α-rhamnosidase gave Kaempferol 3-O-β-D-galatoside (CoPC) and rhamnose (CoPC). Partial acid hydrolrsis of 2 with 0.1N HCl gave Kaempferol-3-O-galactoside-7-O-α-δ-rhamnoside and Kaempferol-7-O-α-δ-rhamnoside (CoPC). On the basis of FABMS and hydrolytic methods the compound (2) was identified as kaempferol-3-O-α-δ-rhamnosyl (1→6)-galactoside-7-O-α-δ-rhamnoside. The structure of 2 was further supported by `HNMR (in DMSO-d_6, 400m Hz).

`HNMR showed two meta coupled and two ortho coupled symmetrical doublets in aromatic region at (δ) 6.48 (1 H,d,J = 1.0Hz), 6.81 (1Hk,d,J = 1.0Hz), 8.14 (2H,d,J = 8.5Hz) and 6.93 (2H,d,J = 8.5Hz) represent H-6, H-8, H-2`/6` and H-3`/5`, respectively of Kaempferol. Three anomeric proton signals at δ5.53 (1H, d, J = 7.5Hz), 5.10 (1H, d, J = 1.0Hz) and 5.60 (1H, d, J = 7.5Hz) for C-3-glucosyl, rhamnosyl attached to C-2`` of C-3-glucosyl and rhamnosyl, C-7 respectively. Two methyl signals appeared at 0.90 (1H, d, J = 6.0Hz) and 1.22 (1H,d,J = 6.0Hz) for H-6``` and H-6```.

Compound **(3)** gave a molecular ion, at m/z 755 (FAB-MS -ve) and other prominent ions identified at m/z 609 (m/z 755-rham.), 447 $(m/z\ 609\text{-glaco.})^-$ and 301 (m/z 447-rham.), supporting the release of two moieties of rhamnose and one molecule of galactose from Quercetin. It has further been supported by complete acid hydrolysis of (3) with 2NHCl and gave Quercetin (CoPC), galactose (CoPC) and rhamnose (CoPC). The `HNMR of sugar region of (2) was found similar with the sugar region of compound (3). Thus, the compound (3) was identified as Quercetin-3-O-α-δ-rhamnopyranosyl (1→6)-galactoside-7-O-α-δ-rhamnopyranoside.

Compound (4): FABM (-ve) of (4) gave a molecular ion at m/z, 747 $(M-H)^-$ and other prominent ions at 463 $(m/z\ 747\text{-sopnrose sugar})^-$ and 301 $(462\text{-gluco})^-$ represent release of three moiety of glucose from Quercetin. Complete acid hydrolysis of (4) gave Quercetin (CoPC) and glucose (CoPC). H_2O_2 oxidation of compound (4) afforded Quercetin-7-O-β-D-glucoside (CoPC) and sophorose sugar (CoPC). Thus, sophorose sugar was released from C-3 of Quercetin. The compound (4) was identified as Quercetin-3-O-sophoroside-7-O-β-D-glucoside. `HNMR of (4) in DMSO-d6 (400 MHz) gave five doublets in aromatic region at 7.69 (1H,d,J = 2.0, 8.5, Hz), 7.51 (1H,d,J = 2.0 Hz) and 6.80 (1H,d,J =

8.5 Hz), 6.74 (1H,d,J = 2.0 Hz) and 6.41 (1H,d,J = 2.0Hz) represent H-6`, H-2`, H-5`, H-8 and H-6 respectively of Quercetin. Three anomeric proton signals appeared at 5.80 (1H, d, J = 7.5Hz), 5.16 (1H, d, J = 7.5Hz) and 4.67 (1H, d, J = 7.5Hz) represent 3-glucose-H-1``, 7-glucose-H``` and 2``-terminal glucose H-1``` respectively. Thus compound (4) was identified as Quercetin-3-O-β-D-glucosyl (1→2) glucoside-7-O-β-glucoside.

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