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ISOLATION AND CHARACTERIZATION OF SECONDARY METABOLITES FROM FLOWER METHANOL EXTRACT OF *CATHARANTHUS ROSEUS*

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ABSTRACT

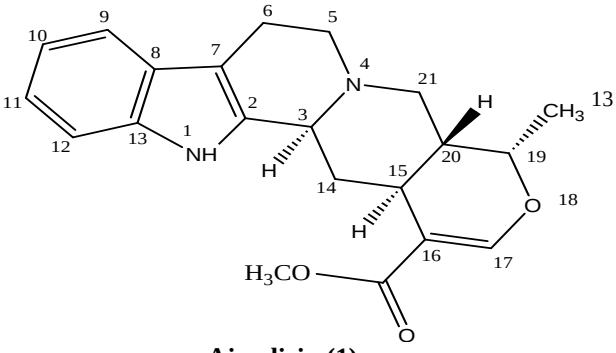
Catharanthus roseus is an important medicinal plant in Bangladesh. The plant is administered as a cooling medicine. From methanol extract 5 Compounds: ajmalicin (**1**), catharanthine (**2**), 3,3',4',5,7-Pentahydroxyflavone or Quercetin (**3**), Quercetin-3-O-rutinose or Rutin (**4**) & benzoic acid (**5**) have been isolated from flowers *Catharanthus roseus*. The structures of the compounds have been established by different spectroscopic data analysis. Compounds Quercetin-3-O-rutinose or Rutin (**4**) and benzoic acid (**5**) were isolated for the first time from flowers as well as from of the plant *C. roseus.*

KEYWORDS: *Catharanthus roseus*, isolation, structure elucidation, spectroscopic methods.

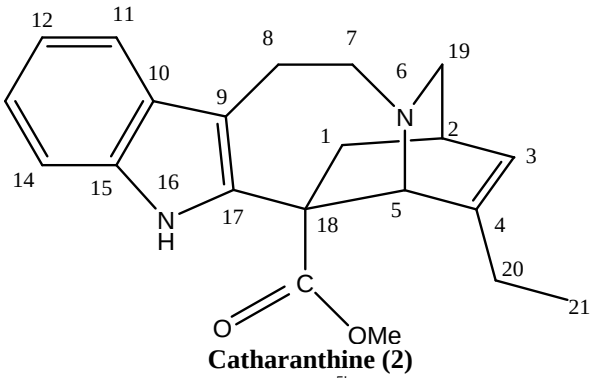
INTRODUCTION

Medicinal plants products have been part of phytomedicine since time immemorial. High plants are sources of drug which have made important contribution to the welfare and quality of life urban as well as rural communities especially in tropics and sub-tropics.[1] Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country.[2] Medicinal plants are a source of great economic value all over the world. Today, according to world health organization (WHO) as many as 80% of the world's people depend on traditional medicine for their primary health care needs.[3] During the early years of human existence, many plants materials by instinct, intuition of trial and error were used to combat different aliments.[4]

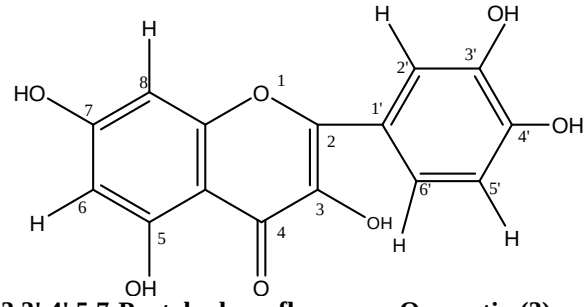
Catharanthus roseus, (Common name-Periwinkle, Vinca; Bengali-Nayantara, Synonyms - Vinca rosea; Family - Apocyanaceae), an important medicinal plant. It is popularly known as madagascar periwinkle is a potential source for anti-leukemic alkaloids. It is cultivated mainly for its alkaloids which are having anticancer activities.[5] It is an evergreen subshrub or harbeceous plant growing up to 1 m tall.[6] This plant is administered as a cooling medicine. It is used for the treatment of diabetes, fever, malaria, throat infection and chest complaints. It is also used for the regulation of menstrual cycles, and as a euphoriant.[7] The plant is an important source of indole alkaloids which are present in all plant parts. The physically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine and reserpine are reported to be present in the roots.[8] Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia.[9,10] These Catharanthus alkaloids are also used for the treatment of both malignant and nonmalignant diseases and in platelet and platelet associated disorder. Previous phytochemical investigations resulted in the isolation of Kaemferol[11], Kaempferol trisaccarides[12], Quercetin[13], Quercetin trisaccarides[11], Syringetin glycosides[14], Malvidin[13], Malvidin 3-*O*-glucosides[15], Malvidin 3-*O*-(6-*O*-p-coumaroyl)[15], Petunidin[13], Petunidin 3-*O*-glucosides[15], Petunidin 3-*O*-(6-*O*-p-coumaroyl)[15], Hirsutidin[13], Hirsutidin 3-*O*-glucosides[15], Hirsutidin 3-*O*-(6-*O*-p-coumaroyl)[15], Catharanthus Plant produce many pharmaceutically important alkaloids. They are antineoplastic medicines and the monoindole alkaloids ajmalicin and serpentine are antihypertension drugs[16-22], Our recent study on methanol part of flower of this plant has led to the isolation of ajmalicin (**1**), catharanthine (**2**), 3,3',4',5,7-Pentahydroxyflavone or Quercetin (**3**), Quercetin-3-O-rutinose or Rutin (**4**) & benzoic acid (**5**) (Figure 1).



