Research Article

Isolation of D-Pinitol: A Bioactive Carbohydrate from the Leaves of *Bauhinia variegata* L.

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ABSTRACT

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures through history. The leaves of *Bauhinia variegata* linn. (Leguminosae) are reported to be of great medicinal importance. In the present investigation isolation of chemical constituent was carried out from the leaves of *Bauhinia variegata*, an evergreen member of the family Leguminosae, using column chromatography. Identification of chemical constituents was done by various techquines viz.MP, TLC, IR, NMR & LC-MS techniques. A bioactive carbohydrate D-pinitol was isolated from the ethanolic extract. These findings are useful in establishing a relationship between chemical composition of the leaf extract and previously reported activities of *Bauhinia variegata* and also may assign a new potential role of *Bauhinia variegata* extract in human health care. The presence of the bioactive compound (+)-pinitol in this plant is being reported for the first time.

Keywords: *Bauhinia variegata*, column chromatography, Leguminosae, NMR, D-Pinitol.

INTRODUCTION

Bauhinia variegata L. belongs to the family Leguminosae, Genus Bauhinia is a genus of shrubs or tree, very rarely climbers, distributed throughout the tropical regions of the world.[1] Rakta Kanchan (*Bauhinia variegata*) is a medium-sized, deciduous tree, found throughout India, ascending to an altitude of 1,300 m in the Himalayas. *Bauhinia variegate* is commonly known as Kanchnar in Sanskrit, Kachnar in Hindi and Mountain Ebony in English.[2] The reported biological activity of this plants are hepatoprotective activity,[3] anti-inflammatory,[4] Anti-tumor activity,[5] antibacterial activity,[6] antimicrobial,[7] haemagglutinating activity,[8] nephrotoxicity,[9] antiulcer activity, antidaibetic activity, haematinic activity, anticarcinogenic activity.[10] Phytochemical analysis of the root bark of plant yielded a flavanone, that is (2S)-5,7-dimethoxy-3y,4y methylene-dioxy flavanone & dihydro-dibenzoxepin,5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2methyl dibenz oxepin.[11] A novel flavonol glycoside 5,7,3',4'-tetrahydroxy-3-methoxy-7-O-alpha-L-rhamnopyranosyl (1--)3)-O-beta galactopyranoside isolated from the roots of *B. variegata*.[12] The stem showed presence of hentriacontane, octacosanol, stigmasterol.[13] The stem yielded a flavonone glycoside characterized as 5, 7-dihydroxyflavonone-4-O-Z-L-rhanmopyranosyl-e-D-glucopyranoside.[14] The isolation of e-sitosterol, lupeol, kaempferol-3-glucoside and a 5, 7-dimethoxyflavonone-4-O-Z-L-rhanmopyranosyl-e-D-glucopyranoside was also reported from the stem of the plant.[15, 16]

The compounds pinitol is belonging to group of Cyclitols (cyclic polyol). Pinitol (3-O-methyl D-chiro inositol)[17] is a natural product of cyclitol group occurring mainly in its (+) form in certain leguminous plants, soya foods and was found to be responsible for hypoglycaemic activities, antidiabetic[18] & its chronic complications obesity; Hyperlipidemia; Dyslipidemia atherosclerosis; Hypertension; cardiovascular disease, malnutrition, stress, aging & other autoimmune disease, Hyperuricemia & Anthelmintic activity.[17] D-pinitol is an active principle of the anti-inflammatory plant. Animal studies showed that D-chiro-inositol is synthesized endogenously in small quantities, while in human most D-chiro-inositol is obtained from dietary pinitol. Literature showed that supplementation with pinitol decreased plasma glucose in normal and diabetic albino mice & suggested that pinitol might have a direct effect on glucose transport that was independent of insulin.

Pinitol and D-chiro-inositol are structurally similar to the inositol phosphates that influence insulin signalling via 121 phosphatidyl inositol 3-kinase. Moreover, it is known for antidiabetic, anti-inflammatory and feeding stimulant activities. D-pinitol is an active principle of the anti-inflammatory plant. Many pharmaceutical preparations of D-pinitol are marketing, the popular D-pinitol products under the trade name Biochem GlucoLean® and Inzitol® can help to facilitate glycogen or circulating sugar into metabolically active tissues.[19]

MATERIALS AND METHODS

Plant Material

The leaf of *Bauhinia variegate* was collected during February 2011 from the farm of CIMAP (CSIR), Lucknow (U.P.). Further taxonomic identification was conducted by Dr. A.K. Gupta, Scientist, botanical & taxonomical department (CIMAP). A plant specimen (RIPS/H/1101) was deposited in the herbarium of Roland institute of pharmaceutical sciences, Berhampur for future reference. The material was shade dried, pulverized and preserved in air tight containers.

