



## ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENTS FROM *ACACIA LEUCOPHLOEA* FLOWERS (ROXB) WILLD

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### ABSTRACT

The study of the chemical constituents of the flowers of *Acacia leucophloea* Roxb Willd has resulted in the characterization of three compounds which were identified as gallic acid (1), ethyl gallate (2) and naringenin (3). These phenolic compounds were isolated for the first time from *A.leucophloea* flowers. The structure elucidation of the isolated compounds was established using chemicals and spectroscopic method of analysis including UV, MS, <sup>1</sup>H and <sup>13</sup>C NMR

**Keywords:** *Acacia leucophloea*, Fabaceae and phenolic compounds.

### INTRODUCTION

*Acacia* (Family Fabaceae) is a cosmopolitan genus containing in excess 1350 species<sup>1</sup>. *Acacia leucophloea* also called safed kikar is a moderate sized tree and it attains a height of about 20 to 30 ft and a girth of 2 to 3 ft<sup>2</sup> belongs to the family Fabaceae under the subfamily Mimosidae<sup>3</sup>. *Acacia leucophloea* native range through south and south East Asia is non contiguous. Its largest continuous distribution is arid in India through Srilanka, Bangladesh, Burma and much of Thailand<sup>4</sup>. Leaves are bi pinnate with spines. Flowers are in yellow heads arranged in terminal panicles. The bark is light yellowish grey to nearly white and light red inside, smooth and exfoliates in irregular scales. The trees yield a gum which is indigenous medicines. The gum is demulcents and used as an emulsifying agent<sup>5</sup>. New leaves appear in April, and yellowish white flower appear from August to October. The tree is very hardly and stands drought well. It is frost hardly except in young age<sup>6</sup>. Chemical investigation on other *Acacia* species has led to the isolation of alkaloids<sup>7</sup>, chalcones glycosides<sup>8</sup>, diterpenes<sup>9</sup> and flavonoids<sup>10</sup>. The isolation of the triterpenes acacigenin B<sup>11</sup>, lupeol, lupenone, lupenyl palmitate and lupenyl cinnamate<sup>12</sup> have also been reported. This genus contains variety of bioactive components which are responsible for numerous biological and pharmacological properties like hypoglycemic, anti-inflammatory, antibacterials, antiplatelet, aggregatory, antihypertensive, analgesic, anticancer and antiatherosclerotic due to their strong antioxidant and free radical scavenging activities<sup>13</sup>. There is no report in the literature regarding the isolation and characterization of phytoconstituents from the flower of this plant and hence forth in the present study the investigations were carried out.

### MATERIALS AND METHOD

#### General

NMR experiments were performed on a Bruker AM X 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in <sup>1</sup>H NMR and 100 and 125 MHz in <sup>13</sup>C NMR. Chemical shifts are given in  $\delta$  values (ppm) using tetramethyl silane as the internal standard and DMSO -d<sub>6</sub> as solvent at room temperature. UV spectral data was measured on Shimadzu 240 spectrophotometer in Methanol. Compounds were visualized by exposure to UV light (254

nm, 366 nm) before and after spraying with vanillin in sulphuric acid.

#### Plant Material

The fresh flowers of *A. leucophloea* Willd were collected from various domestic places in Thanjavur Tamilnadu, India, (August 2010), which was authenticated by Prof. P. Jayaraman, Botanist, Director, Plant anatomy Research Centre, Tambaram, India. A voucher specimen were deposited in CSMDRIA, Arumbakkam, Chennai, India for the future reference.

#### Extraction and Isolation

Shade dried flowers of *A. leucophloea* Willd (500 g) were defatted with petroleum ether and subjected to sequential maceration with chloroform, ethyl acetate, ethanol and water at room temperature. Ethyl acetate extract after concentration to nearly half volume was evaporated under reduced pressure and dried. The dried ethyl acetate (8 g) extract was subjected to column chromatography on silica gel (100 – 200 mesh). The column was eluted with n-hexane, n-hexane chloroform, chloroform and ethyl acetate and their mixtures of increasing polarity. 10 fractions (500 ml) each were collected. The progress of the chromatographic separation was monitored by performing thin layer chromatography of the fractions after removal of the solvent. Fractions showing similar composition were combined together to obtain three major fractions designated as compound 1 (gallic acid), compound 2 (ethyl gallate) and compound 3 (Naringenin) from fraction number 65 - 85 and 86 - 95 respectively. The separation of the isolates were confirmed by UV light (254 nm, 366 nm) before and after spraying with vanillin in sulphuric acid.

