ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article *Article ID: 401*

Isolation and characterization of two anthraquinone derivatives from the roots of *Cassia fistula* Linn.

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Article info

Article History:
Received 4 August 2015
Accepted 24 August 2015

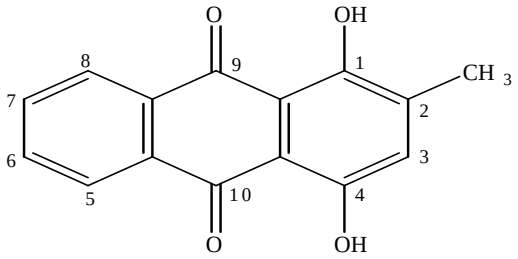
Keywords:
Cassia fistula, Anthraquinone, Roots

Abstract

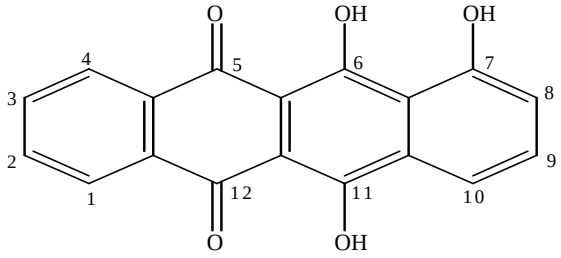
Cassia fistula is one of the important medicinal plants in Bangladesh. Two anthraquinone derivatives: 1,4-dihydroxy-2-methylanthraquinone (**1**) and 6,7,11-trihydroxynaphthacene-5,12-dione (**2**) along with β-sitosterol were isolated from the chloroform extract of the roots of *Cassia fistula*. The compound (**1**) and (**2**) have been isolated for the first time from this plant. The chemical structures of the isolated compounds were established by different spectroscopic analysis.

1. INTRODUCTION

Cassia fistula Linn. (Family: Caesalpiniaceae) commonly known as Sonalu, Bandarlathi in Bangla and popularly called 'Indian Laburnum' in English has been extensively used in Ayurvedic system of medicine for various ailments. It is a native of India, the Amazon and Sri Lanka, and extensively diffused in various countries including Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental plant and widely cultivated as an ornamental tree for its beautiful bunches of yellow flower[1].The whole plant possesses medicinal properties useful in the treatment of skin diseases, rheumatism, anorexia, jaundice and has antitumour, antiseptic, antimicrobial and antifungal activity[2-3]. Stem bark is hypoglycemic, antiviral and anticancer. Pulp of the fruit is laxative, useful in chest and heart diseases and is extremely applied in gout, rheumatism, snake-bite and ringworm. Leaves possess purgative properties and are used in ringworm. Root is prescribed as a tonic, astringent, febrifuge, strong purgative and is useful in fever and heart diseases. The roots are also used in joint pain, migraine and blood dysentery[4-9]. The chemical components of this plant have been extensively investigated. Fatty acids, alcohols, flavonoids, anthraquinones, steroids, glycosides and triterpenoids have been reported from its flowers[10], leaves[11-12], fruits[13-14], stem bark[15-16], heartwood[17], pods[18], seeds[19-20] and roots[21-25].We here in report the isolation and characterization of 1,4-dihydroxy-2-methylanthraquinone (**1**) and 6,7,11-trihydroxynaphthacene-5,12-dione(**2**)from the chloroform extract of the roots of *Cassia fistula* (Figure-1).



1,4-Dihydroxy-2-methylanthraquinone (**1**)



6,7,11-Trihydroxynaphthacene-5,12-dione (**2**)

Figure-1: Structure of the isolated compounds

2. EXPERIMENTAL

Melting points were recorded by thin disc method on fisher john's electro thermal melting point apparatus. UV spectra were recorded in methanol on a Shimadzu UV-Visible spectrophotometer. IR spectra were recorded on a SHIMADZU FTIR spectrometer asKBr disc. ^{1}H NMR spectra were recorded in $CDCl_3$ usingBruker WH 400 MHz NMR spectrometer. Mass spectra were measured on Finnigan Mat SSQ 710 spectrometer with ionization induced by electron impact at 70 eV. Column chromatography was carried out using silica gel 40 (70-230 mesh, E. Merck). Thin layer chromatography was carried out on TLC plastic sheets pre-coated with silica gel 60 F_{254} (E. Merck).

2.1 Extraction of Crude Sample from the Roots of Cassia fistula Linn.

The roots of the matured Cassia fistula were collected from the campus of Jahangir nagar University, Bangladesh. The plant was identified by Bangladesh National Herbarium at Dhaka and a voucher specimen (specimen no. 41562) was deposited at the herbarium.

