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A NOVEL CHEMICAL COMPOUND FROM THE LEAF EXTRACT OF *Vanda tessellata*(Roxb.) Hook. ex G. Don

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ABSTRACT

Vanda tessellata is an endangered and medicinal orchid. Leaves of *V. tessellata* are used in folk medicine for the treatment of cough, edema, respiratory difficulties, fever and paralysis. Its juice is dropped in the ear for the treatment of Otitis media and other inflammatory conditions. Present investigation is conducted to identify the active chemical compound in leaf that is responsible for therapeutic activity by column chromatography. The spectral data study show the presence of, two new active chemical compound 2, 5-dimethoxy-6,8-dihydroxy-Isoflavone and 3,4,5-trihydroxybenzoicacid. This finding of active chemical compound could lead to the development of a commercial future viable and valuable drug.

KEYWORDS: *Vanda tessellata, Otitis,* flavonoid, column chromatography, spectral data.

INTRODUCTION

Orchid symbolize one of the largest, diverse group of flowering plant . It has about 27,800 currently accepted species distributed in about 880 genera (Peter F. Stevens, 2001). Many of the orchids are used as traditional medicine from ancient times. Among the various medicinal orchids, *Vanda tessellata* is used for various ailments from time immemorial. It is an endangered epiphytic medicinal species of orchid occurring from the Indian subcontinent to Indochina.

The whole plant of Vanda tessellata is used for medicinal purposes It is an ingredient of *Rasna Panchaka Quatha*, ayurvedic formulation used in the treatment of arthritis and rheumatism. The root is used as antidote against scorpion sting and remedy for bronchitis (N.S.Chauhan, 1999). The juice of the leaves is used in the treatment of dyspepsia, bronchitis, inflammations, rheumatic ,pains, sciatic, disease of the abdomen, hiccough and tumors (K Biswas, 1973).The leaf paste is applied during fever (R K Kirtikar and B D Basu, 1987). It is used as aphrodisiac, analgesic and nervine tonic [(N S Chauhan, 1999; Singh A P and Raj Nighantu, 2006; M R Uniyal and G C Joshi., 1993). The medicinal value of *Vandaceous* taxon is also discussed in 'Charaka samhita, this form the first record of Indian orchid and their use in ayurvedic medicine. The ancient Indian people were well cognizant of the medicinal values of orchids (K S Manilal and S C Kumar, 1986).

The present study was undertaken to investigate the active chemical constituent present in the leaf extract of *Vanda tessellata* that will help in near future to formulate novel drug.

MATERIAL AND METHOD

Column chromatography

Isolation of Compounds from ethanol extract

The extract was packed to column chromatography applying silica gel (60-120 mesh size), & eluted with the following solvent ratios of Benzene: Ethyl acetate (EA), 100:0, 80:20, 60:40, 40:60, 20:80, 0:100, then with 100:0, 90:10, 80:20, 70:30, 60:40, 50;50, 40:60, 30:70, 20:80, 10:90, 0:100, EA: Ethanol (Eth). Then after it is eluted with 10:90 methanol (Mth): ethanol and finally with 100% methanol. The fractions (25 ml) were collected from the column. The elute collected were monitored by thin layer chromatography (eluent: EA-MeOH, 9:1 & 3:2) for homogeneity & the similar fraction were pooled together. The eleven different fractions were collected & dried. The fraction F1, F2 & F3 were containing waxy material; the fractions F4, F5, F7, F8, F10 & F11 were powder but quantity was very little. The yield of fraction F6 & F9 were 450 mg & 230 mg respectively. Further purification of selected fractions using re chromatographed. The F6 was eluted with the solvent EA-MeOH, 3:2 to yield compound A. The quantity of compound A was 80 mg. The F9 was eluted with the solvent EA-MeOH, 9:1 to yield compound B. The yield of compound B was 60 mg. The compound A and B were further analyzed by different physicochemical methods to determine the nature of compound. The compound B was characterized by interpreting the data obtained from melting points, UV spectra, IR spectra, NMR spectra & Mass spectra.

