PHYTOCONSTITUENTS ISOLATED FROM STEM BARK OF CASSIA FISTULA LINN.

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ABSTRACT
A detailed phytochemical study on the hydro-alcoholic extract of stem bark of Cassia fistula Linn. (Fam. Leguminoseae) led to isolation of five compounds. The structures of the five compounds were elucidated on the basis of analysis of spectroscopic data and physicochemical properties as: 1, 4, 5-trihydroxy-6, 7-dimethoxy-3-methyl-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid [fistulic acid] (CF – 1), 4,5-Dihydroxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid [rhein] (CF – 2), 4, 5-dihydroxy-9, 10-dioxoanthracene-6-methyl-2-carboxylic acid [6-methylrhein] (CF – 3), (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol [+ catechin] (CF – 4) and 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone (CF – 5).

Keywords: fistulic acid, rhein, 6-methyl rhein, (+) catechin, oxyanthroquinone, Cassia fistula, leguminoseae.

INTRODUCTION
Cassia fistula Linn. (Family leguminoseae) also known as golden shower is widely used for its medicinal properties, mainly as a mild laxative suitable for children and pregnant women, also as a purgative due to the wax aloin and a tonic [1]. It has been reported to treat many other intestinal disorders like healing ulcers [2, 3]. The plant exerts an antipyretic, analgesic effect and also anti-inflammatory and hypoglycaemic activity [4, 5]. Traditionally, the plant has been in use against skin diseases, liver troubles, tuberculous glands, in the treatment of haematemesis, pruritus, leukoderma and diabetes [6, 7]. C. fistula extract is useful as an anti-
periodic, in the treatment of rheumatism, leaf extract for its anti-tussive, wound healing properties and in the treatment of hypercholesterolaemia \cite{3, 9, 10, 11}.

The antimicrobial activity has been reported against *Escherichia Coli, Bacillus mycides, Bacillus subtilis, Mycobacterium smegmatis, Klebsiella aerogenes, Pseudomonas aerogenes* and *Proteus vulgaris* \cite{12}. Few more reported activities include antitumor \cite{13}, hepatoprotective \cite{14}, antifertility \cite{15}, antioxidant \cite{16, 17, 18, 19} and inhibitory effect on leukotriene biosynthesis \cite{20}.

Various phytochemical constituents were isolated and characterized from various parts of the plant like oxy-anthraquinone, dihydroxy anthraquinone \cite{21}, Fistulic acid \cite{22}, Chrysophanol \cite{23}.

In the present study, an attempt was made to isolate some of the phytoconstituents from stem bark of the said plant.

**RESULTS AND DISCUSSION**

**CF-1 Fistulic acid**

The compound gave positive reaction for anthraquinones. Reddish brown crystals of CF-1 showed melting range between 158-161°C. In mass spectrometric analysis molecular ion peak was observed at 374. Major bands in FT-IR spectrum include 3327 cm\(^{-1}\) (OH stretching), 2932, 1481 cm\(^{-1}\) (CH stretching), 2796 cm\(^{-1}\) (CH\(_3\) attached to oxygen), 1742 cm\(^{-1}\) (carbonyl stretching), 1593 cm\(^{-1}\) (COO\(^-\) in carboxylic acid), 1510 cm\(^{-1}\) (benzene ring stretching), 1154 cm\(^{-1}\) (C-OH stretching), 1109 cm\(^{-1}\) (C-OH in alcohol), 846 cm\(^{-1}\) (tri substituted benzene). The molecular formula of CF-1 is C\(_{18}\)H\(_{14}\)O\(_9\). The peaks observed in the recorded H\(^1\)NMR spectrum of CF-1 are complying with theoretical spectral values which further confirm the purity \cite{24}. A singlet at 2.42 ppm is assigned to methyl protons. A singlet at 3.62 ppm assigned to methoxy protons. A peak observed at 6.65 ppm is due to phenyl proton. A singlet at 12.14 is assigned to – COOH proton. The three -OH protons are observed at 11.84, 12.24 and 14.02 ppm due to different chemical environment of the molecule. The peaks observed in the recorded C\(^{13}\)NMR spectrum of CF-1 are well in accordance with theoretical spectral values which further confirm the purity \cite{24}. The peaks at 184.6 and 185.8 ppm are assigned to keto carbon at 1 and 8 positions respectively. The peak observed at 171.8 ppm is due to carboxyl carbon. The peaks at 160.8, 156.1, 155.2, 153.1 and 141.2 ppm are due to Ipso carbon at positions 10a, 9a, 3, 6 and 8a respectively. The peak observed at 131.9 ppm is due to methyl substituent carbon at position 4. The peaks observed at 126.8, 122.5 and
121.6 are due to aromatic carbons. The peaks at 140.9 and 184.6 ppm are assigned to carbons C2, C7, C9, and C10. A peak at 102.1 is assigned to carbon at 4a (doublet). The peaks at 60.8, 56.1 and 13.6 ppm are assigned to methoxy carbons at methoxy carbons at position 6, 7 and methyl carbon at 2 respectively. Based on the above spectral data the compound was identified as 1, 4, 5-trihydroxy-6, 7-dimethoxy-3-methyl-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid (fistulic acid).

