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STUDIES ON THE ISOLATION OF 2, 5, 7, 4'- TETRAHYDROXY ISOFLAVONE FROM THE LEAVES OF CASSIA ALATA

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ABSTRACT

Studies were carried out on the leaves of *Cassia alata*. An isoflavone 2, 5, 7, 4'-tetrahydroxy isoflavone was isolated for the first time from the leaves of *Cassia alata* with the help of column and thin layer chromatography by using a gradient mixture of organic solvents with increasing polarity. The compound was characterized on the basis of UV, IR, and ¹H-NMR, ¹³C-NMR and Mass spectrometry and confirmed the compound belonged to the isoflavone series.

KEY WORDS

Cassia alata, Isoflavone, ¹HNMR, ¹³C-NMR and Mass Spectrometry.

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INTRODUCTION

Cassia alata is an indigenous flowering plant which grows almost in all the districts of Bangladesh. The plant is also found to grow in the lower Bengal, Western Peninsula, Burma and Malacca, very probably introduced into India, as it does not appear to occur far away from human dwellings. The tree is often cultivated by the Indians for the sake of its leaves which are held in high esteem as a local application in skin diseases. A belief in their powers of this character prevails also in the West Indies, Brazil, Mauritania, Java and other tropical countries.

According to world Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care needs¹. The common view in the society and the medicinal community is that plant based products are healthier, safer and more reliable than synthetic products². In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections³. Anti-inflammatory activities of heat treated *Cassia alata* leaf of extract and the flavonoid glycoside isolated from *Cassia alata* were studied by comparing their activities of sun dried *Cassia alata* leaf of extract⁴. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids tannins and phenolic compounds⁵. *Cassia alata*, a large tropical genus, found in different parts of Bangladesh is rich in flavonoids and these flavonoids after extraction and purification may be used in the practice of traditional medicine⁶. The use of medicinal plants for alleviating diseases originated from the activities of the most primitive man of the remote past. The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist worldwide⁷. Extracts from the leaves of *Cassia alata* (L) Roxb. have shown several pharmacological properties such as antimicrobial and antifungal activities, antiseptic, anti-inflammatory and analgesic and anti-hyperglycemic⁸. From the *Cassia alata* plant species both solvent and aqueous extracts were prepared and tested its toxicity against certain bio-molecules and metabolic enzymes which showed very high insecticidal activity⁹. *Cassia alata* has very high medicinal values like antimicrobial property particularly against fungal dermatophytes and traditionally it is used in the treatment of skin infections in man, leaf extract is also credited for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes¹⁰. Thirteen pathogenic fungi (6 human, 3 animal and 4 plant) were used in the fungicidal sensitivity test for the isolated pure compound 5, 3', 4'-trihydroxy, 7-

methoxy isoflavone which showed inhibition against most of the human pathogenic fungi active against *Microsporiumcanis* and moderately active against *Trichophytonmentagrophytes* out of three animal pathogenic fungi and showed moderate activity against *Fusariumoxysporum* var. *lycopersici* and *Fusariumsolania* var. *lycopersici* out of four plant pathogenic fungi¹¹. As *Cassia alata* is assumed to be rich in flavonoids and the flavonoids have important medicinal values, so, the present work had therefore, been taken with a view to isolate the flavonoid compounds, from the plant *Cassia alata* and accordingly a new isoflavone compound was isolated. The structure of the isoflavone compound was elucidated by using spectroscopic techniques and the thorough studies were reported in this paper.

MATERIALS AND METHOD

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, ¹HNMR spectra (CD₃OD) on a Bruker AM-500 FT NMR spectrometer (500 MHz) using TMS as internal standard, ¹³C-NMR spectra (CD₃OD) on a Bruker AM-500 FT spectrometers 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 to 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-hexane. The solvent was removed under reduced pressure to yield 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 chromatographic over a silica gel (70-230 mash) column and successively eluted with increasing polarities of ethyl acetate and n-hexane. 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 ⁶⁰GF₂₅₄ (thickness 0.1mm) and eluted with n-hexane: ethyl acetate (8:2 v/v). A distinct single band (R_f 0.60) was observed on the PTLC card. The band was collected and washed out with ethyl acetate to obtain a light yellow solid (compound 1, 11.2 mg, m.p 272-274°C, R_F = 0.60).

