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**Research Article**

# Isolation and characterization of flavonoid from ethanolic extract of leaves of *Naravelia zeylanica*

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**Abstract**

In this study the phytochemical analysis of leaves of *Naravelia zeylanica* (Ranunculaceae) plant in various ether extracts were taken up. The ether layer II was taken for study as it was screened positively for the flavonoid type of compound. The extract was chromatographed by preparative-TLC using ethanol: ethyl acetate 8.5:1.5 as the eluent.One of the flavonoid types of compound was isolated by the chromatographic method. Then, the compound was subjected to the routine chemical and spectroscopic analyses. The compound was found to be8-hydroxy-2(4-hydroxy-5methoxyphenyl)-7-(tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2yloxy)-4H-chromen-4-one.

**Keywords**: Ether layer II, flavonoid, *Naravelia zeylanica*.

## INTRODUCTION

*Naravelia zeylanica* is a small genus woody climber distributed in Himalayas[1]. Roots are tuberous, leaves with two opposite ovate, cordate leaflets and a terminal 3 branched tendril, flowers in pinacles, small with pleasant scent, achenes red with long feathery styles. The plants are propagated by seeds or cuttings. The stems can be twisted into strong ropes. *Naravelia zeylanica* is distributed in the tropical forests of eastern Himalayas, Assam, Bengal, Bihar, Deccan Peninsula [2]. They are also reported to be used as tooth sticks to cure toothaches. Roots when crushed emit a smell which is said to relieve headache. It is used as an astringent,anti-inflammatory, anthelmintic, rheumatic pain, wounds, ulcers, intestinal worm's leprosy and skin diseases[3]. The ethanolic extract of *Naravelia zeylanica* yielded three important benzamides i.e.,3,4-methylene dioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxy benzamide. Beriberine, an alkaloid is isolated from methanolic extract of leaves of *Naravelia zeylanica*[4]. The present study focus to isolate a flavonoid compound ($C_{22}H_{22}O_{11}$) based on various chemical and spectral analysis.

## MATERIALS AND METHODS

The fresh leaves of *Naravelia zeylanica* samples were obtained locally from the Kolli Hills,Trichy. The plant species was verified with authentic specimen at Rapinat Herbarium, Trichy, Tamilnadu, India. The leaves were washed in tap water; shade dried, crushed into pieces and packed in a wide-mouthed bottle. The moisture free ethanol was poured into the bottle to soak the plant material completely. The bottle was closed air-tight and allowed to stand for 3 days. Ethanol was collected in

a pure dry bottle. The ethanolic extract was subjected flash-evaporation to get the concentrated extract[5]. The ethanolic extract was divided into two parts. One part was treated with sodium hydroxide and then with ether. The top organic layer was taken as Ether Layer I. The bottom aqueous layer was neutralized with hydrochloric acid and further extracted with ether to get Ether Layer II[6]. The second part was treated with hydrochloric acid and then with ether. The top organic layer was taken as Ether Layer III. The bottom aqueous layer was neutralized with sodium hydroxide and further extracted with ether to get Ether Layer IV. The extraction scheme is given in Figure 1.

The ether layer II was taken for study as it was screened positively for the flavonoid type of compound. The extract was chromatographed by preparative-TLC using ethanol: ethyl acetate 8.5:1.5 as the eluent and silica gel (100 m mesh size) as stationary phase. A compound was isolated and it was recrystallized from acetone and it's taken for melting point measurement. Then, the compound was subjected to the routine chemical and spectroscopic analyses. The structural characterization was done with UV-VIS, IR, H-NMR, C-13-NMR and mass spectral studies as shown in the table below