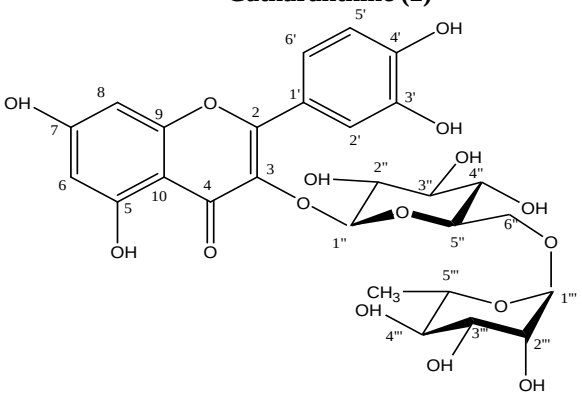
Ajmalicin (1)



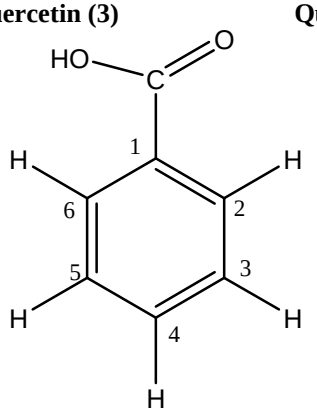
Catharanthine (2)



3,3',4',5,7-Pentahydroxyflavone or Quercetin (3)



Quercetin-3-O-rutinose or Rutin (4)



Benzoic acid (5)

Figure 1: **Structures of the isolated compounds with numbering.**

MATERIALS AND METHODS

Melting points were determined by thin disc method on a Fisher-John's electrothermal melting point apparatus. UV spectra were recorded in methanol on a Shimadzu UV-Visible spectrophotometer. IR spectra were recorded on a Shimadzu FT-IR spectrometer as thin film or KBr disc. NMR spectra were recorded in $CDCl_3$ using Bruker WH 400 MHz NMR spectrometer. Mass spectra of the compounds were measured on Finnigan Mat SSQ 710 spectrometer with ionization induced by electron impact at 70 eV. Separation by column chromatography was carried out using silica gel 40 (70-230 mesh, E. Merck). Thin layer chromatography was carried out on TLC plastic sheets pre-coated with silica gel 60 F_{254} (E. Merck).

Collection of plant material

Fully matured fresh flowers of this plant were collected from the gardens of Chemistry Department of Dhaka university, Bangladesh in June 2013 and identified by the taxonomist of Bangladesh national Herbarium, Dhaka, where a voucher specimen (No. = 39512) has been deposited. The flowers of *C.roseus* were air dried. These dried samples of flowers were powdered using 20 mesh screen in Willey mill and then used for subsequent analysis.

Extraction of the flower parts of *C. roseus*

Flowers dried, grinned and screened powder (200g) was extracted successively with different solvent at room temperature. At first it was extracted with n-hexane for 5 days and the extract was dried to get a gummy mass (7.15 g) using rotary evaporator. Then the residual part of the flower was extracted with dichloromethane for 5 days and the extract was dried to gummy mass (5.80 g). Again the residual part was extracted with methanol and the filtrate was dried under reduced pressure to get methanol extract (22.69 g).

Isolation of compounds from crude extracts

The dry mass of methanol extract (15.0 g) was dissolved in minimum amount of solvent and adsorbed by the column grade silica gel. The adsorbed material was placed on the top of the silica get bed (TLC grade) packed in VLC apparatus. The system was first eluted with 100% n-hexane and then eluted with mixtures of n-hexane and ethyl acetate (EA) and finally with the mixtures of ethyl acetate and methanol with increasing polarity. The eluents were collected in an amount of 200 ml each in 26 conical flasks. The collections were divided into 6 fractions according to their TLC pattern.