#### Experimental Data

##### Compound I

IR: 3282 cm<sup>-1</sup> (hydroxyl), 1654 cm<sup>-1</sup> ( $\alpha,\beta$ - unsaturated carbonyl), 1610 cm<sup>-1</sup> (aromatic system).

<sup>1</sup>H NMR  $\delta$  ppm: 6.96 (H-2, H-6), 8.32 (3H brs)

<sup>13</sup>C NMR  $\delta$  ppm: 168.62 (carbonyl carbon); 144.09 (C3, C5); 109.64 (C2, C6); 137.67 (C4); 121.45 (C1)

**Compound II**

IR: 3449  $\text{cm}^{-1}$  (hydroxyl group), 1700  $\text{cm}^{-1}$  (carbonyl), 1251  $\text{cm}^{-1}$  (ester).

$^1\text{H}$  NMR  $\delta$  ppm: 7.01 (H-2, H-6 s), 1.34 (t, 3H, T, J- 7.2Hz, terminal methyl), 4.28 (q, 2H, J-7.2Hz, methylene protons)

$^{13}\text{C}$  NMR  $\delta$  ppm: 166.76 (ester carbonyl carbon); 14.31 (terminal methyl); 60.55 (methylene carbon); 121.43 (C-1); 137.19 (C-4); 109.46 (C-2, C-6); 144.77 (C-3 and C-5).

**Compound III**

UV: 325 nm, 260 nm, 221 nm.

IR: 3281  $\text{cm}^{-1}$  (hydroxyl), 1628  $\text{cm}^{-1}$  (carbonyl), 1419  $\text{cm}^{-1}$  (aromatic).

$^1\text{H}$  NMR  $\delta$  ppm: 5.28 (t, H-1, J-12.8 and 2.2 Hz), 6.5 (C-3), 3.06 (dd, H-3, J-17.1 and 2.8Hz), 2.74 (dd, H-3, J-17.1 and 2.2 Hz), 6.30 (H-6, H-8 of ring A, J-3Hz), 6.80 (H-2,D,J-8.3Hz, H-3' and H-5'), 7.32 (H-2, D, J-8.3Hz, H-2' and H-6'), 12.07, 10.07, 8.95 (H-1, brs).

$^{13}\text{C}$  NMR at  $\delta$  values: 166.76 (ester carbonyl group); 14.31 (terminal methyl, Q); 60.55 (methylene carbon); 121.43 (C-1); 137.19 (C-4); 109.46 (C-2, C-6, d); 144.93 (C-3 and C-5).

**RESULT AND DISCUSSION**

The flowers extract of *A. leucophloea* was fractionated by silica gel (60 - 120) column chromatography to give several fractions. The compounds were identified as Gallic acid (1), ethyl gallate (2) and naringenin (3) by comparison of their NMR spectral data with the reported data in the literature [15]. The isolated compounds were characterized with the help of physical characters and spectroscopic analysis (UV, IR and NMR).

**Compound 1**

Elution of the column with chloroform : ethyl acetate (1:1) and concentration of the fraction gave compound 1, mp 250°C; lit mp 250°C; yield 80 mg. It answered ferric chloride test for phenols. The IR spectrum showed the presence of hydroxyl (3282  $\text{cm}^{-1}$ ),  $\alpha$ ,  $\beta$ -unsaturated carbonyl (1654  $\text{cm}^{-1}$ ) and presence of aromatic system (1610  $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum showed a singlet at  $\delta$  6.96 corresponding to 2 proton (H-2 and H-6). The phenolic hydroxyl proton appeared as broad singlet at  $\delta$  8.23 corresponding to 3 protons. The  $^{13}\text{C}$  NMR spectrum showed the carbonyl carbon at  $\delta$  168.62. The C-3 and C-5 carbons appeared at  $\delta$  144.09 as singlet. The two equivalent carbons C-2 and C-6 appeared at  $\delta$  109.46 as doublet. Other two carbons C-4 and C-1 carbon appeared at  $\delta$  137.67 and  $\delta$  121.45 respectively. The compound was identified as gallic acid which was further confirmed by comparison with authentic sample (mp, Co TLC, superimposable IR).