![Chemical structures of CF-1 to CF-5]

Figure 1 Chemical structures of CF-1 to CF-5
Table 1 \(^{13}\)C NMR Data for CF-1 to CF-5

<table>
<thead>
<tr>
<th>Carbon position</th>
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**CF-2 Rhein**

The compound gave positive reaction for anthraquinones. CF-2 showed the molecular ion at \(m/z\) 283.2, which was corresponding to the molecular formula \(C_{15}H_8O_6\); its melting point was at 319-322\(^\circ\)C \cite{25}. The \(^1\)H-NMR spectrum revealed five aromatic protons of which two were broad singlets due to meta coupling. In \(^1\)H NMR spectrum, broad signals at \(\delta\) values 11.4 and 11.6 was found due to hydroxyl protons at C-1 and C-8, respectively. Aromatic signals at 8.21, 7.37, 7.76, 7.88 and 7.82 correspond to structure of rhein \cite{13}. In \(^{13}\)C-NMR, the peaks at 160.6 and 160.8 ppm are due to keto carbons at position 1 and 8, respectively. The peaks at 123.8 and 182.4 ppm are assigned to ring fused carbons C2, C7, C9 and C10. The peak observed at 192.3 is due to carboxy carbon. Other peaks including 166.6 (C-3), 118.2 (C-4), 125.3 (C-5), 139.1 (C-6), 129.8 (C-4a), 117.2 (C-8a), 116.9 (C-9a), 134.2 (C-10a), which
corresponded to those reported \[^{25}\]. The active compound was identified as 1,8-dihydroxyanthraquinone-2-carboxylic acid (Rhein).

**CF-3 6-methyl rhein**

The compound gave positive reaction for anthraquinones. Melting range of CF-3 was found to be 147-150\(^\circ\)C. its molecular ion peak was observed at 298. IR absorption pattern shows presence of bands at 3424 (OH) and at 1715 (COOH), 1658 (C=O) and 1619 (chelated C=O)cm\(^{-1}\), respectively. The mass spectrum of CF-3 displayed the parent ion peak at 298 [M]\(^+\). The \(^1\)H NMR spectrum of the compound showed signals for four aromatic protons. A three proton singlet at \(\delta\) 2.42 indicated the presence of an aromatic methyl in the compound. Two phenolic proton signals at \(\delta\) 11.29 and 12.26 were also present. The \(^13\)C NMR spectrum of CF-3 showed the carbonyl carbon signals at \(\delta\)172.1 (COOH), 186.9 (C-9) and 182.4 (C-10), the higher \(\delta\) value for C-9 signal being due to strong intramolecular hydrogen bonding with hydroxyl groups at C-1 and C-8. From the above spectral details and reports available the structure was elucidated as 4, 5-dihydroxy-9, 10-dioxoanthracene-6-methyl-2-carboxylic acid (6-methylrhein).

**CF-4 (+) catechin**

The compound gave positive tests for tannins. In the mass spectra of CF-4 the molecular ion peak was observed at 290. The IR spectra of CF-4 showed broad band around 3400-2600 cm\(^{-1}\) region corresponding to aliphatic and aromatic C-H, phenolic and alcoholic O-H stretching. A band at 1618 cm\(^{-1}\) observed may be due to aromatic C=C stretching. Other stretchings were comparable with IR spectra of authentic (+)-catechin. The \(^1\)H-NMR spectra of (+)-catechin was very clear and understandable. The observed signals in NMR spectra were in good agreement with the authentic (+)-catechin\[^{27}\]. In the \(^13\)C-NMR spectra, signals appeared at \(\delta\) 25.8, 65.6, 80.2, 92.8, 94.9, 113.2, 116.1, 117.6 due to C-4, C-3, C-2, C-8, C-6, C-2, C-5, C-6 carbons respectively and others aromatic carbons showed peaks at \(\delta\) 114.1, 129.4, 146.2, 142.3, 152.1, 157.6 and 158.2. These data were also in good agreement with the authentic sample of (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (+ catechin)\[^{27}\].