Depending on the TLC behavior fraction 2 (collection no. 4-9), 3 (collection no. 10-13), 4 (collection no. 14-16), 5 (collection no. 17-19) and 6 (collection no. 20-25) were selected for further investigation to isolate pure compounds.

Compound **(1)** (7.4 mg) was isolated in pure form from the VLC fraction 2 (collection no. 4-9) by repeated column chromatographic separation using hexane-ethyl acetate solvent system. The compound was found as white amorphous solid and found soluble in chloroform.

VLC fraction 3 (collection no. 10-13) was passed through a column for purification of the compound **(2)**. The column was packed with silica gel-60 and the sample was applied on the top of the column bed. Elution was done with n-hexane-ethyl acetate and ethyl acetate-methanol solvent systems with increasing polarity. The eluted solutions were collected in 45 test tubes. After keeping overnight in room temperature, Needle shaped crystals were separated out from the tubes 21-26 and the crystals were separated by decantation process. Then it was washed with few drops of EA to get as white crystalline compound in pure form and compound **(2)** (6.1 mg) was soluble in methanol. The compound **(2)** (6.1mg) was found as white crystalline solid and was soluble in methanol.

Compound **(3)** (6.4 mg) was isolated from the VLC fraction 4 (collection no. 14-16) using a medium sized column eluted with ethyl acetate-methanol solvent systems in gradient manner. The compound was found as yellow crystalline solid and was soluble in chloroform with few drops of methanol. Compound **(4)** (7.60 mg) was purified from the VLC fraction 5 (collection no. 17-19) by passing through a column eluted with EA & MeOH in different ratios with increasing polarity. The compound was separated out as yellowish crystals from the collections 8 & 9 after allowing the, to stand overnight. The yellow crystalline compound **(4)** (7.6 mg) was finally purified by washing with few drops of n-hexane and EA. The compound was soluble in methanol.

Compound **(5)** (10.07 mg) was isolated in pure form from the VLC fraction 6 (collection no. 20-24) using column chromatography and PTLC methods, respectively. In both the methods, ethyl acetate-methanol solvent system was used. 10% Methanol in ethyl acetate was used in PTLC as mobile phase for the final purification. The compound was found as white needle shape crystalline solid and was soluble in chloroform.

Spectroscopic data of the isolated compounds

Ajmalicin (1)

White amorphous solid; mp 258-260℃ ; **IR (neat)** υ 3053, 2946, 2890, 1687(C=O), 1614, (aromatic C=C), 1493, 1438, 1380, 1159 (C-O), 1106 cm^{-1}; **^{1}H NMR ($CDCl_3$)** δ 8.05 (1H, s, >NH), 7.52 (1H, s, H-17), 7.45 (1H, d, *J*= 7.6 Hz, H-12), 7.29 (1H, d, *J*= 8.0 Hz, H-9), 7.09 (2H, m, H-10 & H-11), 4.41 (1H, m, H-19), 3.72 (3H, s, -OCH_3), 3.39 (1H, d, *J*= 11.2 Hz, H-3), 3.20 (1H, d, *J*= 12.4 Hz, H_a-14), 2.95- 3.12 (2H, m, H-5), 2.71 (2H, m, H-6), 2.41 (1H, t, *J*= 11.2 Hz, H-15), 2.23 (2H, t, *J*= 10.4Hz, H-21), 2.14 (1H, t, ***J***= 11.2Hz, H-20), 1.31 (1H, m, H-H_b-14) 1.17 (3H, d, *J*= 6.8Hz, -CH_3); **^{13}C NMR ($CDCl_3$)** δ 167.5 (C=O), 154.7 (C-17), 136.0, 134.3, 127.2, 121.4, 119.3, 118.0, 110.8, 107.8, 106.6 (C-16), 73.7 (C-19), 60.1, 56.8, 53.2, 51.0 (-OCH_3), 40.9 (C-20), 32.8, 30.6, 21.7, 14.9 (-CH_3) ; MS m/z 352 (M^+), 337, 225, 209, 184, 169, 156 (base peak), 129, 95, 77.

