P-ISSN 2349–8528
E-ISSN 2321–4902
IJCS 2015; 3(1): 35-37

Received: 12-04-2015
Accepted: 14-05-2015

Jaya Gupta
Department of Chemistry,
Agra College, Agra

Amit Gupta
Department of Chemistry,
Agra College, Agra

A.K. Gupta
Department of Chemistry,
Agra College, Agra

Correspondence:
Jaya Gupta
Department of Chemistry,
Agra College, Agra

Extraction and identification of flavonoid natural antioxidant in the leaves of *Rauwolfia serpentina*

Jaya Gupta, Amit Gupta, A.K. Gupta

Abstract
For the presence of flavonoidal constituents, the leaves of Indian medicinal plant *Rauwolfia serpentina* were phytochemically analyzed. From the alcoholic extract, flavonoidal structure was isolated. The structure of the isolated compound was determined by using chromatographic and spectroscopic techniques. Structure of the isolated compound was determined as 3,3',4',5,7- pentahydroxy flavones i.e., Quercetin.

Keywords: *Rauwolfia serpentina*, flavonoid, natural antioxidants, 3,3',4',5,7- pentahydroxy flavones, NMR studies, etc.

1. Introduction
Flavonoids are reducing agents able to interact with free radical species. Falvonoids are low molecular weight polyphenolic compounds available in practically all dietary plants [1]. The common feature of these compounds is phenyl benzopyrone skeleton (C_6-C_3-C_6).Antioxidant flavonoids contribute to retardation or prevention of free radical production. In past few years, interest has increased considerably in finding naturally occurring antioxidants for use in food or to replace synthetic antioxidants. BHT (Butylated hydroxyl toluene) BHA (Butylated hydroxyl anisole) and other synthetic antioxidants are widely used but they are suspected to promote carcinogenic activity [2]. So attention is much shifted towards natural antioxidant.

Rauwolfia serpentina L.Benth of family apocynaceae is known as Sarpagandha, Chandrabhaga, Chota chand in Hindi, Rauwolfia, Indian snake root in English, chevanamalpodi in Tamil, Harkaya, Harki in Marathi, Chandra in Bengali etc [3]. It is evergreen, perennial, glabrous shrub. In India it is found in Northern Himalayas, especially in Gharwal region, Punjab, Gangetic plains etc [4]. *Rauwolfia serpentina* is used in the treatment of high blood pressure [5], cardiovascular diseases [6], breast cancer [7], mental disorder. It is used an antidiuretic, antioxidant, anti-inflammatory, anticancerous, antidiarrhoeal, antifibrillar, antiproliferative etc [8-10]. The main object of this study is to extract and identify flavonoid natural antioxidant in *Rauwolfia serpentina*.

2. Experimental
^{1}H NMR and ^{13}CNMR spectra were recorded on a Bruker Advance 400 MHZ spectrometer. The EI-mass was recorded on Shimadju QP 2000 mass spectrometer. UV-spectra were recorded on Shimadju UV-160 spectrophotometer. The leaves of *Rauwolfia serpentaina* was collected from Agra College, Agra. The leaves were dried under shade for fourteen days.The100gm powdered leaves of *Rauwolfia serpentaina* were subjected to hot extraction in soxhlet apparatus with petroleum ether, ether and chloroform for the removal of fatty materials. Now, remaining powder was extracted with 95% ethyl alcohol in soxhlet apparatus. The extract was evaporated in vaccum to leave a residue. The dried residue was dissolved in ethyl alcohol and subjected to chromatography, using the silica gel as stationary phase and mobile phase containing isopropanol-formic acid-water (2:5:5).

Yellow bands with R_f value 0.18 were located on chromatogram. These yellow bands were carefully eluted and extracted. The solvent was evaporated from the resulting extracts and residue was obtained. It was subjected to various physical and spectral analysis.

2.1 Chemical identification of flavonoids [11]:-

1. **Shinoda Test: -** To a small amount of extract in alcohol, magnesium ribbon was added followed by addition of drops of concentrated hydrochloric acid. Formation of pink color confirms the presence of flavonoids.
2. **Concentrated sulphuric acid Test: -** To a small amount of extract, two drops of concentrated sulphuric acid was added, orange color confirms the presence of flavonoids.
3. **Aluminium Chloride Test: -** To a small amount of extract, two droops of 1% aluminium chloride is added, yellow color confirms the presence of flavonoids.
4. **Zinc-Hydrochloride Reduction Test: -** To the extract add a mixture of Zn-dust and concentrated hydrochloric acid. Heat the solution after few minutes, color of the solution changes to red.