**CF-5 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone**

The compound CF-5 gave positive reaction for anthraquinones. Compound was obtained as a yellow amorphous solid and has molecular ion peak at 315.4. Based on this data, its molecular formula has been deduced as C\(_{17}\)H\(_{14}\)O\(_6\). The IR spectrum showed characteristic
absorption bands of hydroxyl group at 3349 cm\(^{-1}\), aromatic ring at 3121 and 1565 cm\(^{-1}\), a chelating carbonyl at 1617 cm\(^{-1}\) and an unchelating carbonyl at 1657 cm\(^{-1}\). The \(^1\)H NMR spectra exhibited signal at \(\delta\) 12.69, due to a chelated hydroxy proton at C-1. Three aromatic protons exhibited as doublets of both 8.3 Hz at \(\delta\) 7.9 (H-8) and 7.62 (H-7), and a singlet at \(\delta\) 7.12 (H-4) revealing the substitution on the aromatic rings. The two methoxy groups appeared as singlet at \(\delta\) 3.82 and 3.68 while a methyl group showed at \(\delta\) 2.12. On the basis of the above data and by comparison with the reported compound\(^{[28]}\), the structure of CF-5 was assigned as 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone.

**Experimental**

**General**

Melting points were determined on a Perfit melting point apparatus and are uncorrected. FT IR: Jasco FT/IR 5000, UV: Shimadzu UV-VIS (version 2.20) Spectrophotometer, Japan; \(^1\)HNMR: Bruker-DPX 300 NMR spectrophotometer USA, \(^{13}\)CNMR: Advance DRY Bruker, Mass: Shimadzu LC-MS 2010A, Japan and API 2000 Applied Biosystems, Canada; CC: Silica gel, 60-120 silica gel, TLC: Silica gel G. Spots were visualized by exposure to iodine vapors, UV radiation and by spraying reagents.

**Plant materials**

The stem bark of *Cassia fistula* was collected from the provinces around New Delhi, India. The herbarium was submitted and the plant material was authenticated by Dr. K. C. Bhatt, Senior Botanist, National Bureau of Plant and Genomic Research (NBPGR), Pusa Campus, New Delhi (Voucher number: NHCP/NBPGR/2009/1).

**Extraction and isolation**

The stem bark (3.5 kg) was coarsely powdered and extracted in a Soxhlet apparatus with methanol for 72 hours. The methanolic extract was concentrated under reduced pressure to get brown colored powder. 60 g extract was subjected to column chromatography and fractions were eluted with varying proportions of petroleum ether, chloroform and methanol. The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having same \(R_f\) values) were combined together and concentrated. The concentrated fractions were purified by re-crystallization.
CF-1 Fistulic acid
Elution of the column with petroleum ether: chloroform (25:75) mixture gave a solid mass which yielded reddish brown crystals of CF-1 after crystallization from methanol, 57 mg. Rf value: 0.65, melting point 158-161 °C, UV λ_max 280.5 (4.14), IR γ_max (KBr) 3327, 2932, 1481, 2796, 1742, 1593, 1510, 1154, 1109, 846 cm⁻¹, ¹H NMR (CDCl₃) δ 2.38 (1H, s, CH), 3.80 (1H, s, OCH₃), 6.8 (1H, m, H), 12.78 (1H, s, –COOH) 12.16 (1H, s, OH), 13.17 (1H, s, OH), 13.31 (1H, s, OH). For ¹³C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 374 [M]⁺, C₁₈ H₁₄ O₉ (10.6).

CF-2 Rhein
Elution of the column with chloroform: ethyl acetate (80:20) mixture gave a solid mass which yielded orange crystals after crystallization from methanol. It showed the molecular ion at m/z 283.2, which was corresponding to the molecular formula C₁₅H₈O₆ of rhein; its melting range was at 319-322°C. The ¹H NMR spectrum revealed five aromatic protons of which two were broad singlets due to meta coupling. ¹H NMR (75 MHz, DMSO): 11.4 (1H, brs, C1–OH), 11.6 (1H, brs, C8–OH), 8.21 (1H, brs, C2–H), 7.37 (1H, d, J = 9.1 Hz, C5–H), 7.76 (1H, m, C6–H), 7.88 (1H, brs, C4–H), 7.82 (1H, d, J = 7.3 Hz, C7–H) corresponded to rhein [13]. For ¹³C NMR spectroscopic data, see Table 1.

