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A Flavone from the Leaves of *Cassia Alata*

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Abstract

Studies were carried out on the leaves of *Cassia alata*. A flavone 3,5,7,4□-tetrahydroxy flavone was isolated as a new source from the leaves of *Cassia alata* with the help of column and thin layer chromatography using a gradient of organic solvents with increasing polarity. The compound was characterized on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry.

Introduction

Cassia Linn. includes a large tropical genus of 580 species of herbs, shrubs and trees of the leguminosae family, including *Cassia alata* and many of them are found in Indo-Pak-Bangladesh subcontinent.[1,2] *Cassia alata* Linn. is a small tree generally grown in the gardens and not far away from human dwellings.[3] Its use as medicine, especially in the eradication of Herpescircinatus is confirmed by Mekenna *et al.*[4]

Earlier investigations on *Cassia alata* reported the presence of only hydroxymethylanthraquinones (unidentified) and chrysophanic acid as isolated compounds.[5] Hauptmann and co-workers[6] isolated rhein (an antibiotic) and its reduced form with other anthraquinone derivatives which they could not identify. Though some anthraquinones were isolated and characterized by some scientists,[7,8,9] none reported on the isolation of flavonoids. The present work had, therefore, been taken up to make a complete chemical investigation on the plant *Cassia alata*. A flavone was isolated from the plant as a new source. The structure of the flavone was elucidated by spectroscopic techniques.

Materials and Methods

A Reichart micro melting point apparatus was used for recording the melting point. UV spectra (MeOH) was recorded on a Shimadzu UV-240 spectrophotometer, IR Spectra (KBr) on a Shimadzu IR-460 instrument, ^{1}H-NMR spectra(CD_3OD) on a Bruker AM-500 FT NMR spectrometer (500 MHz) using TMS as internal standard, ^{13}C-NMR spectra

(CD_3OD) on a Bruker AM-500 FT NMR spectrometers (100 MHz) and Mass spectra on a Varian-MAT 112S spectrometer. Electron Impact (EI), Peak Matching experiments were performed on a MAT-312A mass spectrometer.

Fresh leaves were collected from the plants grown in the adjoining areas of BCSIR Laboratories, Rajshahi campus during August-September period. The leaves were washed with water to remove extraneous materials and then dried in shade. Care was taken to avoid exposure to sunlight. The dried material was crushed to powder.

The air-dried *Cassia alata* leaf powder (6.6 Kg) was soaked in 80 % ethanol for a week. The ethanolic extract was then filtered and the solvent was removed under reduced pressure to obtain a viscous residue (494 g). The crude residue was then defatted with n-henane. The defatted mass was dried under reduced pressure to give a residue (162g).

The defatted extract was then treated with water, shaken well to resolve into water soluble and water insoluble parts. The water soluble part was extracted with ethyl acetate. The ethyl acetate soluble part was chromatographed over a silica gel (70-230 mash) column and successively eluted using n-hexane and ethyl acetate. Elution of the column with n-hexane : ethyl acetate (40:60 v/v) afforded a compound designated as compound-**1** along with minor impurities.

Purification of the compound by preparative thin layer chromatography (PTLC)

The compound [1] with some impurities was applied to a PTLC card of silica gel $^{60}GF_{254}$ (thickness 0.1mm) and eluted with n-hexane : ethyl acetate (4:1 v/v). A distinct single band (R_f 0.49) was observed on the PTLC card. The band was collected and washed out with ethyl acetate to obtain a yellow solid (compound **1**, 12.5mg, m.p 276-278^O C, R_f = 0.49).

Spectroscopic analysis of compound 1

UVλ_{max} (MeOH)nm : 366, 323, 266, 203, 195 IRυ_{max} (KBr)cm^{-1} : 3490(O - H), 2900 (C - H), 1670(C = O), 1570 -1480(C = C)

EIMS $^m/_z$ (rel. int%) : 286(100), 257(9), 241(1), 229(8), 193(0.85), 184(1), 143(10), 121(20), 105(3), 93(5), 69(8).

Peak matching $^m/_z$(formula) : 286.41360 ($C_{15}H_{10}O_6$).

