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Poly phenolic constituents of *Dalbergia paniculata* roots

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Abstract

Flavonoids are biologically active polyphenolic compounds widely distributed in plants, are biogenetically derived from cinnamoyl-CoA and three molecules of malonyl-CoA, and contain a C₆+C₃+C₆ structural unit. On the basis of an *in vitro* primary screening test many kinds of flavonoids and isoflavonoids, including flavones, flavanones, flavanols, flavanonols, isoflavones, chalcones, and catechins, showed inhibitory effects on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation. The stem bark of *Dalbergia cultrate* Grah. And *D. nigrescens* Kurz (Leguminosae) were found to exhibit a significant sources of flavonoids are vegetables, fruits, nuts, seeds, roots, and beverages like tea and wine. Health effects of tea have been recognized since the ancient times. In a tea polyphenols could play a role in the prevention of cancer and heart disease. To date, the most protective effect is shown against lung cancer. The major antioxidant activity of tea extracts is attributed to Epigallocatechin gallate. Besides the catechin molecule, which is a potent antioxidant, gallate moiety is an important contributor to the antioxidant activity.

Plant belonging to *Dalbergia* genus exhibit different physiological activity, such as general tonic and as cure for blood troubles, inflammation, ulcers, tumors, and leprosy and also employed as a insect repellent and is infectant. The flowers and bark of *Dalbergia paniculata* (Roxb) is widely distributed throughout Southern and Central India. Its vernacular name is "patsaru".

The roots air dried (1Kg) was powdered in a pulveriser and extracted dichloromethane and methanol (1:1) (3lit) in a room temperature for 5 days for each time and 24h soxhlet extractor. The combined extracts were concentrated separately under vacuum to yield gummy semi solids (125gm). This extract was suspended in water and then was further extracted with EtOAc and n-Butanol to yield soluble fraction of EtOAc and n-Butanol (45gm). This fraction was subjected to a chromatography (CC) over silica gel eluted with different solvents of increasing polarity (hexane: acetone and DCM: Methanol from 10:1 to 1:1).

Keywords: Co-TLC, crystallization, aromatic carbon

Introduction

The powdered roots of *Dalbergia paniculata* (1kg) was extracted with dichloromethane and methanol (1:1) (3lit) in a room temperature for 5 days for each time and 24h soxhlet extractor. The combined extracts were concentrated separately under vacuum to yield gummy semi solids (125gm). This extract was suspended in water and then was further extracted with EtOAc and n-Butanol to yield soluble fraction of EtOAc and n-Butanol (45gm). The individual extracts were worked out separately.

The EtOAc: n-BuOH insoluble extract was concentrated under reduced pressure to yield light yellowish gummy material. It was shown to be complex mixture hence not worked out further.

EtOAc: n-BuOH soluble extract

The EtOAc: n-BuOH soluble extract was concentrated under reduced pressure to yield a greenish semi-solid (45g). It showed major compounds in TLC. The semi solid gave a play of colors, changing from green to red, in the Liebermann Burchard test and produce red color in the Salkowishi test indicating the presence of steroids. It gave light brown colouration with methanolic ferric chloride indicating the presence of phenolic compounds. DCM: MOH 1:1 V/V 43-56 columns later crystallized from Acetone gives white colorless needles named DP-1.

Structure elucidation of DP-1

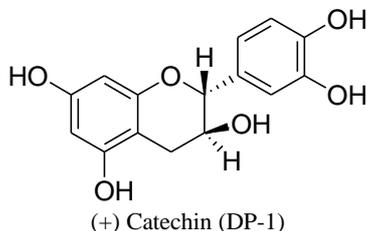
Compound DP-1 was obtained as white crystalline compound and its molecular formula was

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determined to be $C_{15}H_{14}O_6$ by HRMS, M^+ 290 ($[\alpha]_D +16.2$ ($c=1, H_2O$)) which was in agreement with the 1H - and ^{13}C -NMR spectral data. The methanolic solution of DP-1 on heating with hydrochloric acid furnished red color solution. This color Reaction is characteristic of flavan-3,4-diols. It gave deep green color with methanolic ferric chloride indicating the presence of chelated phenolic hydroxyl group. It formed pentaacetate with acetic anhydride pyridine (m.p. $176^\circ C$, $C_{25}H_{24}O_{11}$ M^+ 500. [1H -nmr: δ 2.14, 2.23 and 2.38].



The UV spectrum showed the typical catechin absorption at 224 and 282 nm. Its IR spectra exhibited absorption bands at 3368 cm^{-1} for hydroxyl, at 1745 cm^{-1} and at 1615 cm^{-1} 1525 cm^{-1} and 1448 cm^{-1} for the phenyl functionalities in the molecule.

