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Original Research Paper

ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENTS FROM *BARLERIA MONTANA* NEES (ACANTHACEAE) LEAVES

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ABSTRACT

The methanolic extract of leaf of *Barleria montana* Nees. was studied for isolation of phytoconstituents by thin layer chromatography (TLC), column chromatography and prepa-rative-thin layer chromatography (PLC), and characterized by spectroscopic techniques (Infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry). A rotenoid (Nicouline) and a flavonoid (Delphinidin 3, 5-diglucoside chloride) was isolated from the methanolic extract of leaf and identified with the help of preliminary phytochemical methods and spectroscopic data. Delphinidin 3,5-diglucoside chloride is found to be an anthocyanin glycoside (plant pigments of flavonoid class), abundantly found in many fruits and vegetables. They are the largest and most important group of water-soluble and vacuolar pigments in nature. They are chemically glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation (anthocyanidin group). These compounds are of special interest because of their antioxidant activity and their potential use in the food industry as natural colorants and preserving agents. Nicouline is found to be a rotenoid, having broad spectrum insecticidal and pestiscidal activities.

Keywords: *Barleria montana*, Delphinidin 3, 5diglucoside chloride, Nicouline, Spectroscopy, Column chromatography, Preparative thin layer chromatography.

INTRODUCTION

*Barleria montana, (*Synonym *Barleria purpurea)* commonly known as "Mountain Barleria" is one of the species in the genus Barleria belonging to the family Acanthaceae (Ruellia family).[1] Mountain Barleria is an erect herb found in the mountains of Western Ghats. It exists as herbs or undershrubs, perennial, erect or suffruticose, upto 1m height. Traditionally, it has been used for centuries for treating wounds, diabetes and for its hepatoprotective activity. Literature review suggested that the isolation and characterization of the phytoconstituents from this plant has not been studied and henceforth in the present study the investigations were carried out.

MATERIALS AND METHODS

Preparation of Extract

The leaves of *Barleria montana* Nees. were collected, washed and dried at room temperature, voucher specimen deposited at Dept. of Botany, S.V. University, Tirupati, India. After complete drying, they were powdered and passed through a 60# mesh sieve and stored in air tight container. Dried powdered drug was used to prepare the extracts.

Successive Solvent Extraction

About 100 g of air dried powdered plant material was extracted successively in a soxhlet apparatus, with petroleum ether (60-80^{0}C), followed by benzene, chloroform, acetone and methanol.[2] The marc was air dried below 50 ^{0}C each time, before extracting with next solvent. Finally, marc was macerated with chloroform water to obtain an aqueous extract. Extracts were filtered, the solvent was evaporated and accurate weight of the extracts was taken. Extractive value (%) was calculated with reference to air dried drug. Colour and consistency of extracts were noted.

Phytochemical Screening

Preliminary phytochemical screening of different extracts was performed for the presence of phytoconstituents.

Separation and Isolation of Phytoconstituents by Chromatographic Methods

Since, methanolic extract yielded maximum number of phytoconstituents; it was selected for further fractionation and isolation. Gradient fractionation was performed by eluting the column with n-hexane, chloroform, ethylacetate and methanol. TLC of the fractions were performed by using the solvent system, Methanol : Chloroform (6:4) over silica gel (60-120#), resulting in the isolation of compound-I (CND-I) and the solvent system, Chloroform : Methanol : Glacial acetic acid (2:8:0.5) for isolation of compound-II (CND-II). The separations of isolates were confirmed by spraying with Vanillin–Sulphuric acid reagent. TLC of all the fractions were performed and similar fractions were pooled and labelled. CND-I and CND-II were isolated from fractions no. 137-186 and 192-212, respectively. Fractions (137-186) collected from the main column were pooled and numbered as fraction No.13 with solvent ratio Ethylacetate : Methanol (35:65) confirmed the CND-I of the plant. Fraction No.13 (380 mg light green residue) was further purified by re-column (silica gel 60-120#). The solvents were allowed to flow in the order of increasing polarity. Twelve fractions were collected. Fraction 11 was further kept for crystallization. It yielded 62 mg of needle shaped crystals of CND-I. Fractions (192-212) collected from the main column were pooled and numbered as fraction No.15 with solvent ratio Ethylacetate : Methanol (10:90) confirmed the CND-II of the plant. Fraction No. 15 (120 mg) was further purified by re-column chromatography (silica gel 60-120#). The solvents were allowed to flow in the order of increasing polarity. Thirteen fractions were collected. Fraction No.12 was further subjected to preparative thin layer chromatography by using silica gel (60-120#) as stationary phase and solvent system, Chloroform : Methanol : Glacial acetic acid (2:8:0.5) which yielded 40 mg of CND-II.