Compound -1

Characterization of Compound-C1

Melting point

The melting point of isolated fraction was recorded to be >310°C.

UV/VIS spectrophotometer

The sample solution was diluted with methanol. UV scan was done between 200 – 400 nm. The maximum absorbance λ_{max} obtained for the compound A at 273.8 nm.

Infrared spectrum

The IR spectrum (KBr) indicated the presence of various bonds as given in below.

The IR spectrum of compound 1 showed absorption bands at 3419.16 (O-H, free hydroxyl group), 3356.14 (Cyclic C-H, str), 2908.65 (Cyclic C-H, str), 2843.07 (Ali- C-H, str), 1620.21 (C=O, ester), 1446.61 (C-C ring stretch), 1099.43 (O-H, out of plane bend), 867.97 and 790.81 (monosubstituted in aromatic ring), 659.66 (out of plane ring C=C, bond).

NMR spectra

^{1}H-NMR

The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 5.01 (O-H, 3, 4, 5, s), 9.21 (CO-O-H, s), 7.01 (H–2 & 6, s).

^{13}C-NMR

The ^{13}C-NMR spectrum of compound displayed the characteristic signals at 1-120.43 (C), 2&6-108.84 (C), 3&5-145.24 (C), 4-137.94 (C), C=O-167.70 (C)

Mass spectra

The mass data which showed m/z = 169.06 considered as 170 (100) [M^+] indicative of $C_7H_6O_5$ (Fig.1).

Characterization of Compound-C2

Melting point

The melting point of isolated fraction was recorded to be >340°C.

UV/VIS spectrophotometer

The sample solution was diluted with methanol. UV scan was done between 200 – 400 nm . The maximum absorbance λ_{max} obtained for the compound 2 at 278.3 nm

Infrared spectrum

The IR spectrum (KBr) indicated the presence of various bonds as given in below.

The IR spectrum of compound II showed absorption bands at 3406.29 (O-H, free hydroxyl group), 3290.56 (Cyclic C-H, str), 2908.68 (Cyclic C-H, str), 2839.2 (Ali- C-H, str), 1666.50 (C=O, ester), 1649.19 (C=C stretch), 1458.18 (C-C ring stretch), 1199.72 (C-C stretching), 1091.71 (O-H, out of plane bend), 794.17 and 640.37 (monosubstituted in aromatic ring), 601.79 (out of plane ring C=C, bond).

NMR spectra

^{1}H-NMR

The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 6.24 (H–7, s), 7.75 (H–2' & 6', s), 7.62 (H–3' & 5', s), 7.59 (H–4', s), 6.61 (H–6', s), 6.61 (H–2, s), 5.18 (O-H, s), 3.68 (CH_3–2, s), 3.89 (CH_3–5, s).

^{13}C-NMR

The ^{13}C-NMR spectrum of compound displayed the characteristic signals at 2&3-175.7 (C), 4-163.8 (C), 5-160.6 (C), 6-156.0 (C), 7-98.1 (C), 8-147.6 (C), 4a-102.9 (C), 8a-135.6 (C), 1'-144.9 (C), 2'-146.7 (CH), 3'-121.9 (C), 4'-119.9 (C), 5'-115.5 (CH), 6'-115.0 (CH), OCH_3-39.6, OCH_3-39.8.

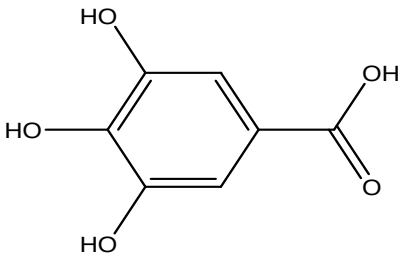
Mass spectra

The mass data which showed m/z = 314 (100) [M^+] indicative of $C_{17}H_{14}O_6$ (Fig.6).