In the 1H NMR spectrum of (+) catechin proton resonance at δ 6.84 (2H, s) and 6.68 (2H,s). The aromatic signals at δ 5.94 and 5.83(each 1H, d, $J=2.0$ Hz) and sp^3 proton signals at δ 4.52, 4.50 and 2.62 indicated that DP-1 had a catechin skeleton. The coupling constant (5.6 Hz) between H-2 and H-3 implied that the relative stereochemistry was a trans. In the ^{13}C NMR spectrum C2-carbon resonated at δ 86.4 and one sp^3 carbon at 18.8 and 13 aromatic carbons (δ 101.2 to δ 176.9) were shown. Colour reactions show that DP-1 is a catechin. The analytical and spectral data of DP-1, its penta-*O*- acetyl derivative and tetra -*O*- methyl ether are identical with that of (+) catechin and its derivatives. Further DP-1 was found to be identical al in all respects with an authentic samples of (+) catechin (m.p., mmp, Co-TLC, The 1H -nmr spectral data of DP-1, its penta-*O*-acetyl derivative and tetra-*O*- methyl derivative are presented in table. The structure (+) catechin is now confirmed by chemical as well as by spectral evidence. This is the first report of (+) catechin from the *Dalbergia paniculata* root.

DP-1 (+) catechin spectral data

MP, $[\alpha]_D$ and spectral characteristics of (+) Catechin and its derivatives DP-1(+ Catechin m.p. $^\circ C$ 176 (lit 177-178)¹, $[\alpha]_D +17.0$ (0.01 mole in methanol); IR (KBr) ν max cm^{-1} ; 3350 (-OH), 1540-1500 (aromatic C=C); UV λ_{max} MeOH, nm (log ϵ); 214(4.12), 230 (sh) 3.80, 280 (3.36); 1H NMR spectra data (2000MHz) values in δ ppm, J in Hz; 9.17(s,2H, D_2O exchangeable, C_5, C_7 -OH), 8.92(s,2H, D_2O exchangeable, C_3 , and C_4 -OH), 6.72-6.62 (m, 3H, C_2, C_5 , and C_6 -H), 5.87 (d, 1H, $J_{8H,6H}= 2.5$, C_8 -H), 5.68(d, 1H, $J_{6H,8H}= 2.5$, C_6 -H), 4.88 (br, 1H, D_2O exchangeable C_3 -OH), 4.52(d, 1H, $J_{2H\beta\text{ax}, 3H, \alpha\text{ax}}= 8.0$, C_2 -H), 3.66 (m, 1H, C_3 -H), 2.82 (m, 2H, C_4 -H); M^+ and other fragments; 290 (35), 272 (5), 153 (10), 152 (50), 139 (100), 124 (30), 123(55).

DP-1a Penta-O-acetyl derivative of (+) catechin spectral data

Penta-*O*-acetyl derivative of (+) Catechin m.p. $^\circ C$ 132 (lit 132-133)¹, $[\alpha]_D +4.5$, (0.01 mole in methanol); IR (KBr) ν max cm^{-1} ; 1740 (acetoxyl C=O), UV λ_{max} MeOH, nm (log ϵ); 218(4.57), 224 (sh) (4.52), 270 (3.84); 1H NMR spectra data

(200MHz, $CDCl_3$) values in δ ppm, J in Hz; 1.90 (s, 3H, C_3 -acetoxyl), 2.24(s, 12H, C_5, C_7, C_3 and C_4 - acetoxyls). 2.80(m, 2H, C_4 -H), 3.76 (m, 1H, C_3 -H), 5.08(d, 1H, $J_{2H\beta\text{ax}, 3H\alpha\text{ax}}= 8.0$, C_2 -H), 6.56 (d, 1H, $J_{6H, 8H}= 2.5$, C_6 -H), 6.49d, 1H, $J_{8H, 6H}= 2.5$, C_8 -H), 7.18 (m, 3H, C_2, C_5, C_6 -H). M^+ and other fragments; 500 (5), 440 (20), 398 (40), 356 (38), 314 (40).

DP-1b: Tetra-O-methyl ether of (+) Catechin

Tetra-*O*-methyl ether of (+) Catechin m.p. $^\circ C$ 1442 (lit 142-144)¹, $[\alpha]_D +8.2$, (0.01 mole in methanol); UV λ_{max} MeOH, nm (log ϵ); 218 (4.48), 228 (sh) (4.44), 272 (3.82); 1H NMR spectra data (200MHz, $CDCl_3$) values in δ ppm, J in Hz; 2.80 (m, 2H, C_4 -H), 3.74 (s, 3H, C_7 -OCH₃), 3.79 (s, 3H, C_5 -OCH₃), 3.81 (s, 6H, C_3, C_4 -OCH₃), 4.65 (d, 1H, $J_{2H\beta\text{ax}, 3H\alpha\text{ax}}= 7.80$, C_2 -H), 3.92 (M, 1H, C_3 -H), 6.12 (bs, 2H, C_6, C_8 -H), 6.84-6.96 (m, 3H, C_2, C_5, C_6 -H).

Results and Discussions

Dalbergia genus consists of 300 species which are rich in flavanoid compound. Moreover, many of them are having medicinal activity in nature. The present work deals with Dalbergia family, namely Dalbergia paniculata root. Already earlier work reveals many poly phenolic compounds. Present work reposting a new source of compound Catechin in Dalbergia paniculata root. Catechin is a flavon-3-ol, a type of natural phenol and antioxidant. Catechin have been used as natural antioxidant in oils and fats against lipid oxidation, supplement for animal feeds, to improve animal health and to protect animal products, an antimicrobial agent, ingredient in various foods and dietary supplements.

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