Spectroscopic analysis of compound 1

UV λ_{max} (MeOH) nm: 287, 243, 228, 204, 198, 193 (Figure No.1).

IR ν_{max} (KBr) cm⁻¹: 3490(O-H), 2980(C-H), 1650(C=O), 1600 (C=C).

EIMS m/z (rel. int %): 286(100), 257(3), 244(1), 229(1), 194(4), 165(2), 153(33), 134(25), 106(11), 93(7), 69(20) (Figure No.2).

Peak matching m/z (formula): 286.06261 (C₁₅H₁₀O₆).

¹H-NMR (500 MHz) δ_{TMS} (CD₃OD) (Figure No.3).

δ 6.22 [H-6, 1 H, d, J_(H-6, H-8) 2.1 Hz]

δ 6.51 [H-8, 1 H, d, J_(H-8, H-6) 2.1 Hz]

δ 7.13 [H-2', H-6', 2H, dd, J_(H-2', H-3') 8.95 Hz, J_(H-2', H-6') 2.1Hz]

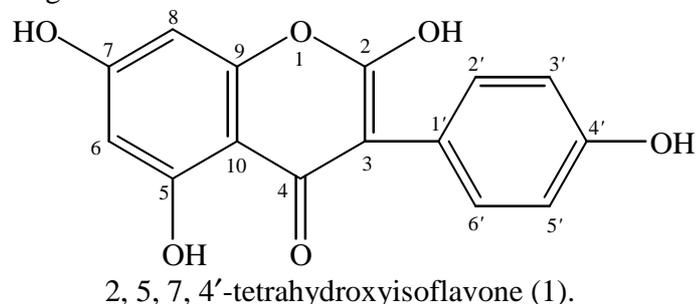
δ 6.86 [H-3', H-5', 2H, dd, J_(H-5', H-6') 8.95 Hz, J_(H-3', H-5') 2.1Hz]

¹³C-NMR (CD₃OD, 100 MHz): 143.3, 129.7, 183.2, 161.5, 99.5, 164.1, 94.1, 156.2, 102.0, 122.4, 122.0, 116.7, 168.1, 116.7, 122.0 (Figure No.4).

RESULTS AND DISCUSSION

The ethyl acetate triturate of the ethanolic extract of *Cassia alata* leaves yielded compound 1 as a light yellow solid after purification by preparative TLC.

Compound 1 may be a flavonoid as it exhibited light yellow appearance on silica gel card and deep yellow color when sprayed with ceric sulphate reagent.



The IR spectrum (KBr) exhibited absorption bands at 1650 and 3490 cm⁻¹, which revealed the presence of carbonyl and hydroxyl functions in the molecule. The IR spectrum also showed absorption at 2980 and 1600 cm⁻¹ due to C-H and C=C functions. The molecular mass was established with the help of E1 mass spectrum as 286. The molecular formula was established with the help of ¹H-NMR, ¹³C-NMR and peak matching experiments as C₁₅H₁₀O₆ corresponding to the mass m/z 286.06261. The broad band of ¹³C-NMR spectrum of compound 1 showed 15 signals including six methine and nine quaternary carbons. Table No.1: ¹³C-NMR (CD₃OD, 100MHz), chemical shifts of 2, 5, 7; 4'-tetrahydroxyisoflavone (1) is given in Table No.1. The multiplicities were determined with the help of DEPT experiment.

The ¹H-NMR spectrum of compound 1 displayed a doublet at δ 6.22 with a coupling constant 2.1 Hz for H-6 proton and another doublet at δ 6.51 having coupling constant 2.1 Hz for H-8 proton. Two protons at 2' and 6' positions gave a double-doublet at δ 7.13 having coupling constants 8.95 and 2.1 Hz respectively. Two protons at 3' and 5' positions gave another double-doublet at δ 6.86 having coupling constants 8.95 and 2.1 Hz, respectively.