2.2 Quercetin: Slightly yellow powder; m. p 316 °C; ^{1}H NMR (400 MHz, Me OD): δ (ppm) = 6.19 (1H, *d, J* = 2.0 Hz, H-6), 6.40 (1H, *d, J* = 2.0 Hz, H-8), 6.88 (1H,*d, J* = 8.2 Hz, H-5'), 7.62 (1H, *dd*, *J* = 8.3; 2.1 Hz, H-6'), 7.75 (1H, *d*, *J* = 2.1 Hz, H-2'),^{13}C NMR (100MHz, Me OD): δ (ppm) = 148.1 (C-2), 137.2 (C-3), 177.5 (C-4),162.5(C-5), 99.4 (C-6), 165.8 (C-7), 94.6 (C-8), 158.1 (C-9), 104.6 (C-10), 124.2 (C-1'), 116.1 (C-2', C-5'), 146.2 (C-3'), 150.4 (C-4'), 121.9 (C-6').

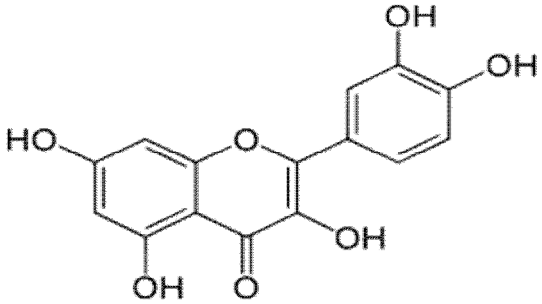

Fig: Structure of Quercetin

3. Results and Discussion

The U.V spectrum of this compound exhibited two major absorption peaks in the region 260- 400nm.Two peaks are at 278nm and 362nm. Peaks of Flavones occurs in the range of 304-350nm.The EI- mass spectrum of the compound showed the molecular ion at m/z 302(M+) corresponding to the molecular formula $C_{15}H_{10}O_7$ and in agreement with the other spectroscopic data. The ^{1}H NMR spectrum showed that the proton H-6 and H-8 appeared as a duplet at δ 6.19 and δ6.40. The proton of H-5' and H-6' and H-2' appears at δ 6.88, δ7.62 and δ7.75 respectively.[13] CNMR spectrum showed a carbonyl group at δ 177.5. The carbon bonded to hydroxyl group appeared at δ137.2, δ146.2, δ150.4, δ162.5, δ165.8. ^{13}CNMR of the compound showed 15 signals for flavonoid skeleton.

The protons of C-6 and C-8 of flavonoids which contain the common 5,7-dihydroxy substitution patterns give rise to two doublets in the range 6.0-6.5. The H-6 doublet occurs consistently at higher field than the signal for H-8.

The position of the C-2' and C-6' doublets appears at lower field (7.1-8.1). The carbonyl C- 4, resonates at around δ175-178, when the carbonyl in not hydrogen bonded but in the presence of hydrogen bonding to a 5-hydroxyl group it moves down field to about δ 182. When 3-hydroxyl group is present as well as 5-hydroxyl group, it resonates at about δ176, but when 3-hydroxy group is alone it resonates at about δ171-173[13]. Two doublet proton were recorded in NMR spectrum at δ6.88 and δ7.75 corresponds toH-5' and H-2'. One double doublet at δ 7.62 corresponds to H-6'. ^{13}C-^{1}H coupling data have been used to good effect in the distinction of C-6 and C-8 signals and C-5 from C-9 signals. The degree of coupling identifies each carbon and demonstrates the C-5 resonated down field from C-9 and C-6 resonates downfield in comparison from C-8. The quercetin has been earlier reported in various plants [14-15].

4. Conclusion

Quercetin is natural flavonoid. Quercetin possesses anticancer, anti- inflammatory [16], anticancer, antiviral activity. It is used in asthma, eczema, inhibits inflammatory leukotriene production, stablizes free radicals etc [17-20]. Quercetin is natural antioxidant and may be useful if it is used in place of artificial or synthetic antioxidants. According to WHO/ FAO synthetic antioxidants are toxic and their use in food has been declining due to suspected action as a promoter of carcinogenesis. Thus, the use of quercetin as a potential natural antioxidant and a possible substitute of artificial antioxidant should be considered. So production of quercetin from *Rauwolfia serpentina* may be of economic benefit.

5. Acknowledgement

We are very grateful to University Grants Commission, New Delhi, India for their financial assistance (Grant No.F.15-39/12 (SA-II)). We are also very thankful to Dr. M.K. Rawat, Principal Agra College, Agra for their support.