Characterization of Isolated Compounds

The isolated compounds were further characterized with the help of physical characters like melting point, solubility and spectroscopic analysis (IR spectroscopy, Mass spectrometry and NMR spectroscopy).

Equipments Used

Shimadzu FTIR 8400S, Sortorius Balance (0.01mg), Shimadzu Bruker AMX 500 (resonance frequencies 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C)

RESULTS AND DISCUSSION

Phytochemical Screening

A qualitative phytochemical analysis was performed for the presence of phytoconstituents in different extracts of the leaves. The phytoconstituents found in the various extracts were phenolic compounds, phytosterols, alkaloids, carbohydrates and flavonoids. Methanolic extract indicated the presence of phenolic compounds, flavonoids, phytosterols and alkaloids.

Separation and Isolation of Phytoconstituents

Methanolic extract of leaf resulted in column chromatographic separation and further purification by Preparative-thin layer chromatography.

Structural Elucidation of Isolated Compounds

CND-I

White needle shaped crystals, odourless, tasteless, Soluble in alcohol (1:300), chloroform (1:3), and ether (1:200), m.p. 165-166^{o} C, Rf value 0.48.

Elemental analysis of the isolated Compound-I was performed by using Flash EA 1112 series. It indicated the presence of nitrogen, carbon and hydrogen to an extent of 6.25, 32.64 and 4.41% respectively.

The IR spectrum (figure 1) showed characteristic bands indicating the corresponding functional groups depicted in Fig.1, indicating 3435 (=OH), 2920 (=C-H st), 2845(-C-H st), 2397(=S-H st), 2254 (=C-H), 1765, 1645 and 1386 (=C=O), 1026 and 1005 (=C-O), 873, 825, 763 and 615 (=C-H).

Proton NMR spectra (figure 2), showed the peaks at δ 1.10 (H-1), 1.14 (H-2), 1.17 (H-3), 1.19 (H-4), 1.22 (H-5), 1.26 (H-6) showing 6 shifts corresponding to alkane = -CH_2.

The peaks at δ 2.82 (H-7), 4.13 (H-8), 4.16 (H-9), 4.20 (H-10), 4.23 (H-11), 4.67 (H-12) and 4.89 (H-13) showing 7 shifts corresponds to hydrogen atom and the peaks at δ 7.11 (H-14), 7.12 (H-15), 7.16 (H-16), 7.17 (H-17), 7.27 (H-18), 7.30 (H-19), 7.32 (H-20), and showing 7 shifts corresponds to protons of aromatic ring (phenyl-H atoms). The signals at δ 7.35 (H-21) and 7.39 (H-22), are protons of –C=C-. The signals at δ 7.42 (H-23) and 7.47 (H-24) belongs to the furan ring.

The ^{13}C-NMR spectrum (figure 3) has a signal at δ 14.13(C-1), 16.23 (C-2), 39.97 (C-3), 39.54 (C-4), 39.15 (C-5) corresponds to C-C=C, C-H unsaturated. 5 signals at δ 55.80 (C-6), 62.68 (C-7), 77.99 (C-8), 78.66 (C-9), 79.33 (C-10) corresponds to C-OR for a carbonyl carbon. 6 Signals at δ 112.28 (C-11), 115.16 (C-12), 120.71 (C-13), 128.93 (C-14), 146.06 (C-15) and 147.46 (C-16) corresponds to the aromatic nature of the compound-I.

The Mass spectrum of isolated constituent depicted in figure4, showed a molecular ion peak at m/z 570 for M^+ ion and Base peak at m/z 168.95, in the negative mode ESI-MS spectrum. Molecular mass was 394.43.

The above data represents that the isolated compound is Nicouline with the molecular formula and structure depicted in figure 5.

CND-II

White prism shaped crystals, odourless, tasteless, does not mix with water, m.p. 465 ^{0}C, Rf value 0.36.

The IR spectrum have shown the characteristic bands indicated in figure 6, 3430 (=OH st), 2915 (=C-H st) stretch of alkenes, 2843 (-C-H st), 1742, 1649, 1621 (=C-H), 1561, 1506, 1457, 1101, 1002 and 887 (=C-H δ).