Table No.1: ¹³C-NMR (CD₃OD, 100MHz), chemical shifts of 2, 5, 7, 4'-tetrahydroxyisoflavone (1)

| S.No | Compound No. | ¹³ C-NMR (δ) | Multiplicity (DEPT) | ¹ H-NMR (δ) | ¹ J _{HH} (Hz) |
|------|--------------|-------------------------|---------------------|------------------------|-----------------------------------|
| 1 | C-2 | C | 143.3 | --- | --- |
| 2 | C-3 | C | 129.7 | --- | --- |
| 3 | C-4 | C | 183.2 | --- | --- |
| 4 | C-5 | C | 161.5 | --- | --- |
| 5 | C-6 | CH | 99.5 | 6.22 | d, J=2.1 |
| 6 | C-7 | C | 164.1 | --- | --- |
| 7 | C-8 | CH | 94.1 | 6.51 | d, J=2.1 |
| 8 | C-9 | C | 156.2 | --- | --- |
| 9 | C-10 | C | 102.0 | --- | --- |
| 10 | C-1' | C | 124.4 | --- | --- |
| 11 | C-2' | CH | 122.0 | 7.13 | dd, J=8.95, 5.1 |
| 12 | C-3' | CH | 116.7 | 6.86 | dd, J=8.95, 5.1 |
| 13 | C-4' | C | 168.1 | --- | --- |
| 14 | C-5' | CH | 116.7 | 6.86 | dd, J=8.95, 2.1 |
| 15 | C-6' | CH | 122.0 | 7.13 | dd, J=8.95, 2.1 |



Figure No.1: UV spectra of 2, 5, 7, 4'-tetrahydroxyisoflavone (1)