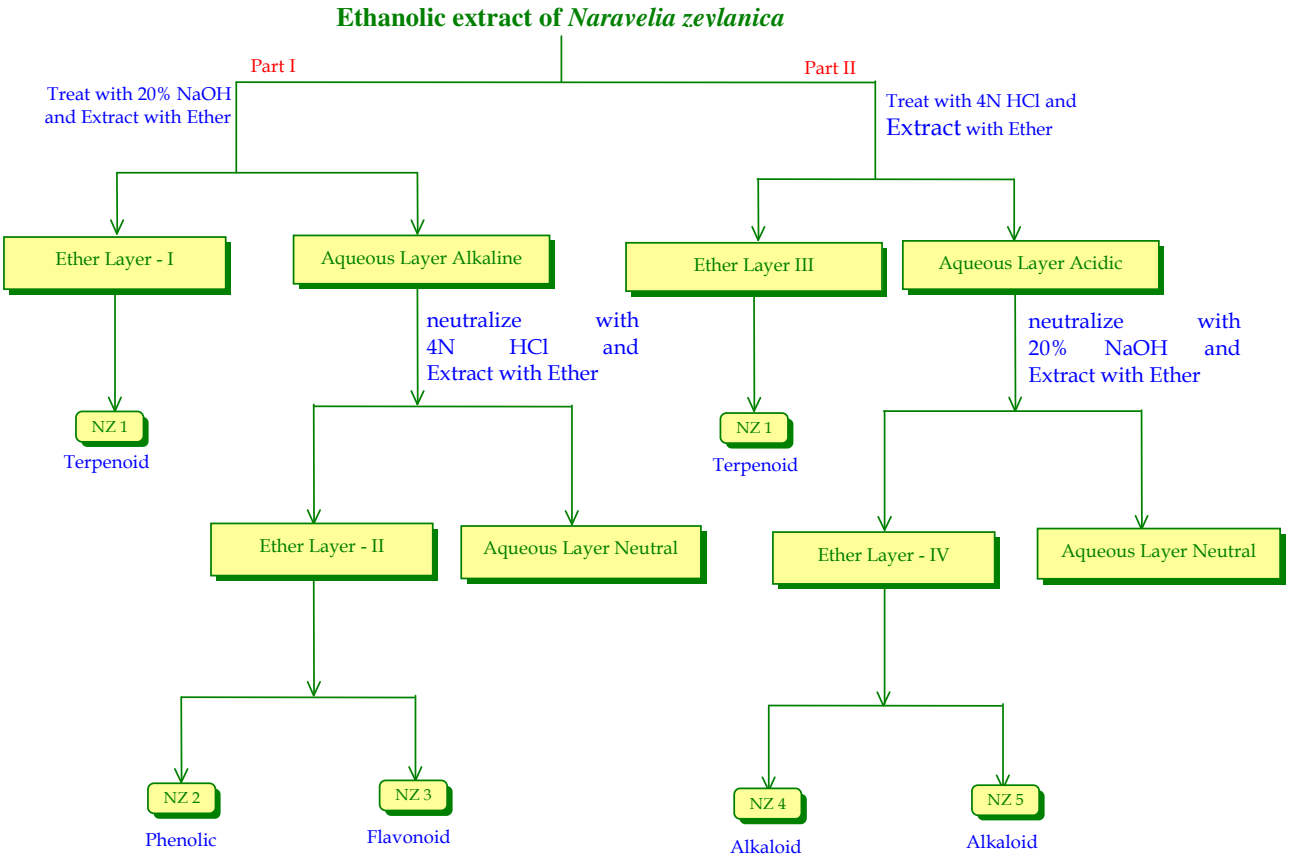
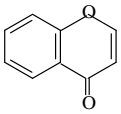
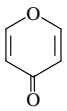



**Figure 1:Extraction scheme of phytoconstituents from Naravelia zeylanica**

**Table :Experimental data of compound**

| S. No | Characteristics | Observation |
|---|---|---|
| 1 | Molecular formula | $C_{22}H_{22}O_{11}$ |
| 2 | Molecular mass | 462.40 Dal. |
| 3 | Melting point | 297.8 ± 2°C |
| 4 | Solubility | Soluble in ethanol, DMSO, chloroform |
| 5 | **Chemical tests** | |
| | (i) Test for aromatic nature | A small amount of the extract was ignited in the flame. |
| | (ii) Test for carbonyl group | A small amount of extract was treated with Borsches re-agent. |
| | (iii) Test for phenol | A small amount of extract was treated with neutral $FeCl_3$. |
| | (iv) Test for flavonoid | A small amount of extract was treated with 10% $NH_4OH$. |
| | (v) Test for glycoside group | A small amount of extract was treated with chloroform and ammonia solution. |
| 6 | **Spectral data** | |
| | UV-VIS spectral data $\lambda_{max}$ nm, ($\varepsilon_{max}$) | 255(8600), 2678(14800)<br>314(2800), 348(180) |
| | IR spectral data $\overline{\nu}_{max}$ $cm^{-1}$ | 2958m, 2836m, 1244s, 1043s, 785s, 3026m, 3001m, 3064m, 3048m, 3000m, 1625s, 3016m, 1682s, 3595s, 3548s, 3619s, 3480 s, 1394s, 1368s, 1136s, 1062s, 1128s, 3395s, 3155, 1229s, 1218s, 1186s, 2925s, 2841s, 1465s, 1349s, 729s. |
| | H- NMR spectral data $\delta_{(ppm)}$ | 3.378 d, 3.511 s, 3.578d, 3.619 m, 3.719 d, 3.833 m, 4.113 s, 5.667 s, 6.317 d, 6.536 d, 6.715 s, 6.762, 7.804, 8.452 s, 9.131s. |
| | C-13 NMR spectral data $\delta_{(ppm)}$ | 55.87, 67.79, 85.09, 85.14, 89.48, 91.78, 103.49, 109.82, 112.08, 113.24, 113.91, 116.57, 127.59, 128.22, 129.28, 146.35, 147.21, 150.57, 152.51, 151.31, 154.96, 197.21 |
| | Mass spectral data *(m/z)* | $M^+$**462.403**, 431.37, 339.27, 314.25, 299.26, 253.23, 246.26, 186.20, 177.13, 163.15, 152.10, 134.13, 138.08, 132.16, 123.13, 104.10 |