6. References

1. Ren W, Qiao Z, Zhu L, Zhang L. Flavonoids: promoting anticancer agents. Med. Res. Rev 2003; 23(4):519-534.
2. Lalas S, Tsakins J. Extraction and identification of natural antioxidant from the seeds of *Moringa oleifera* tree variety of Malawi. J. Am. Oil. Chem. Soc 2002; 79(7):677-683.
3. Mallick SR, Jena RC, Samal KC. Rapid *in vitro* multiplication of an endangered medicinal plant sarpgandha (*Rauwolfia serpentina*).American Journal of Plant Sciences 2012; 3:437-442.
4. Singh P, Singh A, Shukla AK, Singh L, Pande V, Naliwal TK. Somatic embryogenesis and in vitro regeneration of an endangered medicinal plant sarpgandha (*Rauwolfia serpentina*.L.), Life Science Journal 2009; 6(3):74-79.
5. Singh PK, Kumar V, Tiwari RK, Sarma A, Rao CV, Singh RH. Medico- Ethnobotany of 'Chatara'Block of District Sonebhadra, Uttar Pradesh, India. Advances in Biological Research 2010; 4(1):65-80.
6. Anitha S, Kumari BDR. Stimulation of reserpine biosynthesis in the callus of *Rauvolfia tetraphyla* L.by precurson feeding. African Journal of Biotechnology 2006; 5:659-661.
7. Stanford JL, Martin EJ, Brintin LA, Hoover RN. Rauwolfia use and breast cancer: A case- control study.Journal of the National Cancer Institute 1986; 76:817-822.
8. Harisaranraj R, Suresh K, Babu SS, Achudhan VV. Phytochemical based stratagies for pathogen control and antioxidant capacities of *Rauwolfia serpentina* extracts. Recent Research in Science and Technology 2009; 1:67-73.
9. Deshmukh SR, Dhanashree SA, Patil BA. Extraction and evaluation of indole alkaloids from *Rauwolfia serpentina* for their antimicrobial and antiproliferative activities. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(5):329-334.

10. Ezeigbo I, Ezeja M, Madubuike K, Ifenkwe D, Ukweni I, Udeh N, Akomas S. Antidiarrhoeal activity of leaf methanolic extract of *Rauwolfia serpentina*. Asian Pacific Journal of Tropical Biomedicine 2012; 2(6):430-432.
11. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis.IIIrd ed., London, Chapmann and Hall, 1984.
12. Marby TJ, Markham KR, Thomas MMB. The systematic identification of flavonoids, Springler-Verglar Publishers: New York 1970, 1-58.
13. Markham KR, Chari VM. Carbon-13 NMR spectroscopy of flavonoids. In the flavonoids: Advances in research; Harborne JB, Marby TJ. Eds Chapmann and Hall; London United Kingdom 1982, 19-35.
14. Gupta J, Gupta A, Gupta AK. Studies on chemical constituents of leaves of *Phyllanthus emblica* (L.) Oriental Journal of Chemistry 2014; 30(4):2069-2071.
15. Petrus K, Schwartz H, Sontay G. analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry. Anal Bioanal Chem 2011; 400(8):2553-2563.
16. Park HJ, Lee Cm, Jung JD, Lee JS, Jang YI, Chang JH *et al*. Quercetin regulate Th1/Th2 balance in urine model of asthma. Int. Immunopharmacol 2009; 9(3):261-267.
17. Park C, So HS, Shin CH, Baark SH, Moon BS, Shin SH *et al*. Quercetin protects the hydrogen peroxide induced apoptosis via inhibition of mitochondrial dysfunction in H9c2 cardiomyoblast cells. Biochem Pharmacol 2003; 66(7):1287-1295.
18. Galisteo H, Garcia-Saura MF, Jimenez R, Villar IC, Zarzuelo A, Vargas F *et al*. Effects of chronic quercetin treatment on antioxidant defense system and oxidative states of deoxycorticosterone acetate-salt-hypertensive rats. Mol Cell Biochem 2004; 259(1-2); 91-99.
19. Qin TC, Chen L, YU LX, Gu ZL. Inhibitory effect of quercetin on cultured neonatal rat cardiomyocytes hypertrophy induced by angiotensin.Acta Pharmacol Sin 2001; 22(12):880-886.
20. Hackl LP, Cuttle G, Dovichi SS, Lima-Landman MT, Nicolau M. Inhibition of angotesin- converting enzyme by quercetin alters the vascular response to brankykinin and angiotensin 1. Pharmacology 2002; 65(4):182-186.