CF-3 6-Methylrhein
Elution of the column with chloroform: ethyl acetate (10:90) mixture gave a solid mass which yielded colorless solid after crystallization from methanol, 65 mg, Rf value 0.42, melting point 147-150, UV λ_max 231 (4.4), 251 (3.08), 281 (4.18), 448 (4.06), 472 (4.08), IR γ_max (KBr) 3424, 1715, 1668, 1658, 1619, 1430 cm⁻¹, ¹H NMR (CDCl₃) δ 2.42 (s, 3H, Ar-CH₃), 7.44 (brs, 1H, ArH-7), 7.68 (brs, 1H, ArH-5), 7.32 (d, J=1.8 Hz, 1H, ArH-2), 8.11 (d, J=1.6 Hz, 1H, ArH-2), 11.29 and 12.26 (s, 2 x OH). For ¹³C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 298 [M]⁺, C₁₆ H₁₀ O₆.

CF-4 (+) Catechin
Elution of the column with ethyl acetate: methanol (98:2) mixture gave a solid mass which yielded pale yellow colored product after crystallization from methanol, 83 mg, Rf value 0.22, melting point 134-136 °C, UV λ_max 277nm and 220 nm, IR γ_max (KBr) 3400-2600 (broad), 1618, 1520, 1470, 1380, 1280, 1240, 1150, 1120, 1080, 1020, 820, 744 (brs, 1H, Ar-H-7), 7.68 (brs, 1H, Ar-H-5), 7.32 (d, J=1.8 Hz, 1H, Ar-H-2), 8.11 (d, J=1.6 Hz, 1H, Ar-H-2), 11.29 and 12.26 (s, 2 x OH). For ¹³C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 298 [M]⁺, C₁₆ H₁₀ O₆. 
16.10 Hz], 2.58 [H-4e, dd, J(H-4e, H-3a) 5.50 Hz, J(H-4e, H-4a) 16.10 Hz], 5.42 [H-6, d, J(H-6, H-8) 2.3 Hz], 5.71 [H-8, d, J(H-8, H-6) 2.3 Hz], 6.66 [H-2’, d, J(H-2’, H-6’) 1.95 Hz], 6.79 [H-5’, d, J(H-5’, H-6’) 8.07 Hz], 6.63 [H-6’, dd, J(H-6’, H-2’) 1.94 Hz, J(H-6’, H-5’) 8.19 Hz] and 8.00 (phenolic protons, m), For $^{13}$C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 290 [M]$^+$, $^{1}$H NMR (DMSO) δ 7.9 [H-4, s], 7.62 [H-7, d, (J= 8.3)], 7.12 [H-8, d, (J= 8.3)]. 12.69 [1-OH, s], 2.12 [2-CH3, s], 3.82 [5-OCH3, s], 3.68 [6-OCH3, s]. For $^{13}$C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 315.4 [M+H]$^+$, $^{1}$H NMR (DMSO) δ 7.9 [H-4, s], 7.62 [H-7, d, (J= 8.3)], 7.12 [H-8, d, (J= 8.3)], 12.69 [1-OH, s], 2.12 [2-CH3, s], 3.82 [5-OCH3, s], 3.68 [6-OCH3, s]. For $^{13}$C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 315.4 [M+H]$^+$, $^{1}$H NMR (DMSO) δ 7.9 [H-4, s], 7.62 [H-7, d, (J= 8.3)], 7.12 [H-8, d, (J= 8.3)]. 12.69 [1-OH, s], 2.12 [2-CH3, s], 3.82 [5-OCH3, s], 3.68 [6-OCH3, s]. For $^{13}$C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 315.4 [M+H]$^+$, $^{1}$H NMR (DMSO) δ 7.9 [H-4, s], 7.62 [H-7, d, (J= 8.3)], 7.12 [H-8, d, (J= 8.3)]. 12.69 [1-OH, s], 2.12 [2-CH3, s], 3.82 [5-OCH3, s], 3.68 [6-OCH3, s]. For $^{13}$C NMR spectroscopic data, see Table 1. ESI MS (+ve mode) m/z (rel. Int.) 315.4 [M+H]$^+$, $^{1}$H NMR (DMSO) δ 7.9 [H-4, s], 7.62 [H-7, d, (J= 8.3)], 7.12 [H-8, d, (J= 8.3)]. 12.69 [1-OH, s], 2.12 [2-CH3, s], 3.82 [5-OCH3, s], 3.68 [6-OCH3, s].

REFERENCES


