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Phytochemical screening and isolation of a pure phytoconstituent from the leaves of *Callistemon salignus*

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ABSTRACT

The methanol extract of *Callistemon salignus* was subjected to different phytochemical tests and gives positive results for glycosides, tannin, carbohydrate, volatile oil, flavonoid and steroid. This extract leads to the isolation of 3′, 4′, 5, 7-tetra-hydroxy flavonol by chromatographic separation through a graded elution. The identity of the isolated compound was checked by preliminary phytochemical test, TLC study, solubility, melting point determination. Finally the structure was elucidated by different spectroscopic methods such as UV, IR, NMR ($^{13}_{C}$ NMR and Proton NMR) and LCMS. On the basis of this chemical and spectral evidence and upon comparison with the literature data, the isolated compound is identified .The compound was isolated first time from this plant extract.

Keywords:*Callistemon salignus***,** 3′, 4′, 5, 7-tetra-hydroxy flavonol, graded elution, spectroscopic methods.

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INTRODUCTION

Callistemon is a genus of around several species in the family Myrtaceae. Callistemons are commonly known as "bottlebrushes" because of the cylindrical, brush-like shape of the flower spike. Callistemon is a genus of 34 species of shrubs in the family Myrtaceae. Since this plant belongs to the same myrtaceae family as clove, eucalyptus, it is expected that it might also be a store- house of many chemicals of medicinal and pharmacological interest. Several research works on the various parts of the plant have been reported for their anti-thrombin [1], anti mycobacterium tuberculosis properties [2]**.** The phytochemical studies revealed the presence of betulinic acid [3]. In the present work we have isolated and characterized a flavonol compound from the methanol extract of dried leaves of *Callistemon salignus.*

MATERIALS AND METHODS:

Plant material:

Fresh leaves of *Callistemon salignus* were collected from Kolkata, West Bengal and authenticated from the Botanical Survey of India, Howrah [CNH / I- I (87) /005-Tech II/ 1326]

General Instrument details:-

Melting points were determined with capillary melting point apparatus. UV spectophotometer Shimazdu Pharmaspec -1700, made in Japan; IR: The infrared spectra were recorded on IR spectrophotometer (Shimadzu 8201 PC) in KBr phase ;1H and 13C NMR spectra were recorded in DMSO d_6 as internal standard on a Bruker 300MHz and 600MHz DPX spectrometer and the chemical shifts are reported in δ units ; Mass spectra (positive mode) were obtained using LC-ESI-Q-TOF micro mass spectrometer.

Extraction and isolation:

The leaves were shade dried and powdered 185 g of this powdered leaves were first defatted with n hexane and then macerated in methanol in a beaker for 48hrs. Occasional shaking and stirring was done. Then it was filtered through muslin cloth for the methanol extract. Then filtrate was concentrated to dryness under the vacuum. The percentage yield extractive was calculated with references to the air-dried drug. Several chemical tests like Alkaloids, Carbohydrates ,Glycosides; Fixed oils and Fats, Gums and Mucilage, Phenolic compounds and Tannins, Proteins and free Amino acids; Saponin, Sterols; Volatile oils etc were done to detect several and different group of compounds present in the extractions[4]. For isolation purpose, the dried methanol extract was subjected to column chromatography over silica gel (60 – 120 mesh) and graded elutions were carried out with eluents CHCl3 followed by different CHCl3-Ethyl acetate

mixtures (90:10, 65:35, 35:65, 25:75). A total of 73 fractions were collected. Each fraction was collected in 25ml portion and was monitored by TLC. Fraction (34 – 51) eluted from CHCl3 :EtOH (35:65) mixture were homogeneous. They were, therefore, combined together and concentrated to one fourth of its volumes ultimately and then kept in refrigerator for overnight. Finally it afforded a yellow amorphous powder to furnish the pure isolated compound. The isolated compound was again confirmed of their identity by chemical test .It was subjected to TLC and its solubility and melting point was determined. For further characterization UV, IR, NMR (^{13} C and ^{H} NMR), Mass Spectra was done. On getting the results of IR, NMR and MASS spectra interpretation of the isolated compounds were done in respect of their structures [6-10].

RESULTS AND DISCUSSION:

After running different phytochemical tests the methanol extract gives positive results for glycosides, tannin, carbohydrate, volatile oil, flavonoid and steroid.