The dried powered plant materials (1kg) were extracted with chloroform at room temperature. At first, the plants were cut into small pieces, dried well under the shade and then powdered by using a grinder machine. The plant materials were soaked in chloroform at room temperature for 72 hrs. The extraction solvent was then removed by filtration and fresh distilled solvent was added to the plant material. The extraction process was repeated three times and the combined filtrate was evaporated and dried completely on rotary evaporator under reduced pressure to get brownish crude chloroform extracted (3g).

2.2 Isolation of the Compounds from Crude Chloroform Extracts

The chloroform extract (3g) was fractionated on a silica gel column eluted with Petroleum Ether, Petroleum Ether-chloroform, chloroform-ethyl acetate and ethyl acetate-methanol in gradient manner. The collections from the column were divided into twelve fractionsaccording to their TLC behavior.

Fraction number 1(577 mg) of the column was rechromatographed to separate the compound (**1**) by using a medium sized silica gel column. The column was eluted successively with Petroleum Ether and pet. Ether-chloroform solvent systems.The collections from the column were divided into five fractionsaccording to their TLC behavior andthe compound (**1**) [11.6 mg] found in pure form from the third fraction of the column. Compound (**2**) was separated from the column fraction 2 (472 mg) of the crude chloroform extract using further column chromatographic method eluted with pet. Ether-chloroform solvent gradient and found the compound (25 mg) in pure form.

2.3 1, 4-Dihydroxy-2-methylanthraquinone (1)

Orange crystal (11.6 mg); mp174-175°C; **UV**(MeOH)λ_{max}432, 290, 280, 256 nm; **IR** (KBr disc) υ 3442 (O-H), 3064, 2958, 2859, 1627 (C=O), 1561 (C=C, aromatic), 1462, 1382, 1272, 1205 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 12.11 (1H, s, -OH), 12.00 (1H, s, -OH), 7.82 (1H, dd, *J*= 1.2& 7.5 Hz, H-5 or H-8), 7.65 (2H, m, H-6 & H-7), 7.29 (1H, dd, *J*= 1.2& 8.1 Hz, H-5 or H-8), 7.09 (1H, s, H-3), 2.46 (3H, s, -CH_3); **^{13}C NMR**($CDCl_3$) δ 192.5 (C=O), 182.0 C=O), 162.7 (C-OH), 162.4 (C-OH), 149.3, 136.9, 133.7, 133.3, 124.5, 124.3, 121.3, 119.9, 115.9, 113.8, 22.2 (CH_3); **MS** m/z 254 (M^+), 239, 237, 226, 198, 183, 181,132, 122, 104, 76.

2.4 6, 7, 11-Trihydroxynaphthacene-5, 12-dione (2)

Orange solid (25 mg); mp>300°C; **UV**(MeOH)λ_{max}433, 291, 281, 261, 234 nm; **IR** (KBr disc) υ 3441 (O-H), 3066, 1628 (C=O), 1600 (C=C, aromatic), 1270, 1206, 1160 cm^{-1}; **^{1}H NMR** ($CDCl_3$) δ 12.10 (1H, s, -OH), 12.00 (1H, s, -OH), 7.81(1H, br. s, -OH), 7.72 (1H, dd, *J* = 1.3 &8.4 Hz, H-1 or H-4), 7.67 (2H, m, H-2 & H-3), 7.52 (1H, dd, *J* = 1.6 & 8.0 Hz, H-8) 7.29 (1H, dd, *J* = 1.3& 8.5 Hz, H-1 or H-4), 7.10 (2H, m, H-9 & H-10); **^{13}C NMR**($CDCl_3$) δ 192.6 (C=O), 182.0 C=O), 167.7 (C-OH), 162.8 (C-OH), 162.5 (C-OH), 149.3, 136.9, 133.7, 133.4, 132.5, 130.8, 128.8, 124.5, 124.3, 121.3, 119.9, 115.9, 113.8; **MS** m/z306 (M^+), 289, 278, 250, 233, 174, 132, 104, 76.

3. RESULTS AND DISCUSSION

The dried powdered roots of *Cassia fistula* were extracted with chloroform at room temperature. Compound (**1**) and (**2**) were isolated and purified from the chloroform extract by repeated column chromatographic separation (Figure-1).

The mass spectrum of the compound (**1**) showed a molecular ion peak at m/z 254 which is corresponding to the molecular formula $C_{15}H_{10}O_4$. The UV spectrum of the compound showed the absorption bands at 432, 290, 280, 256 nm indicated the presence of large conjugation and chromophoric groups in the molecule.The infrared spectrum showed the absorption bands at 3442 and 3064 cm^{-1} due to O-H and aromatic C-H stretching vibration, respectively. It also showed sharp absorption band at 1627 cm^{-1} due to >C=O stretching vibration chelated to the hydroxyl group.