Catharanthine (2)

White crystals: mp 137-138℃ ; **IR (neat)** υ 3376 (N-H), 3080, 2970, 2850, 1713(C=O), 1642(C=C), 1459, 1362, 1266, 1094 (C-O) cm^{-1}; **^{1}H NMR (CD_3OD)** δ 7.39 (1H, d, *J*= 7.6Hz, H-11), 7.23 (1H, d, *J*= 7.6Hz, H-14), 6.99 (2H, m, H-12 & 13), 5.92 (1H, d, *J*= 5.2 Hz, H-3), 4.06 (1H, s, H-5), 3.71 (3H, s, -OCH_3), 3.42 (2H, m, H-7), 3.25 (2H, m, H-8), 2.90 (2H, m, H-19), 2.81 (1H, d, *J*= 13.2Hz, H_a-1), 2.70 (1H, m, H-2), 2.25 (1H, m, H_a-20), 2.08 (1H, m, H_b-20), 1.68 (1H, d, *J*= 13.2Hz, Hb-1), 1.06 (3H, t, *J*= 7.2Hz, H-21); **^{13}CNMR (CD_3OD)** δ 174.4 (C=O), 149.8, 137.9, 137.0, 129.8, 125.1, 122.2, 119.8, 118.5, 111.7, 111.1, 63.7, 56.2, 54.7, 52.8, 50.6, 38.3,31.8, 27.2, 21.6 10.8 (C-21); MS m/z 336 (M^+), 229, 214, 168,154, 135 (base peak), 121, 107, 93.

3,3',4',5,7-Pentahydroxyflavone or Quercetin (3)

Yellow crystals; mp 310-314℃; **UV (CH_3OH)** λ_{max} 375, 320 nm; **IR (neat)** υ 3253 (O-H), 1652 (C=O), 1106 (C-O) cm^{-1}; **^{1}H NMR ($CDCl_3$ + CD_3OD)** δ 7.66 (1H, d, *J*=2.0 Hz, H-2), 7.56 (1H, dd, *J*=8.4 & 2.0 Hz, H-6), 6.83 (1H, d, *J*=8.4 Hz, H-5), 6.32 (1H, d, *J*=2.0 Hz, H-6), 6.15 (1H, d, *J*=2.0 Hz, H-8); **^{13}C NMR ($CDCl_3$ + CD_3OD)** δ 179.6 (C=O), 167.8, 164.6, 160.8, 151.1, 150.5, 148.6, 139.6, 126.7, 124.6 (C-6), 119.0 (C-2), 118.7 (C-5), 107.3, 102.2 (C-8), 97.6 (C-6); MS m/z 302 (M^+, base peak), 274, 245, 229, 153, 137, 109, 91, 69.

Quercetin-3-O-rutinose or Rutin (4)

Yellow crystals; mp 239-241°C; **UV (CH_3OH)** λ_{max} 362, 324 nm; **IR (neat)** υ 2358, 1681 (C=C), 1633 (C=)), 1537 (aromatic C=C), 1427, 1362, 1296, 1204, 1150, 1094, 1061, 1042, 1015, 1001(C-O) cm^{-1}; **^{1}H NMR (CD_3OD)** δ 7.67 (1H, d, *J*= 2.0 Hz, H-2'), 7.63 (1H, dd, *J*= 8.4 & 2.0Hz, H-6'), 6.88 (1H, d, *J*= 8.4 Hz, H-5'), 6.40 (1H, d, *J*= 2.0 Hz, H-6), 6.21 (1H, d, *J*= 2.0 Hz, H-

8), 5.10(1H, d, *J*= 7.2 Hz, H-1"), 4.52 (1H, d, *J*= 1.2Hz, H-1'"), 3.80 (1H, d, *J*= 10Hz, H-5"), 3.63 (1H, m, H-5'"), 3.24-3.56 (8H, m, other protens of sugar moiety), 1.12 (3H, d, *J*= 6.0Hz, -CH_3) ; **^{13}CNMR (CD_3OD)** δ 178.0 (C-4), 164.6, 161.5, 157.9, 157.1, 148.4, 144.4, 134.2 (C-3), 122.1, 121.7(C-2), 116.3, 114.6, 104.2, 103.3 (C-1"), 101.0 (C-1'"), 98.5, 93.4, 76.7, 75.8, 74.3, 72.5, 70.8, 70.7, 70.0, 68.3, 67.1 (C-6"), 16.5 (-CH_3).

Benzoic acid (5)

White needle shaped crystals; mp 120-122° C; **IR (neat)** υ 3071-2560 (br., O-H), 1684 (C=O), 1602, 1583 (aromatic C=C), 1128 (C-O) cm^{-1}; **^{1}H NMR ($CDCl_3$)** δ 11.06 (1H, br. s, -OH), 8.05-8.24 (2H, m, H-2 & H-6), 7.56-7.70 (1H, m, H-4), 7.41-7.55 (2H, m, H-3 & H-5); **^{13}C NMR ($CDCl_3$)** δ 172.2 (C=O), 133.8 (C-4), 130.2 (2C), 129.3 (C-1), 128.5 (2C); MS m/z 122 (M^+), 105, 77 (base peak), 51, 45.