Proton NMR Spectra (figure7), indicated the peaks at δ 0.0645(H-1), 1.1846 (H-2) and 2.4484 (H-3) showing 3 shifts corresponds to alkane = -CH_3. The peaks at δ 2.5- 5.0 i.e., 2.507 (H-7), 2.5948 (H-8) and 3.0828 (H-9) showing 3 shifts corresponding to –OH atom and the peaks at δ 5.0 – 7.0 i.e., 6.4936, 6.5326 and 6.9913 showing 3 shifts corresponding to protons of aromatic carbon. The signals at δ 7.02, 7.21, 7.24, 7.25, 7.29, 7.34, 7.38, 7.41, 7.45, 7.48, 7.67, 7.70, 7.86, 7.90, 7.98, 8.02 showing 16 signals indicating the presence of aromatic –OH groups.

The ^{13}C-NMR spectrum (figure 8), peaks at δ 39.39(C-1), 39.82 (C-2) and 40.24 (C-3) corresponds to –C-H saturated alkanes. The signal at δ value 78.66 indicates the presence of C-OH group. 10 shifts at δ 116.01, 121.59, 122.29, 124.26, 124.59, 126.08, 128.93, 134.36, 153.95 and 160.71 corresponds to the aromatic carbon. 6 Signals at δ 112.28 (C-11), 115.16 (C-12), 120.71 (C-13), 128.93 (C-14), 146.06 (C-15) and 147.46 (C-16) corresponds to the aromatic nature of the CND-II.

The Mass spectrum of isolated constituent depicted in figure 9, showed a molecular ion peak at m/z 662.125 for M^+ ion, base peak at m/z 157.10, in the negative mode ESI-MS spectrum. Molecular mass was 662.978.

The above data represents that the isolated compound is Delphinidin 3, 5-diglucoside chloride, with the structure depicted in figure10.

Nicouline is found to be a rotenoid, having broad spectrum insecticidal and pesticidal activities. Earlier, it has been isolated from many plants belonging to genera Lonchocarpus and Derris. Emmanuel Geoffroy first isolated rotenone from a specimen of *Robinia nicou*, now called *Lonchocarpus nicou*, while travelling in French Guiana. Researchers later determined that the

substance termed nicouline was identically rotenone.[3] Delphinidin 3,5-diglucoside chloride is found to be an anthocyanin glycoside (plant pigments of flavonoid class), abundantly found in many fruits and vegetables. Anthocyanins are one of the main polyphenols present, especially in the peels of the fruits. They are the largest and most important group of water-soluble and vacuolar pigments in nature. They are chemically glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation (anthocyanidin group).[4] These compounds are of special interest because of their antioxidant activity and their potential use in the food industry as natural colorants[5] and preserving agents.[6]

CONCLUSION

Phytochemical investigation of MEBM led to isolation of two compounds. CND-I was confirmed as Nicouline and CND-II was confirmed as Delphinidin 3,5-diglucoside chloride. The structure of these compounds were confirmed by melting point, Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR and ^{13}C NMR), Infra Red Spectroscopy (IR) and Liquid Chromatography-Mass spectrometry (LC-MS).

ACKNOWLEDGEMENT

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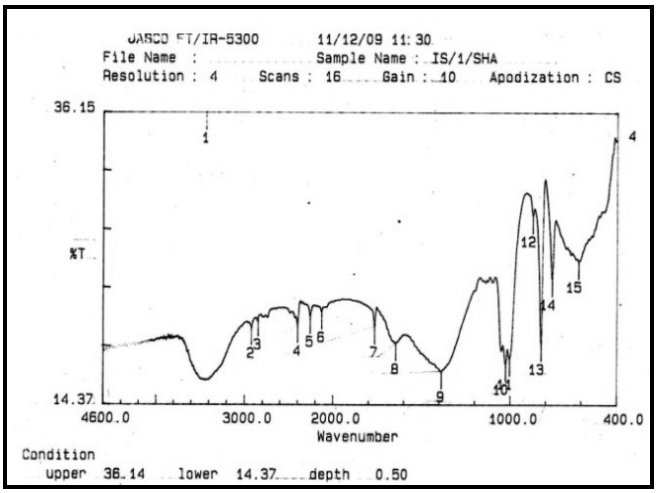