RESULT AND DISCUSSION

Compound 1 was isolated and its molecular formula was determined as $C_7H_6O_5$ (m/z = 170 (100) [M^+]). The IR spectrum indicated the presence of hydroxyl (3419.16 cm^{-1}) and carbonyl functions (1620.21 cm^{-1}). The presence of the aromatic hydroxyl group and carbonyl were confirmed from the ^{13}C NMR spectra exhibited peaks at δ 145.24 to 137.94 and 167.70 respectively. The ^{1}H-NMR spectra exhibited peaks at δ 5.01, it suggested the presence of aromatic hydroxyl group. The ^{13}C NMR revealed higher δ 167.70, while absence of signal in ^{1}H-NMR inferred that carbon atom is free from proton. This statement confirmed the presence of carbonyl group at first position in ring. The ^{1}H-NMR exhibited peaks at δ 7.01 indicate the presence of benzene CH. It confirmed from the ^{13}C-NMR peaks from 108.84. The ^{1}H-NMR displayed the signal at δ 9.21 it demonstrated presence of OH at Carboxyl group(Fig.1). The hypothesis can be clearly justified from the signal of ^{13}C NMR, it exhibited

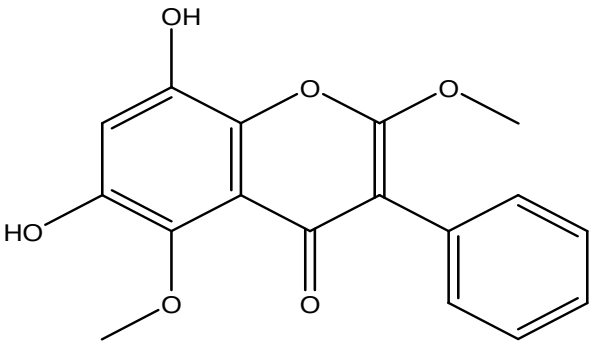
signal at 167.70(Fig.2). The structures was identified as Galic acid on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature. **The compound characterized as 3,4,5-trihydroxybenzoic acid.**



3,4,5-trihydroxybenzoic acid

Compound 2 was isolated and its molecular formula was determined as $C_{17}H_{14}O_6$ (m/z = 314 (100) [M^+]). The IR spectrum indicated the presence of hydroxyl (3406.29 cm^{-1}) and carbonyl functions (1666.50 cm^{-1}). The presence of the aromatic hydroxyl group and carbonyl were confirmed from the ^{13}C NMR spectra exhibited peaks at δ 147.6 to 156.0 and 163.8 respectively(Fig.5). The ^{1}H-NMR spectra exhibited peaks at δ 5.18, it suggested the presence of aromatic hydroxyl group (Fig.4). The ^{13}C NMR revealed higher δ 175.7, while absence of signal in ^{1}H-NMR inferred that carbon atom is free from proton. This statement confirmed the presence of carbonyl group at fourth position in ring. The ^{1}H-NMR exhibited peaks at δ 6.61, 7.59, 7.62, 7.75 and 6.24 indicate the presence of benzene CH. It confirmed from the ^{13}C-NMR peaks from 115.0 to 121.9. The ^{1}H-NMR displayed the signal at δ 3.68 and 3.89 it demonstrated presence of CH_3. The hypothesis can be clearly justified from the signal of ^{13}C NMR, it exhibited signal at 39.6**.**

The structures was identified as flavonoids on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature. The compound characterized as 2,5-dimethoxy-6,8- dihydroxy -Isoflavone.



2,5-dimethoxy-6,8-dihydroxy-Isoflavone

Phytochemicals like phenol, flavanoids, tannin, alkaloids, glycosides and terpenoids present in whole plant extract of *Vanda tessellata* (Bhattacharjee *et al*. 2015). A novel aprodiasic compound were isolated from its flowers (2, 7, 7-tri methyl bicyclo [2.2.1] heptane) (Subramoniam A et al., 2013). Roots of *Vanda tessellata* contain tetracosyl ferrulate and β-sitosterol-D-glucoside (Ghani, 2003 and Rastogi & Mehrotra, 1990).