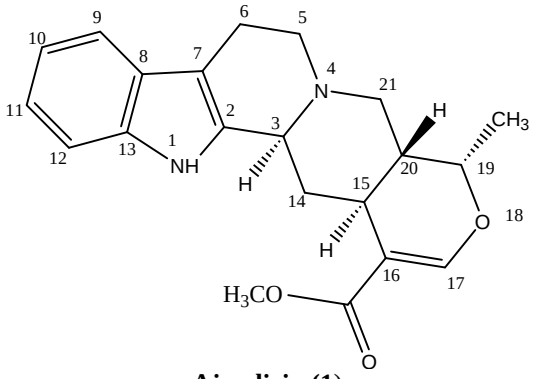
RESULTS AND DISCUSSION

The compound **(1)** (7.4 mg) was found as white amorphous solid and was soluble in chloroform. Melting point of the compound was found as 258-260℃. It showed single spot on TLC plate with R_f value 0.75 in 2% methanol in Chlroform.

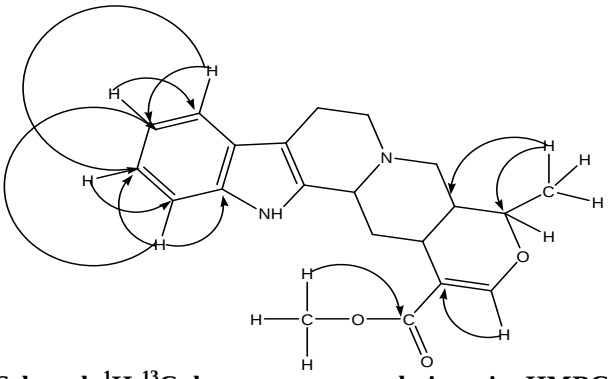
The mass spectrum of the compound **(1)** showed a molecular ion peak at m/z 352 corresponding to the molecular formula $C_{21}H_{24}N_2O_3$. The IR spectrum of the compound showed the absorption bands at 1687, 1614 and 1159 cm^{-1} indicated the presence of C=O, aromatic C=C and C-O stretching vibrations, respectively. In the ^{1}H NMR spectrum, three peaks at δ 7.45 (1H, d), 7.29 (1H, d) and 7.09 (2H, m) indicated four aromatic protons in indole nucleus. The 1H singlet at δ 7.52 showed the olefinic proton at C-17 which is directly attached to oxygen. The >NH proton of indole nucleus indicated by the singlet at δ 8.05. The methine proton at C-19 attached to oxygen clearly showed by the peak at δ 4.41. The 3H singlet at δ 3.72 and the 3H doublet at δ 1.17 confirmed the presence of methoxyl and methyl group (attached to C-19), respectively in the molecule. The ^{1}H-^{1}H correlations between H-5 & H-6, H-14 & H-3/H-15, H-20 & H-19/H-21, H-19 & -CH_3 clearly indicated by COSY spectrum.

The presence of 21 carbons in the molecule showed by 21 signals in the ^{13}C NMR spectrum. The peak at δ 167.5 was found due to the carbonyl carbon of eater group. Total eight aromatic carbons of indole nucleus and two olefinic carbons ascertained by the 10 peaks at the region of δ 106.6 to 154.7 in the ^{13}C NMR spectrum. The peaks at δ 73.7 & 14.9 were found due to C-19 & methyl carbon respectively. The analysis of ^{1}H NMR, ^{13}C NMR and DEPT-135 spectrum confirmed that the structure of the compound contains two methyl, four methylene, nine methine and six quartenary carbons. Finally the structure of the compound was confirmed by the fragment ions present in the mass spectrum and ^{1}H-^{13}C long range correlations indicated by the HMBC spectrum.

Based on all spectroscopic data, literature values[23] and melting point of the compound, it was confirmed that the compound **(1)** is an alkaloid named ajmalicin. The structure of the compound **(1)** is given below:



Ajmalicin (1)



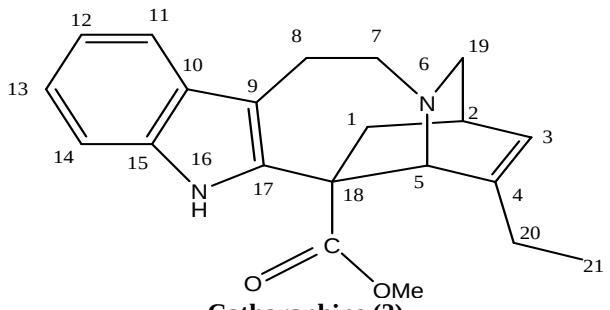
Selected ^{1}H-^{13}C long range correlations in HMBC spectrum of Ajmalicin (1)