Figure 1: IR spectrum of CND-I

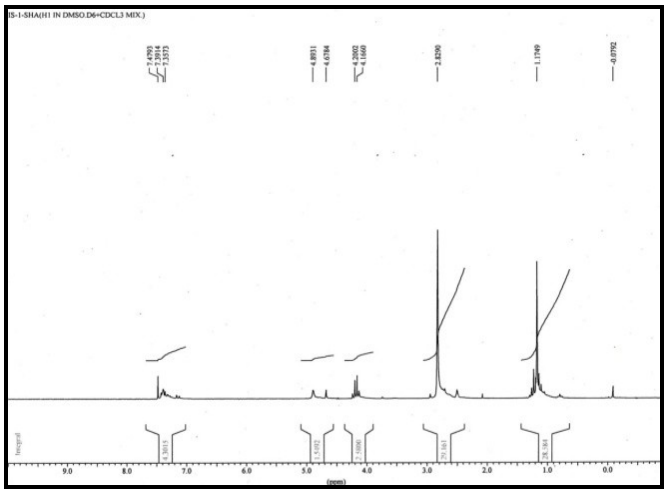

Figure 2: ^{1}H NMR Spectrum of CND-I

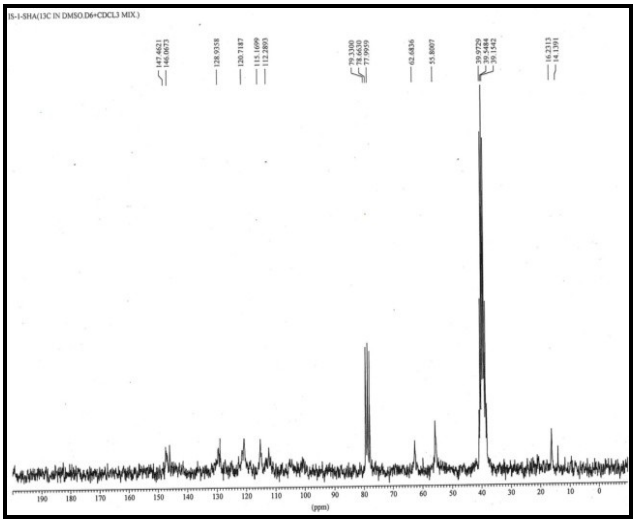

Figure 3: ^{13}C NMR Spectrum of CND-I

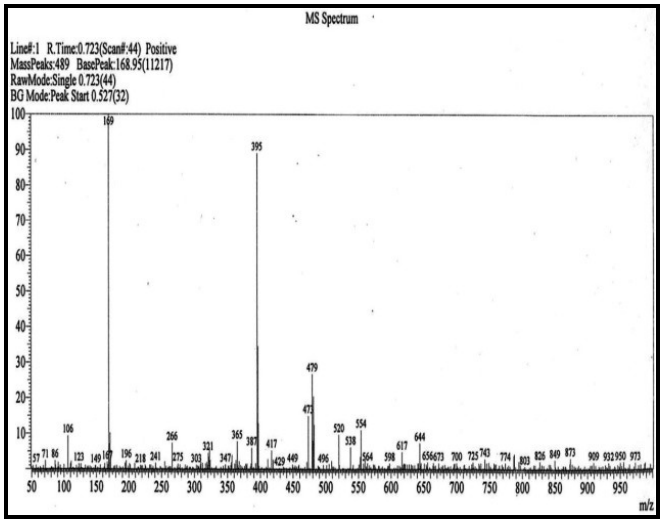

Figure 4: LC-MS Spectrum of CND-I

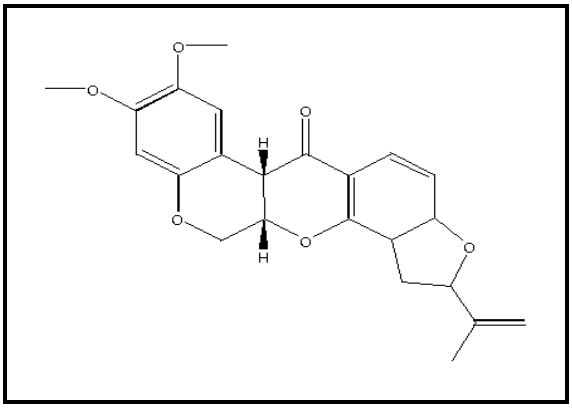

Figure 5: Structure of CND-I (Nicouline)