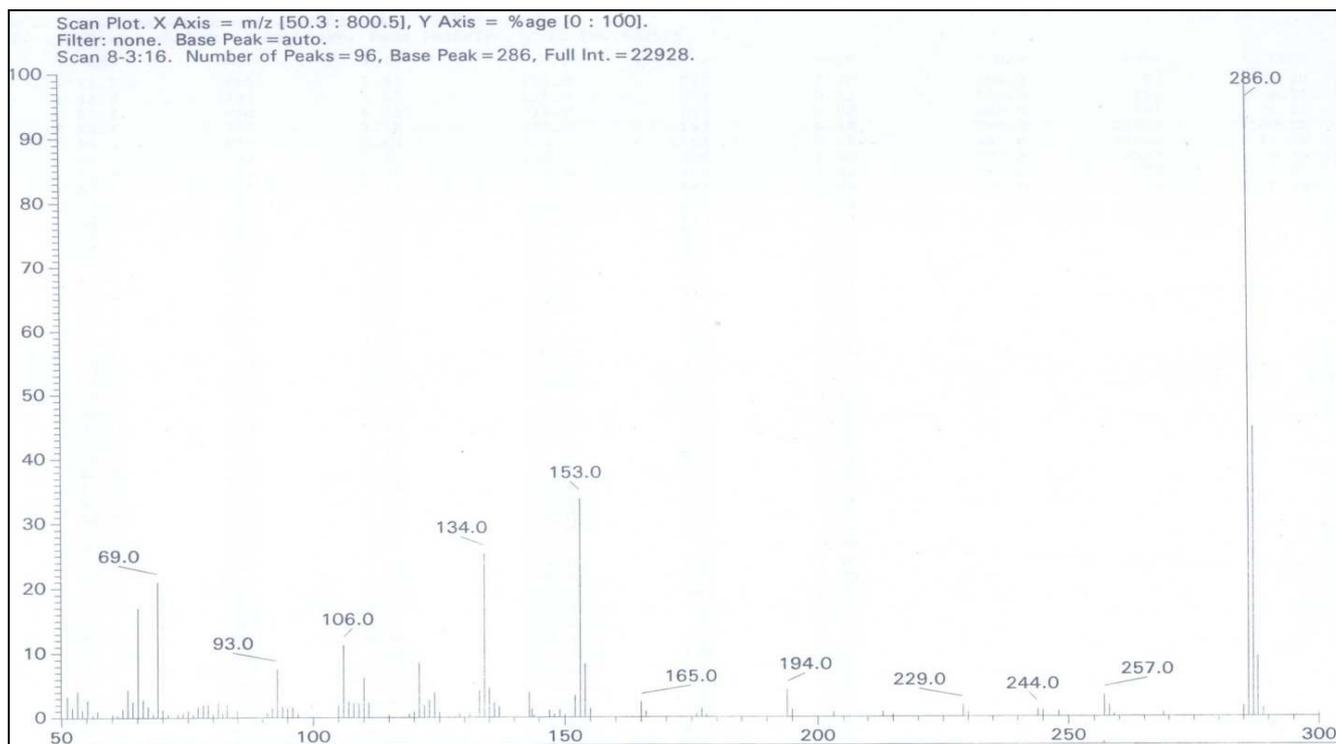


Figure No.2: Mass spectra of 2, 5, 7, 4'-tetrahydroxyisoflavone (1)

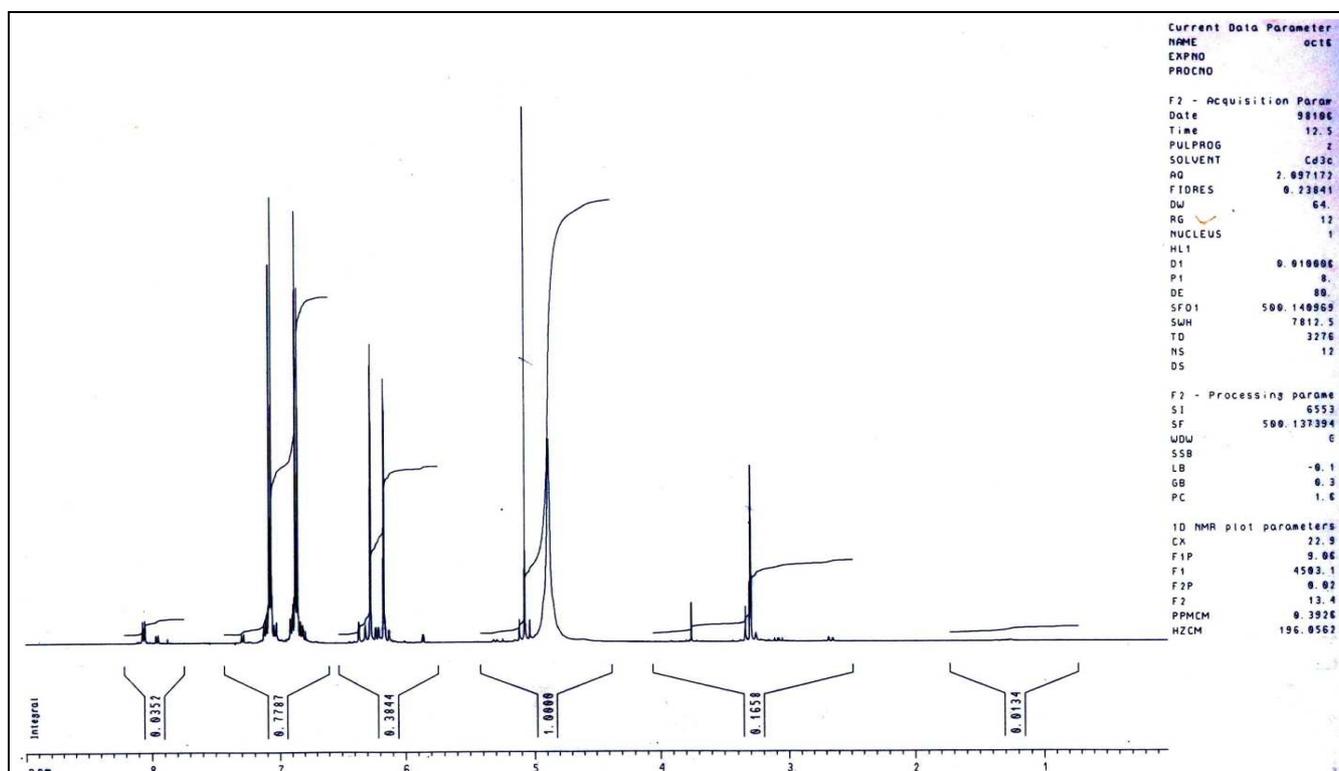


Figure No.3: ¹H-NMR spectra of 2, 5, 7, 4'-tetrahydroxyisoflavone (1)