The compound **(2)** (6.1 mg) was found as white crystalline solid and was soluble in methanol. Melting point of the compound was found as 137-138℃. It showed single spot on TLC plate with R_f value 0.57 in 100% EA which indicated that the compound was isolated in pure state. The mass spectrum of the compound F7 showed a molecular ion peak at m/z 336 which it corresponding to the molecular formula $C_{21}H_{24}N_2O_2$. The IR spectrum of the compound showed an absorption band at 1713 cm^{-1} indicated the presence of C=O stretching vibrations. The absorption bands at 1642 and 1094 cm^{-1} were found due to C=C and C-O stretching vibrations, respectively. A broad absorption band at 3376 cm^{-1} also showed in the IR spectrum due to the N-H stretching vibrations.

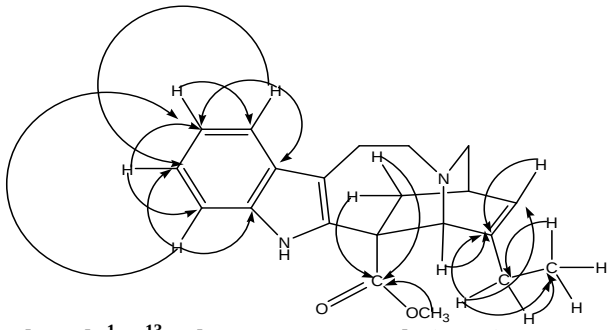
In the ^{1}H NMR spectrum, two one-proton doublets at δ 7.39 & 7.23 and a two-proton multiplet at δ 6.99 indicated the presence of four aromatic protons in the molecule. The olefinic proton at C-3 was clearly showed by the doublet at δ 5.92 (*J* = 5.2 Hz). The 1H singlet at δ 4.06 and the 3H singlet at δ 3.71 were found due to H-5 and methoxyl protons, respectively. The methylene proton at C-20 indicated by two multiplets at δ 2.25 & 2.08 and the triplet integrated for three protons indicated the terminal methyl group at C-21.

The ^{13}C NMR spectrum clearly showed 21 signals for 21 carbons, the signal at δ 174.4 was found due to the carbonyl carbon of ester group. Eight aromatic carbons and two olefinic carbons were ascertained by the 10 peaks at the region of δ 149.8-111.1 in the ^{13}C NMR spectrum. The analysis of ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data showed the presence of two methyl, five methylene, seven methine and seven quaternary carbons in the structure. The ^{1}H-^{1}H correlations between aromatic protons, H-20 & H-21, H-3 & H-2, H-2 & H-1/H-3/H-19 clearly showed in the COSY spectrum. The ^{1}H-^{13}C long range correlations were indicated by the HMBC spectrum. Finally the structure of the compound was confirmed by the fragment ions present in the mass spectrum.

Based on all spectroscopic data, literature values[23] and melting point of the compound, it was confirmed that the compound **(2)** is an alkaloid named catharanthine. The structure of the compound **(2)** is given below:



Catharanhine (2)

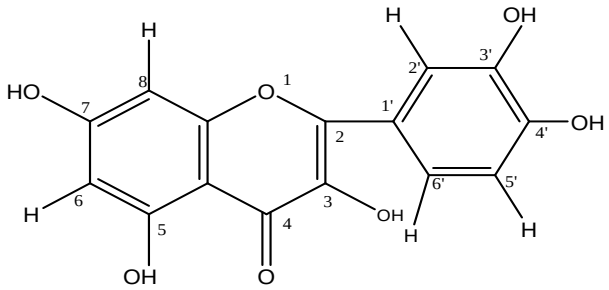


Selected ^{1}H-^{13}C long range correlations in HMBC spectrum of Catharanthine (2)

The compound **(3)** (6.4 mg) was found as yellow crystals and was partially soluble in chloroform and completely soluble in methanol. Melting point of the compound was found as 310-314° C. It gave positive test with $FeCl_3$ solution indicated the presence of phenolic hydroxyl group in the molecule. The compound showed single spot on TLC plate with R_f value 0.70 in the solvent system, 10% methanol in ethyl acetate. The mass spectrum of the compound showed a molecular ion peak at m/z 302 which is corresponding to the molecular formula $C_{15}H_{10}O_7$. The absorption bands at λ_{max} 375 and 320 nm suggested the presence of conjugation and chromophoric groups in the molecule. The λ_{max} values were found very similar with that of flavonoid compounds. The IR spectrum of the compound showed absorption band at 3253 cm^{-1} due to the O-H stretching vibrations. The absorption band found at 1652 cm^{-1} due to the C=O stretching and band at 1106 cm^{-1} due to the C-O stretching vibrations, respectively.