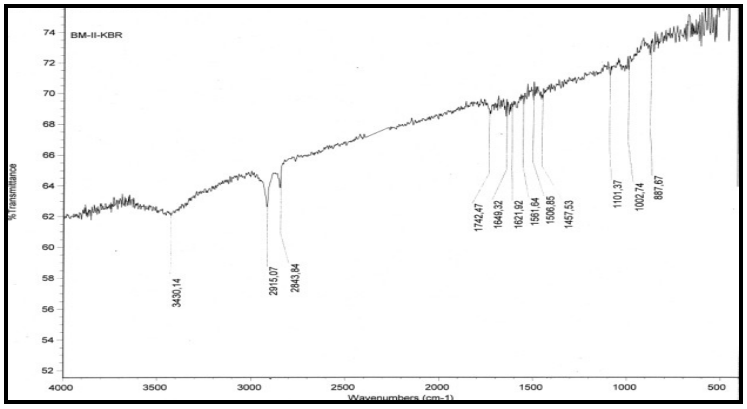

Figure 6: IR Spectrum of CND-II

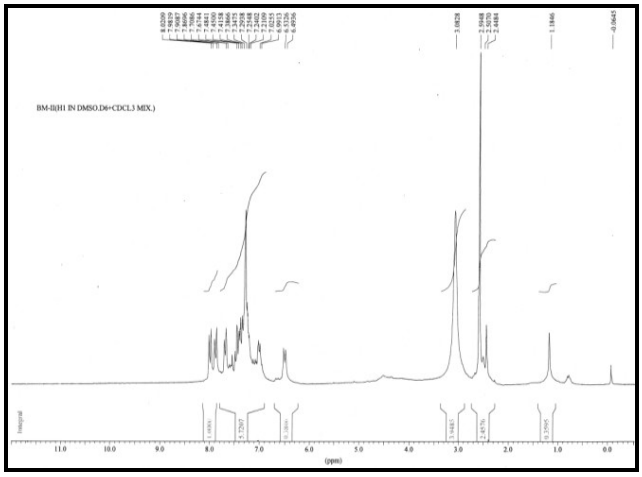

Figure 7: ^{1}H NMR Spectrum of CND-II

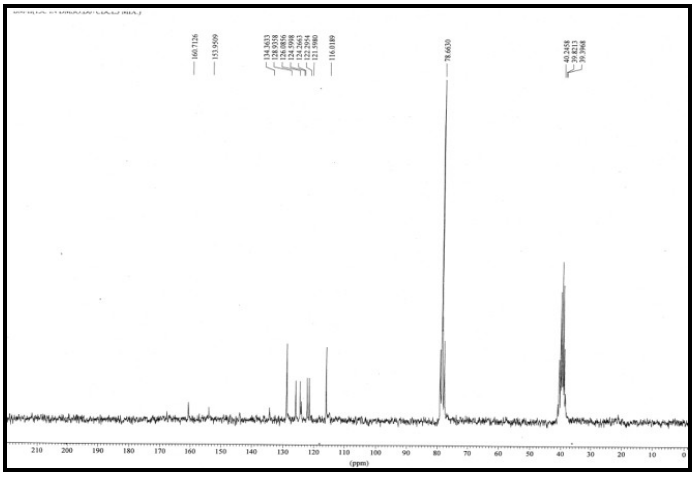

Figure 8: ^{13}C NMR Spectrum of CND-II

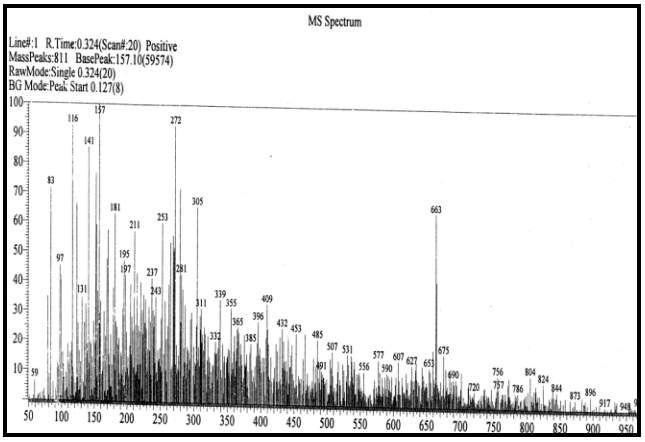

Figure 9: LC-MS Spectra of CND-II

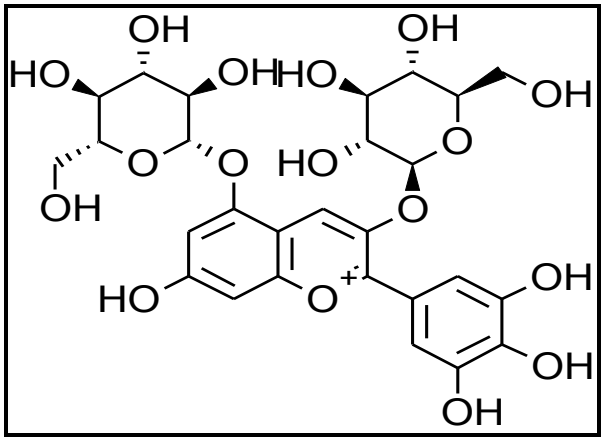

Figure 10: Structure of CND-II
(Delphinidin 3,5-diglucoside chloride)

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