The two singlets at δ 12.11 and 12.00 in the ^{1}H-NMR spectrum indicated the presence of two phenolic -OH groups. The chemical shift value of these two hydroxyl protons confirmed that the hydroxyl groups are attached to C-1 and C-4 those are chelated with two carbonyl groups of the anthraquinone moiety.The ^{1}H NMR spectrum showed three peaks at δ 7.82 (1H, dd, *J*= 1.2& 7.5 Hz, H-5 or H-8), 7.65 (2H, m, H-6 & H-7) and 7.29 (1H, dd, *J*= 1.2&8.1 Hz, H-5 or H-8)for the aromatic protons. All these protons are situated in the same ring proved by ^{1}H-^{1}H COSY spectrum which showed the correlations between the peaks atδ 7.82 (H-5 or H-8) & 7.65 (H-6, H-7),δ 7.29 (H-5 or H-8) & 7.65 (H-6, H-7)and δ 7.65 (H-6, H-7) & 7.29/7.82 (H-5 or H-8). It was indicated that the ring has no other substitutions except carbonyl groups which was further supported by the fragment ion at m/z 132 in the mass spectrum. The aromatic proton attached to C-3 indicated by the one proton singlet at δ 7.09.The three proton singlet at δ 2.46 showed the presence of methyl group attached to the position C-2.

The ^{13}C-NMR spectrumclearlyindicated the presence of fifteen carbons in the molecule. It showed two peaks at δ 192.5 and 182.0 for two carbonyl carbon of anthraquinone skeleton. The peaks at δ 162.7 and 162.4 indicated C-1 and C-4 those are attached with hydroxyl groups. The only methyl carbon indicated by the peak at δ 22.2. Moreover the fragment ions at m/z 226 and 198 in the mass spectrum confirmed that the compound (**1**) is an anthraquinone derivative.

Based on all spectral data and melting point[26-27], it was confirmed that the compound (**1**) is 1,4-dihydroxy-2-methylanthraquinone.

The mass spectrum of the compound (**2**) showed a molecular ion peak at m/z 306 which is corresponding to the molecular formula $C_{18}H_{10}O_5$. The UV spectrum of the compound showed the absorption bands at 433, 291, 281, 261, 234 nm indicated the presence of large conjugation and chromophoric groups in the molecule. The bands are very similar to the bands of compound (**1**) indicated that both the compounds contain same structural moety.TheIR spectrum of the compound (**2**) showed the absorption bands at 3441 and 3066 cm^{-1} due to O-H and aromatic C-H stretching vibration, respectively. It also showed sharp absorption band at 1628 cm^{-1} due to >C=O stretching vibration chelated to the hydroxyl group.

The three singlets at δ 12.10, 12.00 and 7.81 in the ^{1}H-NMR spectrum indicated the presence of three phenolic -OH groups which is strongly supported by the three signals at δ 167.7, 162.8 and 162.5 in the ^{13}C NMR spectrum. The chemical shift value of the three hydroxyl protons confirmed that two of these (δ 12.10 & 12.00) are attached to C-6 and C-11which are chelated with two carbonyl groups.The three peaks at δ 7.72 (1H, dd, *J* = 1.3& 8.4 Hz, H-1 or H-4), 7.67 (2H, m, H-6 & H-7) and 7.29 (1H, dd, *J* = 1.3&8.5 Hz, H-1 or H-4) indicatedfour aromatic protonsthose are situated in the same ring proved by ^{1}H-^{1}H COSY spectrum.It was indicated that the ring has no other substitutions except carbonyl groups which was further supported by the fragment ion at m/z 132 in the mass spectrum.The ^{1}H-^{1}H correlations showed in the COSY spectrum between the peaks atδ 7.72 (H-1 or H-4) & 7.67 (H-2, H-3),δ 7.29 (H-1 or H-4) & 7.67 (H-2, H-3),δ 7.67 (H-2, H-3) &7.29/7.72 (H-1 or H-4), 7.52 (H-8) & 7.10 (H-9, H-10). These correlations also indicated that the three aromatic protons (H-8, H-9 & H-10) showed by the peaks at δ 7.52 (1H, dd, *J* = 1.6 & 8.0 Hz, H-8) and 7.10 (2H, m, H-9 & H-10) are present in another ring.

The ^{13}C-NMR spectrumclearlyindicated the presence of eighteen carbons in the molecule. It showed two peaks at δ 192.6 and 182.0 for two carbonyl carbonsand other sixteen peaks in the region at δ 113.8 to 167.7 for aromatic carbons.

Based on all spectroscopic data analysis[28]and comparison with the spectral data of compound (**1**), it was confirmed that the compound (**2**) is 6, 7, 11-trihydroxynaphthacene-5, 12-Dione.

4. Conclusion

Isolation of two anthraquinone derivatives from the roots of *Cassia fistula* has been reported in the present study. Literature study of this plant showed that not enough phytochemical studies on the roots of this plant have been done yet. So, there is a scope for the scientist to do extensive phytochemical works on the roots as well as other parts of the plant *Cassia fistula*.

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