In the ^{1}H NMR spectrum, the two one-proton doublets at δ 6.32 & 6.15 with coupling constant value 2.0 Hz clearly indicated the meta-coupled protons at C-6 & C-8 in the structure. The three protons attached to C-2′, C-5′ and C-6′ in another aromatic ring undoubtedly showed by the three peaks at δ 7.66 (1H, d, *J* = 2.0 Hz, H-2′), 7.56 (1H, dd, *J* = 8.4 & 2.0 Hz, H-6′) and 6.83 (1H, d, *J* = 8.4 Hz, H-5′) in the spectrum. The coupling patterns between the three protons indicated by their coupling constant values which confirmed their positions in the same ring. In the ^{13}C NMR spectrum, fifteen peaks showed the presence of fifteen carbons in the molecule. The carbonyl carbon indicated by the peak at δ 179.6 and comparatively lower chemical shift value of this carbon was due to the conjugation and chelation with hydroxyl group. The five =CH- carbons were precisely attributed by DEPT-135 and the carbon-hydrogen direct correlations showed by HSQC spectrum. The structure of the compound F8 was finally confirmed by the fragment ions present in the mass spectrum.

Based on all spectroscopic data, literature values[24] and melting point of the compound **(3)**, it was confirmed that the compound is 3,3′,4′,5,7-Pentahydroxyflavone or Quercetin. The structure of the compound **(3)** is given below:



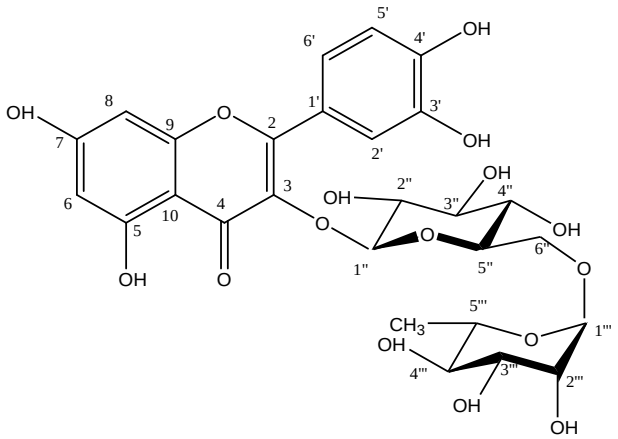
3,3',4',5,7-Pentahydroxyflavone or Quercetin (3)

The compound **(4)** (7.6 mg) was found as yellow crystalline solid and was soluble in methanol. Melting point of the compound was found as 239-241°C. It showed single spot on TLC plate with R_f value 0.70 in the solvent system, EA:Me (90:10). The absorption bands at λ_{max} 362 and 324 nm suggested the presence of conjugation and chromophoric groups in the molecule. The λ_{max} values were found very similar with that of flavonoid compounds. In the IR spectrum, the absorption bands at 1681 and 1537 cm^{-1} indicated the presence of olefinic and aromatic C=C in the structure. The presence of carbonyl group showed by the band at 1633 cm^{-1}. The lower value of the band was due to the conjugation and chelation with hydroxyl group attached to C-5. The bands in the region of 1296 to 1001 cm^{-1} were found due to different C-O stretching vibrations.

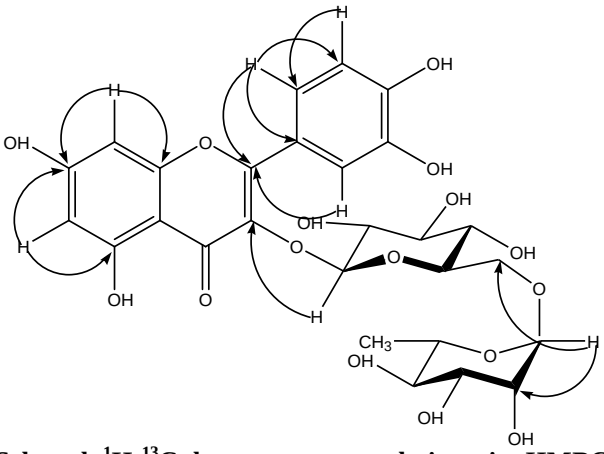
In the ^{1}H NMR spectrum, three peaks at δ 7.67 (d, *J* = 20.Hz), 7.63 (dd, *J* = 8.4 & 2.0 Hz) and 6.88 (d, *J* = 8.4 Hz) indicated three aromatic protons of the same ring at C-2', C-6' and C-5', respectively. Two 1H doublets at δ 6.40 and 6.21 with coupling constant, *J* = 2.0 Hz clearly showed two meta coupled aromatic protons of other ring attached to C-6 and C-8, respectively. The ^{1}H-^{1}H correlations between H-6' & H-5'/H-2' and H-6 & H-8 present in the COSY spectrum easily confirmed the above facts. Two 1H doublets at δ 5.10 (*J* = 7.2 Hz) and 4.52 (*J* = 1.2 Hz) indicated two anomeric protons at C-1" and C-1"' of sugar moiety, respectively. The coupling constants of anomeric protons clearly indicated the β-glycoside linkage with C-1"and α-glycoside linkage with C-1"' of disaccharide rutinose. Other protons of rutinose moiety were found in the region of δ value 3.24 to 3.80. The methyl group attached to C-5"' easily assigned by the 3H doublet at δ 1.12.