After isolating the yellow colored compound, it gives positive results for flavonoid and a single spot in TLC (Chloroform: Ethyl Acetate: 40:60). The compound is Soluble in Glacial acetic acid, in DMSO, Sparingly soluble in ethanol, insoluble in water. The melting point was found 316 ^{o} C.

UV spectrum of isolated compound exhibited major peaks at 212,257,375 nm which showed the presence of a flavonol skeleton [11]. UV spectrum is shown in Figure 1.

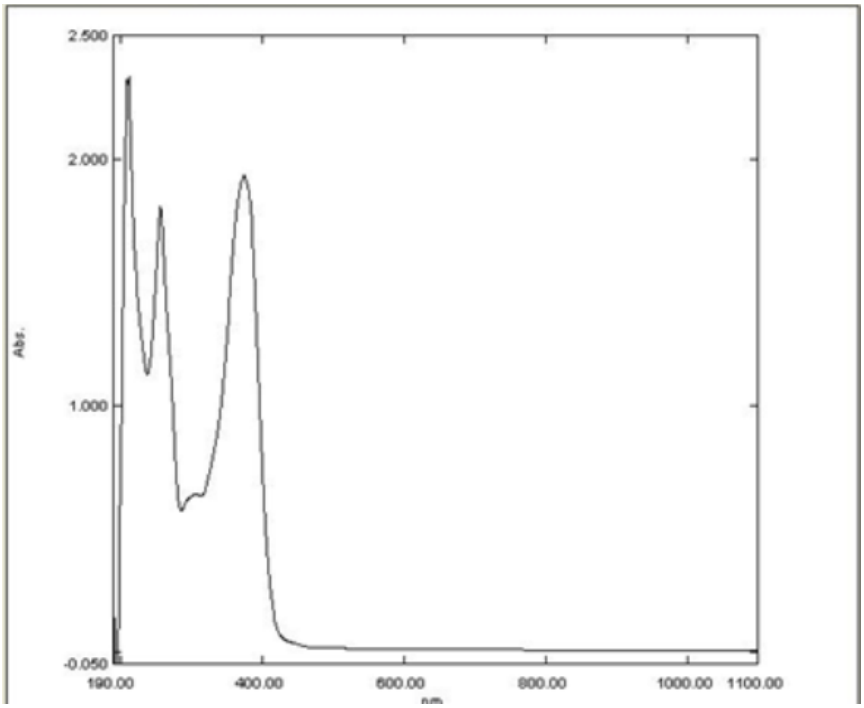

Figure 1:- UV spectra of the isolated compound

IR Spectrum is shown in Figure 2 and the spectral data is tabulated with proper justification of presence of individual functional group in table 1 [12].

Table 1: Interpretation of IR data of isolated compound:

Position (Wave Number in cm $^{-1}$ at which band appeared)	Comment
3408.57, 3322	O-H Stretching Vibration of Phenol
1663.3	C=O Aryl Ketonic Stretch
1609.31, 821.527, 1456.96	C---C Aromatic Ring Stretch
1382.71	In plane OH Bending of Phenol
1319.07	In plane bending of C-H Bond in aromatic hydrocarbon
1264.11	C-O Stretch of Aryl Ether
1203.36	C-O Stretch of Phenol
1168.65	C-CO-C Stretch and Bending Ketone
937.235,821.527, 681.713,	Out of plane C-H Bending of Aromatic Hydrocarbon