^{1}H-NMR (500MHz) δ_{TMS} : (CD_3OD) :

δ6.28 [H-6, 1H, d, $J_{(H\text{-}6,\ H\text{-}8)}$ 2.1 Hz]

δ6.54 [H-4, 1H, d, $J_{(H\text{-}8,\ H\text{-}6)}$ 2.1Hz]

δ8.09 [H-2′, H-6′, 2H, dd, $J_{(H\text{-}2',\ H\text{-}3')}$ 9.0Hz, $J_{(H\text{-}2',\ H\text{-}6')}$ 2.1 Hz]

δ7.01 [H-3′, H-5′, 2H, dd, $J_{(H\text{-}5',\ H\text{-}6')}$ 9.0Hz, $J_{(H\text{-}3',\ H\text{-}5')}$ 2.1 Hz]

^{13}C-NMR (CD_3OD, 100MHz) : 147.9, 137.0, 177.2, 160.4, 99.2, 162.4, 94.4, 158.1,104.5, 123.7, 130.6, 115.2, 165.4, 116.2, 130.6 (Table I).

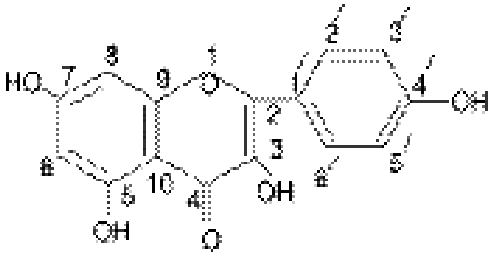
The absorption at 3490 and 1670 cm^{-1} in the IR spectrum (KBr) of the compound were indicative of hydroxyl and carboxyl functions respectively. The IR spectrum also

Table I. ^{13}C-NMR (CD_3OD, 100 MHz) chemical shifts of 3,5,7,4′-tetrahydroxy flavone.

C. No.	Multiplicity (DEPT)	^{13}C-NMR (δ)	^{1}H-NMR (δ)	$^{1}J_{HH}$(Hz)
C-2	C	147.9		
C-3	C	137.0		
C-4	C	177.2		
C-5	C	160.4		
C-6	CH	99.2	6.28	d, *J* = 2.1
C-7	C	162.4		
C-8	CH	94.4	6.54	d, *J* = 2.1
C-9	C	158.1		
C-10	C	104.5		
C-1′	C	123.7		
C-2′	CH	130.6	8.09	dd, *J* = 9.0, 2.1
C-3′	CH	116.2	7.01	dd, *J* = 9.0, 2.1
C-4′	C	165.4		
C-5′	CH	116.2	7.01	dd, *J* = 9.0, 2.1
C-6′	CH	130.6	8.09	dd, *J* = 9.0, 2.1

Results and Discussion

The ethyl acetate triturate of the ethanolic extract of *Cassia alata* leaves aforded compound **1** after purification by preparative TLC. Compound **1** was suggested to be a flavonoid as it exhibited light yellow appearance on silica gel card and deep yellow colour when sprayed with ceric sulphate reagent.



3,5,7,4′ - tetrahydroxy flavone (1)

showed absorption at 2900 and 1570-1480cm^{-1} due to CH and C=C functions. The EI mass spectrum showed the molecular ion as well as base peak at m/z 286.

The moleular formula was established with the help of ^{13}C-NMR, ^{1}H-NMR and peak matching experiments as $C_{15}H_{10}O_6$ corresponding to the mass $^{m}/_{z}$ 286.41360. 15 signals appeared in the broad-band spectrum of copound-**1**, which were resolved with the help of DEPT experiment into six methine and nine quaternary carbons.

The ^{1}H-NMR spectrum of compound **1** displayed a doublet at δ6.28 with coupling constant 2.1 *Hz* for H-6 proton and another doublet resonated at δ6.54 having coupling constant 2.1 *Hz* for H-8 proton. Two protons at 2′ and 6′ positions gave a double doublet at δ8.09 having coupling constant 9.0 and 2.1 *Hz* respectively. Two protons at 3′ and 5′ positions gave another double doublet at δ7.01 having coupling constants 9.0 and 2.1 *Hz*.

From the spectral informations, it became apparent that the compound 1 belonged to the flavone series and was characterized as 3,5,7,4′ tetrahydroxy flavone.

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