Antimicrobial studies on leaf extract and whole plant extract of vanda tessellata was studied (Chhavi Gupta and SS Katewa, 2012, Bhatacharjie *et al*., 2015).In all the previous work structural information about the active constituent present in the leaf of *vanda tessellata* had not been reported.

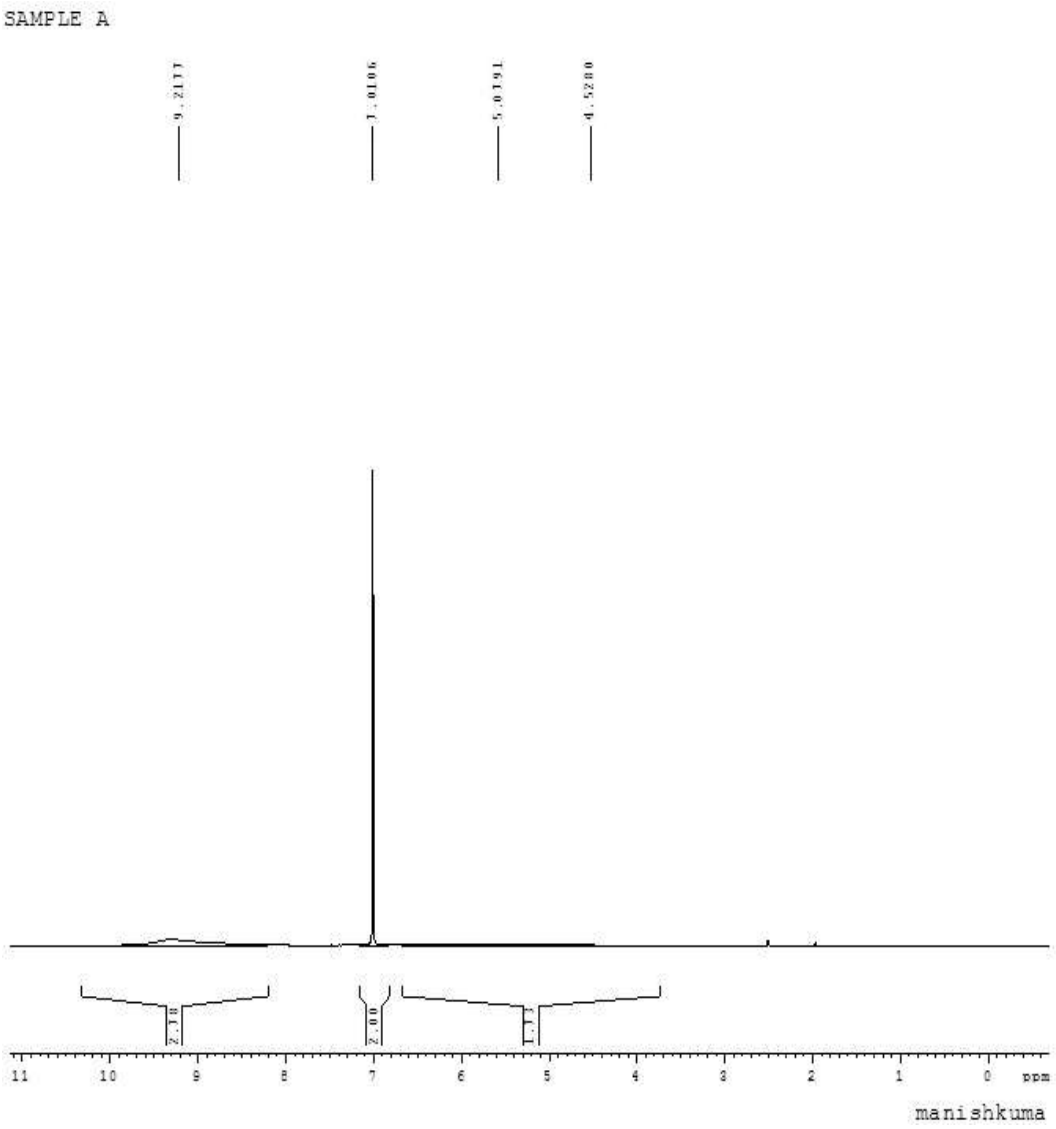


Figure.1: ^{1}H-NMR spectra of Compound-C1 isolated from *Vanda tessellata*(Roxb.) Hook. ex. G.Don extract.

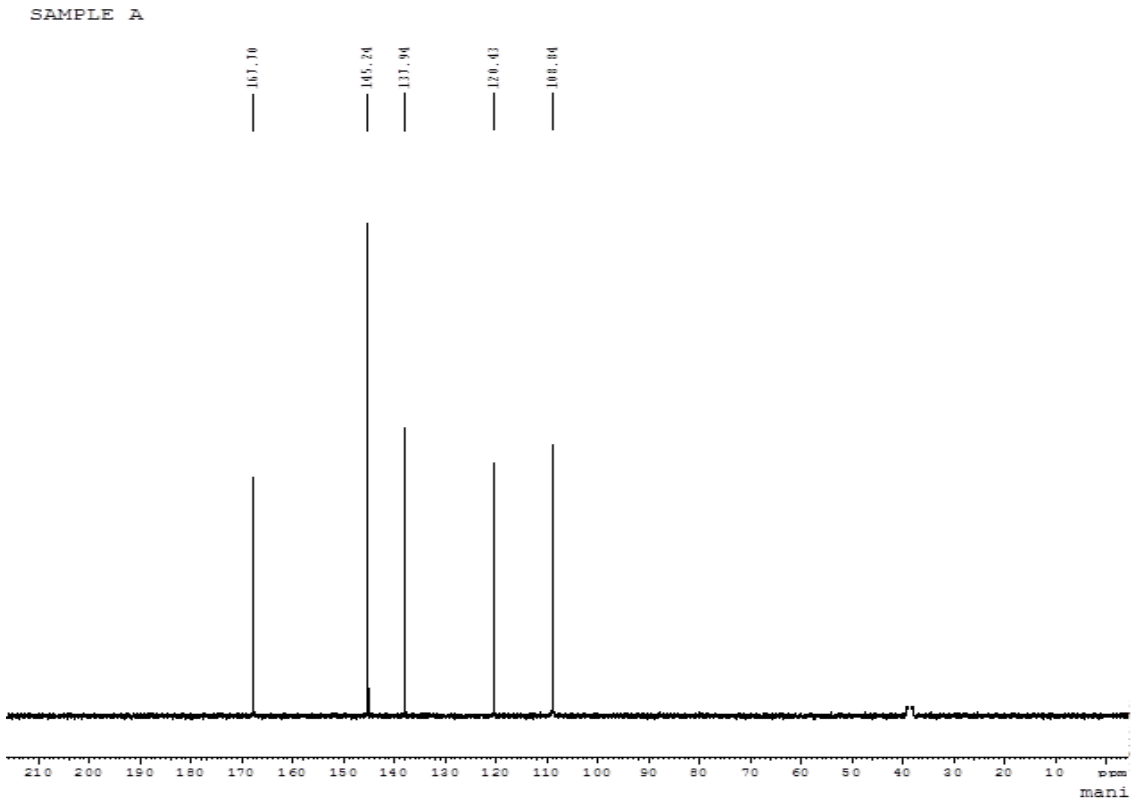


Figure.2: ^{13}C-NMR spectra of Compound-C1 isolated from *Vanda tessellata* (Roxb.) Hook. ex. G.Don extract.

