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Jaya Gupta
Department of Chemistry,
Agra College, Agra, India.

Amit Gupta
Department of Chemistry,
Agra College, Agra, India.

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

Isolation and identification of flavonoid rutin from *Rauwolfia serpentina*

Jaya Gupta, Amit Gupta

Abstract
In the present work, methanolic extract from the leaves of *Rauwolfia serpentina* were analyzed phytochemically for the presence of flavonoids. Phytochemical studies revealed the presence of flavonoidal structure, by using chromatographic and spectroscopic techniques, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one i.e., rutin is identified.

Keywords: *Rauwolfia serpentina,* flavonoids, IR, NMR, etc.

1. Introduction
Rauwolfia serpentina is an evergreen, woody glabrous shrub belongs to family apocynaceae. It is known as sarpagandha in Hindi, Indian snake root in English, amalpori in Malayalam, chandra in Bengali etc [1]. It is an herb of medicinal value described in ayurvedic, western system of medicine. In India, it is found in Northern Himalayas especially in gharwal region, gangetic plains etc. Its leaves, seeds, roots, fruits are used in treatment of various ailments [2]. It is used in the treatment of hypertension [3], arrhythmia [4], high blood pressure [5], human promyelocytic [6] leukemia, fever [7], malaria [8], eye diseases, pneumonia [9], asthma [10], AIDS, spleen disorder [11], skin diseases etc.
Dietary flavonoids are natural antioxidants [12]. Flavonoids are good antioxidants because of more number of target sites for free radicals [13]. Over 4000 structurally unique flavonoids have been found in plants. These compounds have common features i.e., phenyl benzopyrone skeleton (C_6-C_3-C_6). Flavonoids have existed for over one billion years and possess anti-inflammatory [14], antioxidant [15], anti-thrombic [16], anti- hypertensive [17] activity. Flavonoid serves as antioxidant by scavenging singlet oxygen [18], superoxide anion [19] and lipid peroxy radicals [20].
The main object of this study is to extract and characterize flavonoid antioxidant in the leaves of *Rauwolfia serpentina*.

2. Experimental
^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker Advance 300MHZ spectrometer. The EI-mass was recorded on Shimadzu QP 2000 mass spectrometer. The FT-IR spectrum was recorded on Perkin Elmer, spectrum 100 instrument. UV-spectra were recorded on Shimadzu UV-160 spectrophotometer. The leaves of *Rauwolfia serpentina* was collected from Agra College, Agra. The leaves were dried under shade for fifteen days. The fine powder was obtained from dried leaves using kitchen mixer grinder. The 50gm powdered leaves were subjected to extraction using 500ml 80% methanol in a soxhlet extractor for 24 hours. In a rotatory vaccum evaporator, the extract was concentrated by evaporation to 50-60-ml.In 100 ml of distilled water, the concentrated methanolic extract was suspended and ether was added. Ethereal layer was separated. The hydro alcoholic layer was again extracted with the ether and again ethereal layer was separated. The hydro alcoholic layer was evaporated under reduced pressure to10 ml. Again, this process was repeated with chloroform. The concentrated residual liquid is cooled at 0-5^{o}C, a crystalline solid substance was obtained.
The crystalline substance was subjected to thin layer chromatography using the silica gel as stationary phase and methanol: glacial acetic acid: water (90:5:5) and benzene: acetic acid: water (60:35:5) as mobile phase. Yellow bands with R_f value 0.45 and 0.31 were obtained. On the basis of phytochemical and spectroscopic studies the characterization and structural elucidation of the compound was done.

Chemical identification of flavonoids [21]:-

1. Shinoda Test: - To a small amount of test solution 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. Zn- Hydrochloride Reduction Test: - To the test solution add a mixture of zinc dust and concentrated hydrochloric acid. Heat the solution, after few minutes, color of the solution changes to red.
3. Aluminium Chloride Test: - To a small amount of test solution, two drops of 1% aluminum chloride was added, yellow color was obtained.

Rutin, light yellow powder, m. p 190 ^{o}C, molecular formula C_{27} H_{30} $O_{16.}$ IR (KBr) V_{max}cm-^{1}:3408, 3321(OH-stretching), 2483 (CH_2-stretching), 2714 (C-H bonding), 1462 (C=O groups) and 1383 (C-OH vibrations). ^{1}HNMR(300 MHz, CD_3OD) δppm = 6.18 (*d, J=2.0* HZ, 1H,H-6), 6.36 (*d*, *J*=2.0Hz,1H,H-8), 7.66 (*d*,*J*=2.05Hz,1H,H-2'), 6.88 (*d*, *J*=8Hz,1H,H-5'), 7.62 (*dd*, *J*=8.5 & 2.2Hz, 1H, H-6'), 5.12 (*d*, *J*=7.5Hz, 1H,H-1"), 4.50 (*d*,*J*=1.5Hz,1H,H-1"'), 1.12 (*d*,*J*=6.3Hz,3H,H-6"'), 3.32-3.86(m). ^{13}CNMR (75.5 MHz,CD_3OD) δppm=158.6 (C-2), 135.8 (C-3), 179.5 (C-4), 162.6 (C-5,) 100.1 (C-6), 166.1 (C-7), 94.9 (C-8), 159.3 (C-9,) 105.7 (C-10,) 123.2 (C-1'), 117.7 (C-2'), 145.8 (C-3'), 149.8 (C-4') 116.1 (C-5'), 123.4 (C-6'), 104.8 (C-1'') 75.7 (C-2''), 77.3 (C-3''), 71.5 (C-4''), 78.1 (C-5'') 68.7 (C-6''), 102.5 (C-1'''), 72.1 (C-2'''), 72.2 (C-3''') 73.9 (C-4'''), 69.8 (C-5'''), 18.0 (C-6''')