**Interpretation of UV-Visible spectral data of the compound (below)**

| Band at wavelength (nm) | Electronic transition | Chromophore |
|---|---|---|
| 255 (8600) | π →π* |  |
| 2678 (14800) | π →π* | |
| 314 (2800) | n →π* |  |
| 348 (180) | | |

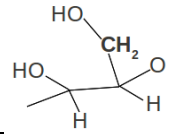
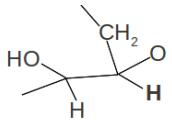
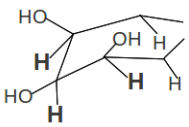
## RESULT AND DISCUSSION

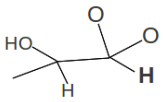
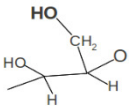
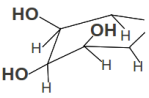
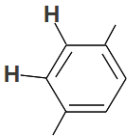
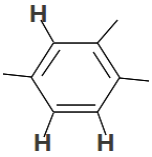
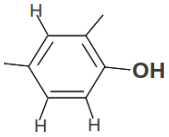
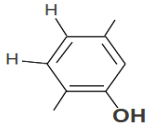
The recrystallized sample was yellow coloured needles and soluble in polar solvents like acetone,ethanol and DMSO. The mass spectral studies of the compound were done using the JEOL GC mate.The molecular mass of the compound was found to be 462.40 Dal. The melting point was 297.8 ± 2°C. It burned with a sooty flame confirming the aromatic nature.Yellow precipitate with Borsches reagent showed the presence of carbonyl functionality. Purple coloration with neutral ferric chloride revealed the presence of phenolic nature.Yellow fluorescence with ammonium hydroxide inferred the flavonoid type of compound.Pink colouration with Borntrage's reagent indicated the presence of glycosides[8].

**Interpretation of IR spectral data of the compound [7] (below)**

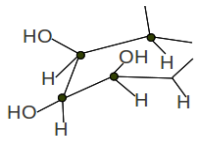
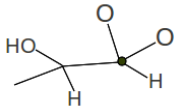
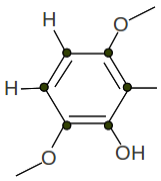
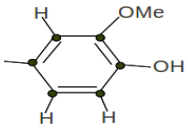
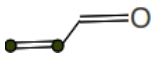
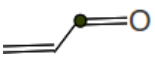
| Band at frequency ($cm^{-1}$) | Type of bond |
|---|---|
| 3395strong, broad | O-H stretching due to free hydroxyl group |
| 3480 – 3201 broad, strong | O-H stretching due to free hydroxyl group involved in hydrogen bonding |
| 3026 medium<br>1601medium<br>786 medium | C-H stretching of aromatic ring<br>C=C stretching of aromatic ring<br>C-H bending of aromatic ring |
| 1682 strong | -C=O stretching |
| 1244 strong | Asymmetric C-O-C- stretching |

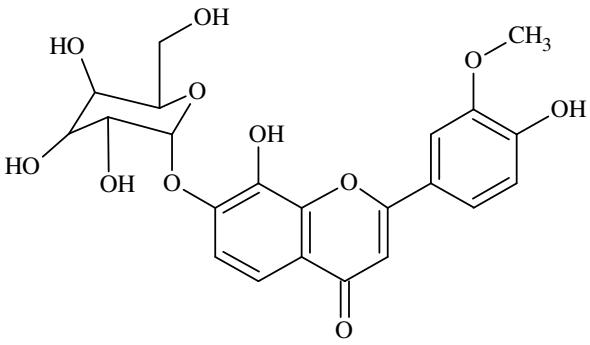
**Interpretation of H-NMR spectral data of the compound (below)**