The ^{13}C NMR spectrum of the compound showed 27 signals for 27 carbons in the molecule. The carbonyl carbon at position-4 indicated by the peak at δ 178.0. Twelve aromatic and two olefinic carbons were observed in the region of δ 93.4-164.6. Two anomeric carbons of sugar moety were indicated by the peaks at δ 103.3 (C-1") and 101.0(C-1"'). The two signals at δ 67.1 & 16.5 were found in the ^{13}C NMR spectrum due to the presence of one methylene carbon at position-6" and one methyl carbon at position-6"', respectively. There are one methyl, one methylene, fifteen methine and ten quarternary carbon present in the molecule which was confirmed by the analysis of DEPT-135 spectrum. Finally the stucture of the compound **(4)** was confirmed by the ^{1}H-^{13}C long range correlation indicated in the HMBC spectrum.

Based on all spectroscopic data, literature values[25] and melting point of the compound, it was confirmed that the compound **(4)** is flavonoid glucoside named rutin. The compound is isolated for the first time from the flower as well from the plant of *C. roseus*. The structure of the compound **(4)** is given below:



Quercetin-3-O-rutinose or Rutin (4)



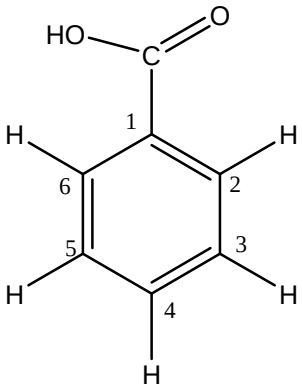
Selected ^{1}H-^{13}C long range correlations in HMBC spectrum of Rutin (4)

Compound **(5)** (10.0 mg) was found as white needle shape crystals and was soluble in chloroform. Melting point of the compound was found as 120-122° C. A distinct single spot was observed on TLC plate with R_f value 0.69 in 100% $CHCl_3$.

The mass spectrum of the compound showed a molecule ion peak at m/z 122 corresponding to the molecular formula $C_7H_6O_2$. The IR spectrum of the compound showed a broad absorption band at 3071-2560 cm^{-1} due to the O-H stretching vibration of the carboxyl group and the absorption band at 1128 cm^{-1} due to the C-O stretching vibration. The band at 1684 cm^{-1} indicated the C=O stretching and bands at 1602, 1583 cm^{-1} due to the aromatic C=C stretching vibrations in the molecule.

The broad peak at δ 11.06 in the ^{1}H NMR spectrum indicated the proton of carboxyl group. The two multiplets at δ 8.05-8.24 and 7.41-7.55 integrated with two protons each clearly showed the presence of two pairs of equivalent protons attached to C-2 & C-6 and C-3 & C-5, respectively. Another multiplet at δ 7.56-7.70 indicated the aromatic proton at C-4. The ^{13}C NMR spectrum gave five peaks for seven carbons. Two pairs of equivalent carbons showed by the peaks at δ 130.2 and 128.5 which was supported by the ^{1}H NMR spectral data. The carbon of the carboxyl group was indicated by the peak at δ 172.2. All the data was supported again by the DEPT-135 spectrum. Finally the structure of the compound was confirmed by the fragment ions present in the mass spectrum.

Based on all spectroscopic data, literature values[26] and melting point of the compound **(5)**, it was confirmed that the compound is benzoic acid. The compound was found from the flowers as well as from the plant *C. roseus* for the first time. The structure of the compound **(5)** is given below:



Benzoic Acid (5)

CONCLUSION

Literature survey showed that very little phytochemical studies have been done on flower methanol extract of the plant *Catharanthus roseus*. The isolation and characterization of five compounds from flower part of the plant have been reported here. And the compound benzoic acid was found from the flowers as well as from the plant *C. roseus* for the first time. We believe, there is a scope to do more detailed phytochemical and biological study on this plant in future.

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