3. Result and Discussion

The UV spectrum of this compound exhibited two major absorption peaks in the region 360nm and 258nm, which indicates the presence of flavonol structure.

Mass spectra of isolated compound show molecular ion m/z 610 [M^{+}] corresponding to the molecular formula C_{27} H_{30} O_{16}.

IR spectra showed OH-stretching at 3408 cm-^{1}, 3321 cm-^{1},CH_2-stretching at 2843 cm-^{1},CH-bonding at 2714 cm-^{1},COgroup at 1462 cm-^{1},C-OH vibration at 1383 cm-1

The ^{1}HNMR spectrum showed a two doublet proton at the region δ6.18 and δ6.36 corresponding to H-6 and H-8 protons respectively. The protons at C-6 and C-8 of flavonols which contain the common 5,7-dihydroxy substitution pattern give rise to two doublet in the range 6.0-6.5ppm.The H-6 doublet occur consistently at higher field than the signal for the H-8 [22]. Four doublets proton occurs at δ4.50, δ5.12, δ6.88 and δ7.66 corresponding to H -1''', H-1''.H- 5' and H-2'protons respectively. One double doublet proton found at the region 7.62 corresponding to H-6' proton ^{13}CNMR showed 27 carbon signals, double bond between C-2 and C-3.The carbonyl carbon appears at δ179.5. The carbonyl carbon resonates at around 175-178 ppm, when the carbonyl is not hydrogen bonded, but in the presence of hydrogen –bonding to a 5-hydroxyl group it moves down field to about 182 ppm [23].

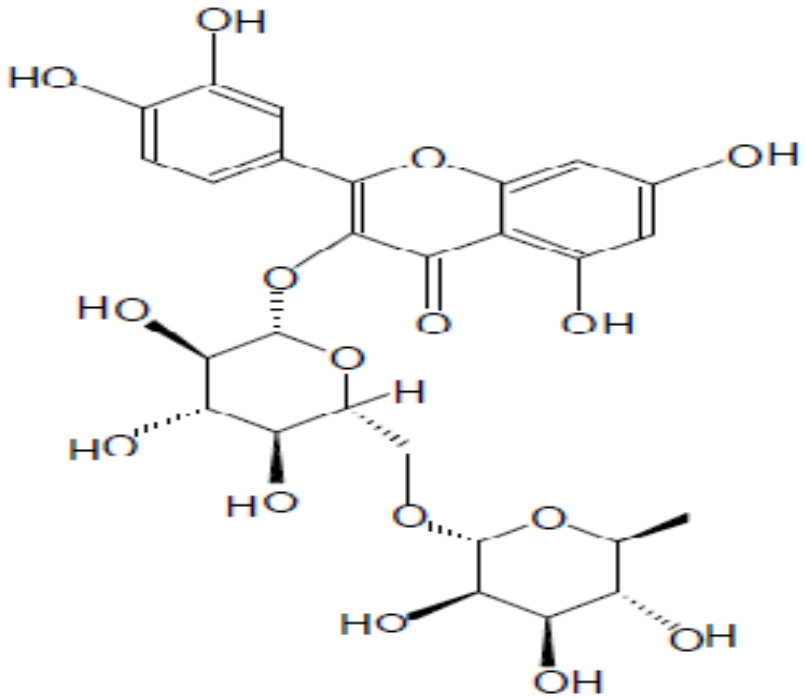

Fig 1: Structure of Rutin

From the physical, chemical and spectral characteristics it was confirmed that the isolated compound was 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one i.e., rutin. The rutin has been earlier reported in other plants [24].

4. Conclusion

Rutin protects against DNA damage [25]. Rutin is protective against carcinogensis [26]. Rutin inhibits low-density lipoprotein (LDL) peroxidation [27]. Rutin possess antioxidant activity [28]. Rutin is used in the treatment of various conditions related to capillary bleeding and increased capillary fragility and permeability. Flavonoids are a part of human diet. *Rauwolfia serpentina* contains rutin which is a flavonoid. The production of rutin from *Rauwolfia serpentina* may be of economic benefit.

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