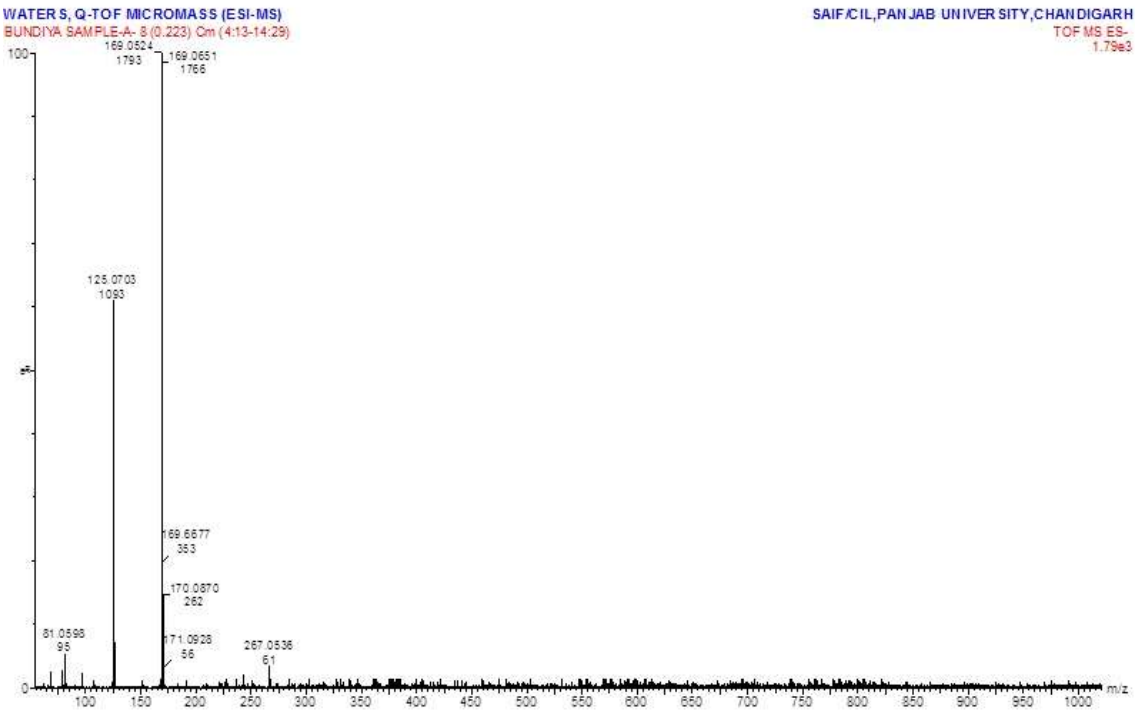


Figure.3: Mass spectra of Compound-C1 isolated from *Vanda tessellata* (Roxb.) Hook. ex. G.Don (Roxb.) Hook. ex. G.Don extract.