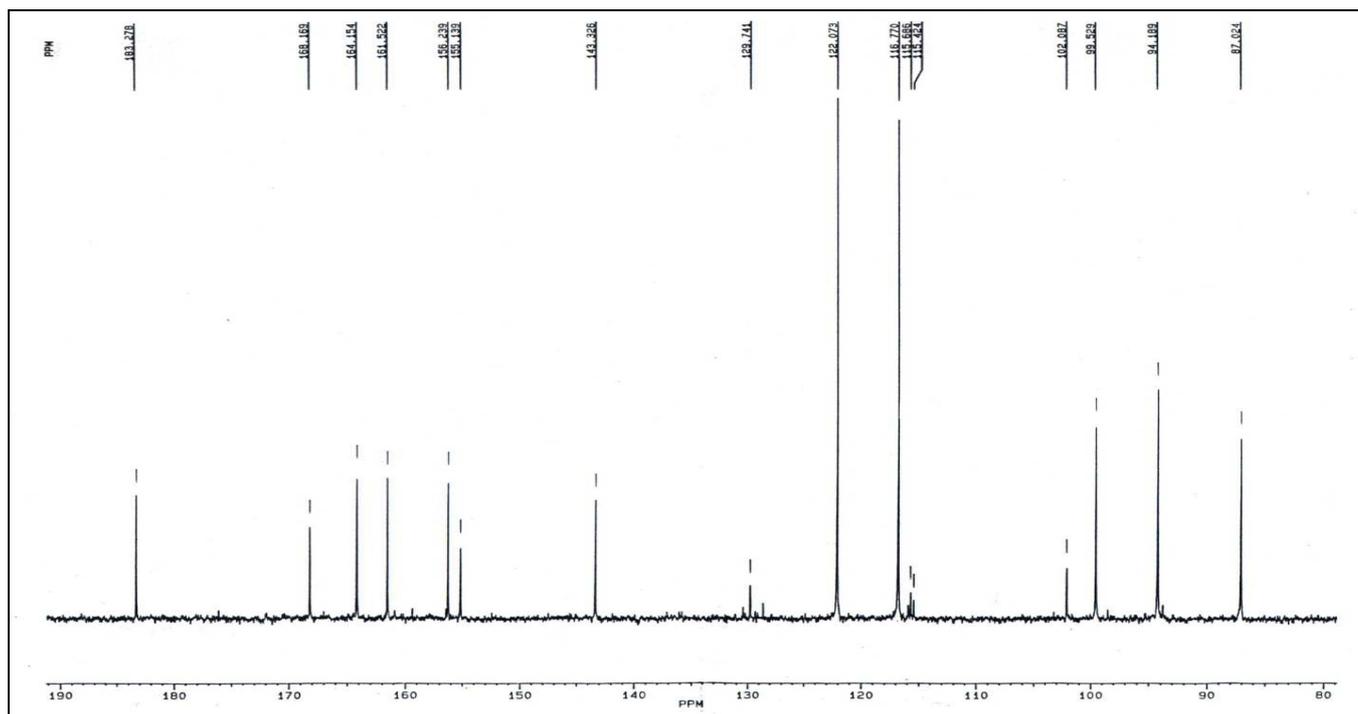


Figure No.4: ^{13}C -NMR of 2, 5, 7, 4'-tetrahydroxyisoflavone (1)

CONCLUSION

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientists worldwide. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. One of the most important bioactive compounds of plants is flavonoids. The flavonoid compound which had been isolated from *Cassia alata* belonged to the isoflavone series and was characterized as 2, 5, 7, 4'-tetrahydroxyisoflavone on the basis of the spectral evidences. As the flavonoids have bioactive compounds, so the isoflavone compound isolated from *Cassia alata* demands the investigation of its anti-microbial antifungal, antibacterial and citotoxic activities. It is assumed that these investigations may open up a new era in the public health sector of Bangladesh in particular and the world as a whole in protecting the people from the adverse effects of different infectious diseases.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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