Chemicals

The chemicals for isolation were obtained from Merck and SD fine chemicals.

Silica gel (60-120), n-Hexane, chloroform, ethyl acetate, ethanol obtains from Loba chemie Pvt Ltd, Mumbai.

Extraction & Isolation

The ethanolic extract of coarsely powder (5 kg) of the leaves was prepared by using Soxhlet apparatus. The crude extract was evaporated to dryness in a rotavapour to give dark brown mass. The ethanolic extract (5 g) was subjected to column chromatography on silica gel (60-120 mesh) using solvents of varying polarities, starting from n-hexane, chloroform, ethyl acetate and methanol to yield several sub fractions. The column was eluted firstly with low polar solvent n-hexane & 20 fractions were collected. Then successively with n-hexane: ethyl acetate in different ratio (gradually increased) and fraction no. 21-100 were collected and monitored by TLC. Those all fractions are only mixture of compounds. Then Column was eluted successively with ethyl acetate: methanol in different ratio (gradually increased) and fraction no. 101-130were collected and checked TLC. On TLC plate (EtOAc: MeOH, 3:2) fraction no. 115-120 gives 2-3 spots respectively which was eluted at EtOAc:MeOH (4:1). Finally Column washed by Methanol (100%). After some time of elution, a white color amorphous solid was precipitated at the bottom of the test tube (Fraction 115-120) which was filtered with the help of vacuum filter. This compound showed one prominent single spot on TLC & assigned as B-1(Wt. - 126mg).

RESULTS AND DISCUSSION

The structure of isolated compound (B-1) was elucidated on the basis of spectral data. Isolated compound was obtained as white color amorphous powder and had a M.P. 180-183^{0}C. This compound was found as UV inactive. TLC profile of compound R_f = 0.42 (n-Butanol:Acetic acid:Water-7:2:1) (fig.-1) & R_f = 0.6 (EtOAc:MeOH - 3:2) as also reported in literature.[18] The isolated compound has molecular formula $C_7H_{14}O_6$ which was confirmed by its mass spectra MS (M^+ 194). IR(KBr cm-1):Ali C-H(str) 2909.98; OH(str) 3402.12, 3319.36(H-bonded) ; C-C(str) 1126.31; C-O(str) 1251.35; C-H (ben) 1450.46; OH(ben) 1072.86. ^{13}C NMR ($CDCl_3$)(δ ppm) spectrum revealed the presence of methoxy group(OCH_3) at 60.07, 83.12 (C-3), 72.47 (C-5), 72.02(C-1), 71.81 (C-6), 70.88 (C-2) and 70.17 (C-4). In its ^{1}H NMR spectrum (D_2O, 300MHz), H-1 at 3.880 (*m,3H*), H-2 at 3.676 (d, J=9.9,2.4), H-3 at 3.249 (*d, J=5.7*), H-4 at 3.709 (d, J=9.9),H-5 at 3.557 (*m,1H, J=9.6*), H-6 at 3.624 (*d,1H, J=9.6*) and the methoxy group as a singlet at 3.471

(*3H*). COSY and HSQC spectra established the assignments of each protons and carbons. Their in HMBC spectrum δc 83.12 shows cross peak at δ_H 3.880 (C_3-H), 3.624 (C_6-H) and δc 70.88 shows cross peak with δ_H 3.709 (C_4-H), 72.02 shows cross peak with 3.557 (C_5-H) which shows the assignment of proton and ^{13}C values are correlating and perfect. Dept spectra shows the presence of CH in downfield.

Analysis by various techniques viz. melting point, TLC, IR, NMR and LC-MS & their data, isolated compound B-1 is identified as (+)pinitol (fig.-2) which is a cyclic polyol.

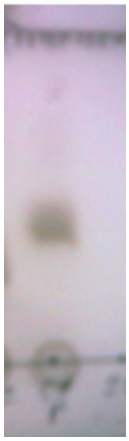

Figure 1: TLC profile of isolated compound

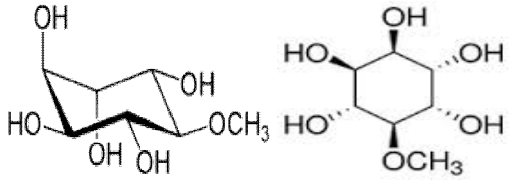

Figure: 2 - Structures of isolated compound BD-1 (D-pinitol)

CONCLUSION

In the present investigation on the basis of physical (M.P.) and spectral (IR, NMR and Mass) data, it was concluded that the isolated compound B-1 from the ethanolic extract of *Bauhinia variegata* is a D-Pinitol. These compound Pinitol is belonging to group of Cyclitols (cyclic polyol). Pinitol was isolated for the first time from plant *Bauhinia variegata*.

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