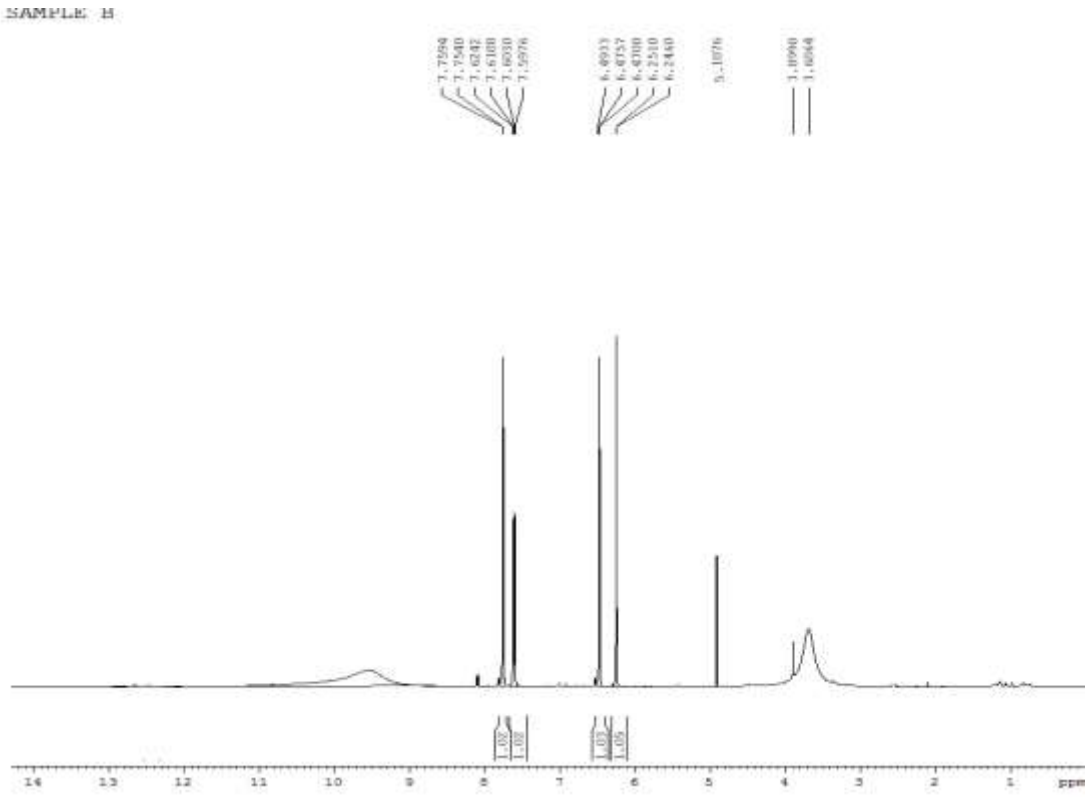


Figure. 4: ^{1}H-NMR spectra of Compound-C2 isolated from *Vanda tessellata* (Roxb.) Hook. ex. G.Don extract.