| Chemical shift (ppm) Signal pattern, Number of protons, J value in Hz | Environment of protons | Type of protons |
|---|---|---|
| 3.378 doublet (2H,*J7.4Hz*) | Methylene protons deshielded due to the attachment of electronegative oxygen atom. It has one neighbouring proton. |  |
| 3.511 singlet (3H) | Methoxy proton deshielded due to the bonding with oxygen atom.<br>It has no neighbouring proton. | **$CH_3O$-** |
| 3.578 doublet of triplet<br>(1H, *J 8.6, 7.4Hz*) | One methynic proton that is deshielded by the presence of electronegative oxygen atom(s). It has three neighbouring protons – one on side and two on the other side. |  |
| 3.619 doublet of doublet (1H, *J 9.4, 10.3 Hz*)<br>3.833 doublet of doublet<br>(2H *J 8.6, 9.4.Hz*) | Three methynic protons each deshielded due to the attachment of the electronegative oxygen atom of the hydroxyl group.<br>Each proton has two neighbouring protons, one on each side. |  |

| | | |
|---|---|---|
| 3.719 doublet (1H, *J 9.4Hz*) | One methynic proton that is deshielded due to the hydroxyl oxygen atom. It has one neighbouring proton on one side only. |  |
| 3.919 singlet (1H) | Hydroxyl proton deshielded due to the attachment of electronegative oxygen atom.<br>It has no neighbouring proton. |  |
| 4.113 singlet (3H) | Three hydroxyl protons deshielded due to the attachment of electronegative oxygen atom.<br>It has no neighbouring proton. |  |
| 5.667 singlet (1H) | Methynic proton deshielded due to the pi-bonded electron of the ring and attachment of electron withdrawing carbonyl group.<br>It has no neighbouring proton. | -C-**CH**-C=O |
| 6.317 doublet (1H, *J 7.8 Hz*)<br>6.536 doublet (1H, J 7.8 Hz) | A set of two aromatic protons that are deshielded due to the pi-cloud of aromatic ring. Each one is at *ortho-* to the other. |  |
| 6.716 singlet (1H) | One aromatic proton that has no neighbouring proton. |  |
| 6.762 doublet (1H, *J 6.4 Hz*)<br>7.804 doublet (1H, *J 6.4 Hz*) | A set of two aromatic proton that are positioned *ortho-* to each other | |
| 8.452 singlet (1H) | A hydroxyl proton that is significantly deshielded due to pi cloud of aromatic ring and the bonding with the electronegative oxygen atom |  |
| 9.131 singlet (1H) | A hydroxyl proton that is highly deshielded due to pi cloud of aromatic ring and carbonyl oxygen atom in its close proximity. |  |

**Interpretation of Carbon -13 NMR spectral data of the compound (below)**

| Chemical Shift (ppm) | Environment of the Carbons | Type of Carbon |
|---|---|---|
| 55.87 | A methoxy carbon that is deshielded due to the presence of the electronegative oxygen atom. | **-$OCH_3$** |
| 67.79 | Methylene carbon deshielded by the bonding with electronegative oxygen atom | -CH-**$CH_2$**-O |
| 85.09, 85.14, 89.48, 91.78 | Four methynic carbons deshielded due to the presence of electron withdrawing oxygen atom |  |
| 109.82 | Methynic carbon deshielded by the bonding with two electronegative oxygen atoms. |  |
| 103.49, 113.91<br>116.57,147.21<br>151.31,152.51 | Aromatic carbons deshielded significantly due to pi-cloud of aromatic ring and the presence of hydroxyl groups at some positions of the ring. |  |
| 112.08, 113.24<br>127.59, 128.22<br>146.35, 154.96 | Aromatic carbons deshielded remarkably due to pi-cloud of aromatic ring and the presence of hydroxyl groups at some positions of the ring. |  |
| 129.28, 150.57 | A set of two alkenic carbons that is greatly deshielded due to the pi-bonded electrons, the attachment of the electronegative oxygen atom and the presence of the carbonyl group. |  |
| 197.21 | Carbonyl carbon strongly deshielded due to bonding with the electronegative oxygen and also the alkenic part |  |



**8-hydroxy-2(4-hydroxy-5methoxyphenyl)-7-(tetrahydro-3,4, 5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2yloxy)-4H-chromen-4-one.**

On the basis of the chemical studies and spectral studies –UV-Visible, Infra-red, $^{1}$H-NMR, 13C-NMR and mass spectra studies of the compound was proposed to have the above structure

## CONCLUSION

The ethanolic extract was fractioned using alkali, acid and ether to get ether layer I, II, III and IV. The ether layer II was taken for chromatographic separation.The compound was isolated and it was taken for characterization studies. It was further confirmed by the chemical methods , phytochemical screening and spectral studies. Thus, the compound was flavonoid named as 8-hydroxy-2(4-hydroxy-5methoxyphenyl)-7-(tetrahydro-3,4, 5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2yloxy)-4H-chromen-4-one. XRD studies of the compound can be further studied for future work.

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