**Characterization of Compound 2**

The elution of the column with 90 % of ethyl acetate in chloroform gave compound 2, melting point 157°C and it answered ferric chloride test for phenols. The IR spectrum showed the presence of hydroxyl group (3449  $\text{cm}^{-1}$ ) carbonyl (1710  $\text{cm}^{-1}$ ) and ester group (1251  $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum showed two equivalent aromatic protons H-2 and H-6 appeared at  $\delta$  7.01 as singlet. The presence of ethyl ester moiety was confirmed by the terminal methyl appearing as three proton triplet at  $\delta$  1.34 (J- 7.1 Hz). The methylene proton appeared as two proton quartet at  $\delta$  4.28 (J- 7.1 Hz). The  $^{13}\text{C}$  NMR spectrum showed the ester carbonyl at  $\delta$  166.76. The terminal methyl appeared at  $\delta$  14.31 as quartet. The methylene carbon appeared at  $\delta$  60.55. C-1 appeared at  $\delta$

121.43 and the C-4 carbon appeared at  $\delta$  137.19. The two equivalent carbons C-2 and C-6 appeared at  $\delta$  109.46 as doublet. Similarly, other two equivalent carbons at C-3 and C-5 appeared at  $\delta$  144.77. The IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR confirmed the compound to be ethyl gallate. The identity was confirmed by comparison of the physical and spectroscopic data. The identity as ethyl gallate was further confirmed by comparison with an authentic sample (mp, Co-TLC and superimposable IR)

**Characterization of Compound 3**

The compound molecular formula  $\text{C}_{15}\text{H}_{12}\text{O}_5$ , mp 250 - 251°C (lit.247°C-250°C) showed positive colour with magnesium and concentrated hydrochloric acid characteristic of a flavone. The presence of free hydroxyl was indicated by the brown colouration of the compound with ferric chloride. The IR spectrum of the compound had absorption at 3281  $\text{cm}^{-1}$  for hydroxyl, carbonyl (1628  $\text{cm}^{-1}$ ) and for aromatic system (1419  $\text{cm}^{-1}$ ). The compound showed UV absorption at 325, 260, 221 nm. The compound showed bathochromic shift in  $\text{AlCl}_3$  at 308, 228 nm and in sodium acetate at 325, 292, 220 nm. Two of the hydroxyls could at position 5 and 7 of the ring A. The compound showed the presence of a methine proton at  $\delta$  5.28 (t, 1H, J = 12.8 and 2.2 HZ) which suggested that it could be a flavanone. There was no signal at  $\delta$  6.5 characteristic of C-3 proton in flavones signal at indicating the compound belongs to flavanone group. H-3 axial proton of  $-\text{CH}_2-$  appeared at 3.06 as double doublet with J = 17.1 and 2.8 HZ and H-3 equatorial appeared at  $\delta$  2.74 as double doublet, J = 17.1 and 2.2 Hz. There were two meta coupled protons at  $\delta$  6.30 (J = 3 Hz) corresponding to H-6 and H-8 of ring A. The presence of a 4' substituted B ring was observed from the A2B2 pattern at  $\delta$  6.80 and 7.32, 2H, D, J = 8.3 Hz, H-3' and H-5', H-2' and H-6' respectively. Three phenolic hydroxyl protons appeared as broad singlet at  $\delta$  12.07, 10.07 and 8.95 as each one proton respectively. These data suggested that the compound to be a 5, 7-dihydroxy-2-(4-hydroxyphenyl)-2, 3-dihydrochromen-4-one (Naringenin). The  $^{13}\text{C}$  NMR spectrum data were 195.81(C-4), 166.92(C-7), 164.13(C-5), 163.15(C-9,C-1), 129.12(C-1'), 127.0(C-2',C-6'), 115.71(C-3',C-5'), 102.36 (C-10), 96.61(C-6), 95.58(C-8) and 78.93(C-2).

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