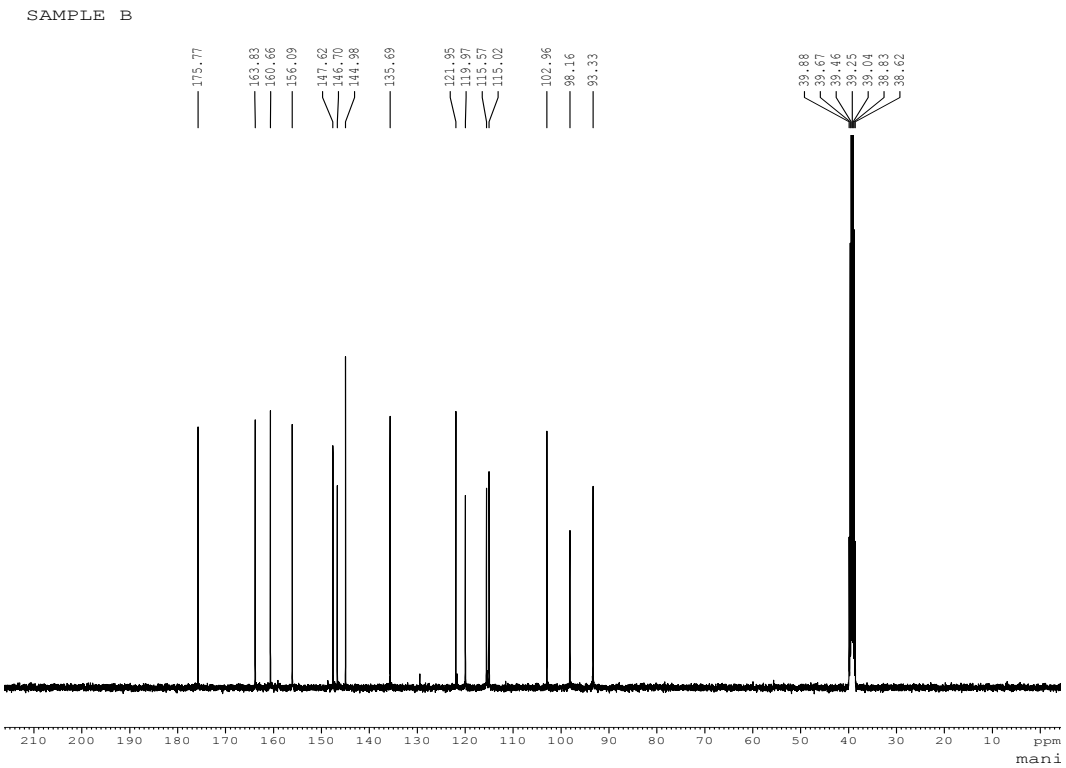


Figure .5: ^{13}C-NMR spectra of Compound-C2 isolated from *Vanda tessellata* (Roxb.) Hook. ex. G.Don extract.

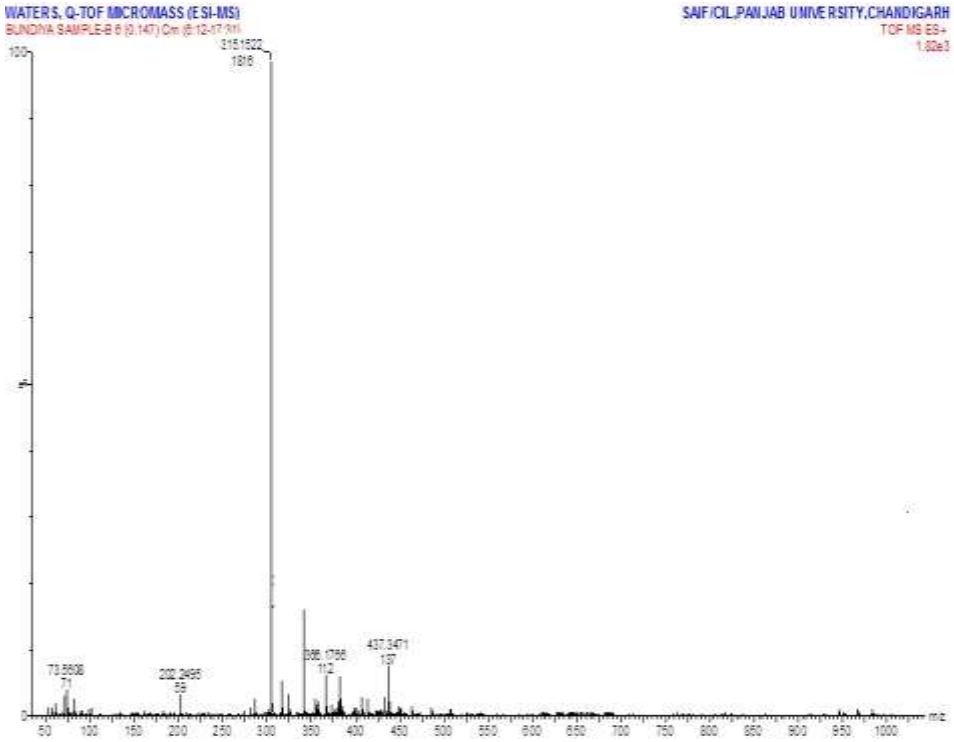


Figure. 6: Mass spectra of Compound-C2 isolated from *Vanda tessellata* (Roxb.) Hook. ex. G. Don extract.

CONCLUSION

The present research work is carried out to study the active compound that is accountable for therapeutic activity present in the leaf extract of *Vanda tessellata* , as till date no reports on active constituent in its leaf have been reported only the active constituent present in its root and flower has been documented. The active chemical compounds derived from its leaf is 3,4,5-trihydroxybenzoic acid and 2,5-dimethoxy-6,8-dihydroxy-Isoflavone. Since the leaf of *Vanda tessellata* is having ample of medicinal properties as reported in earlier works . Consequently, discovery of active chemical compound will need further research so that new drug can be formulated.

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