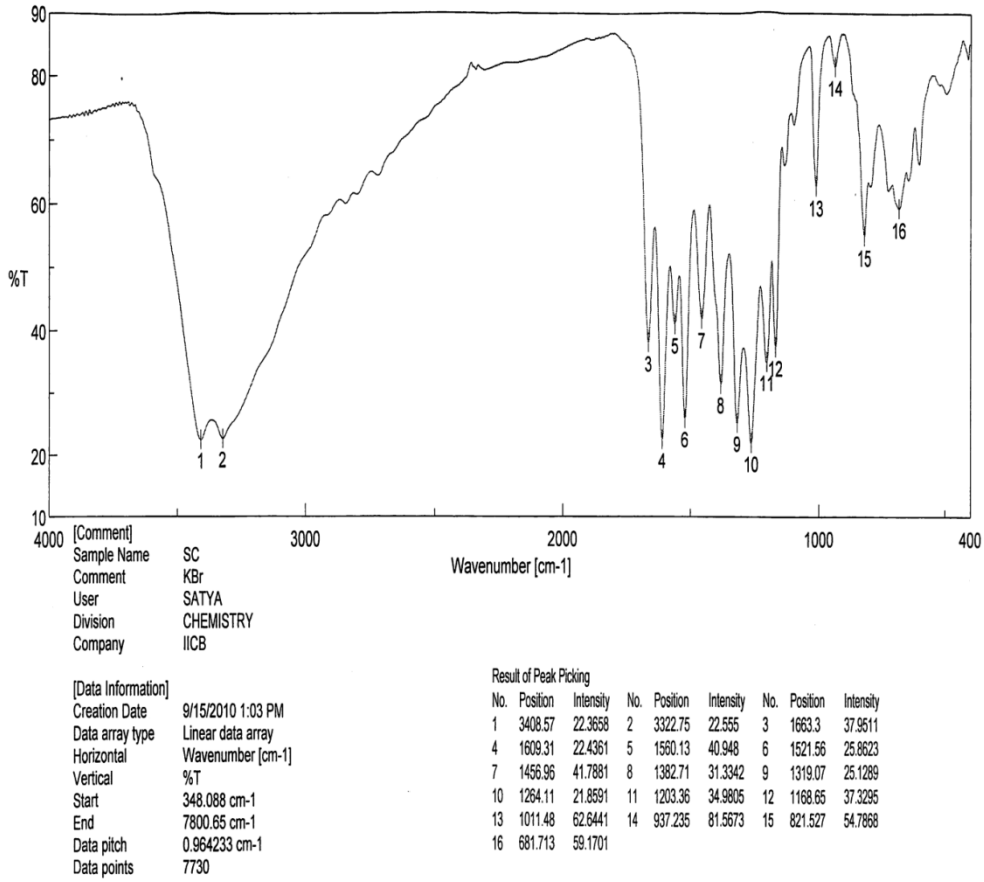

Figure 2: IR spectra of isolated compound

Mass spectrum reveals a molecular ion peak at 301.05 m/z which is quite similar with the literature data [13]. Peaks are visible with m/z ratios larger than the molecular ion peak due to isotope distributions, called isotope peaks [14]. The mass spectrum of this specific compound also showed two isotope peaks at 324.94 and 325.77 m/z. The mass spectrum is shown in Figure 3.

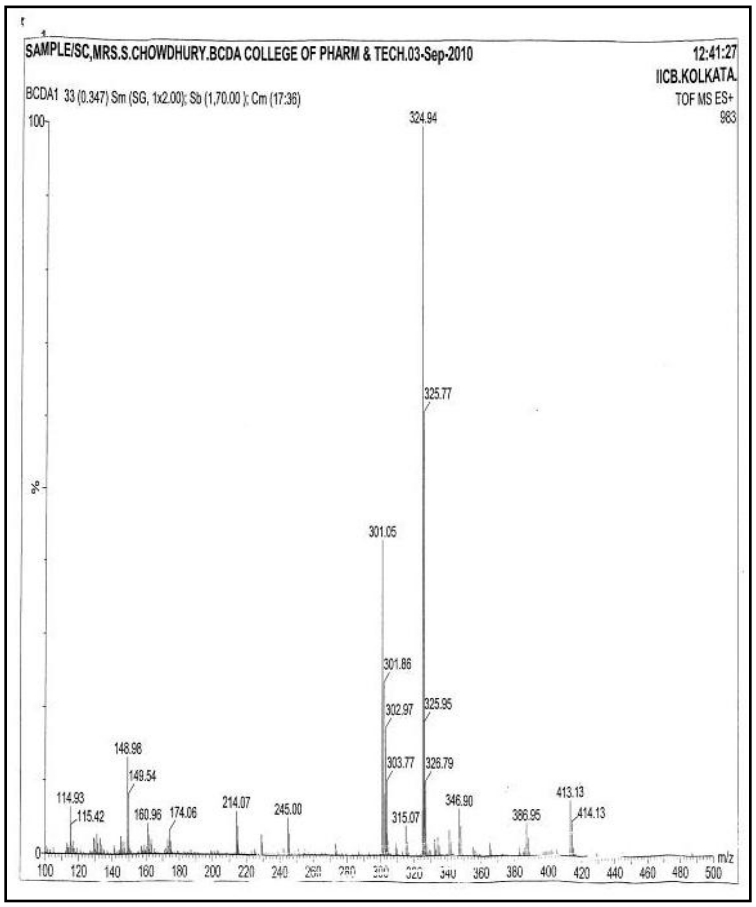

Figure 3:- Mass spectra of isolated compound

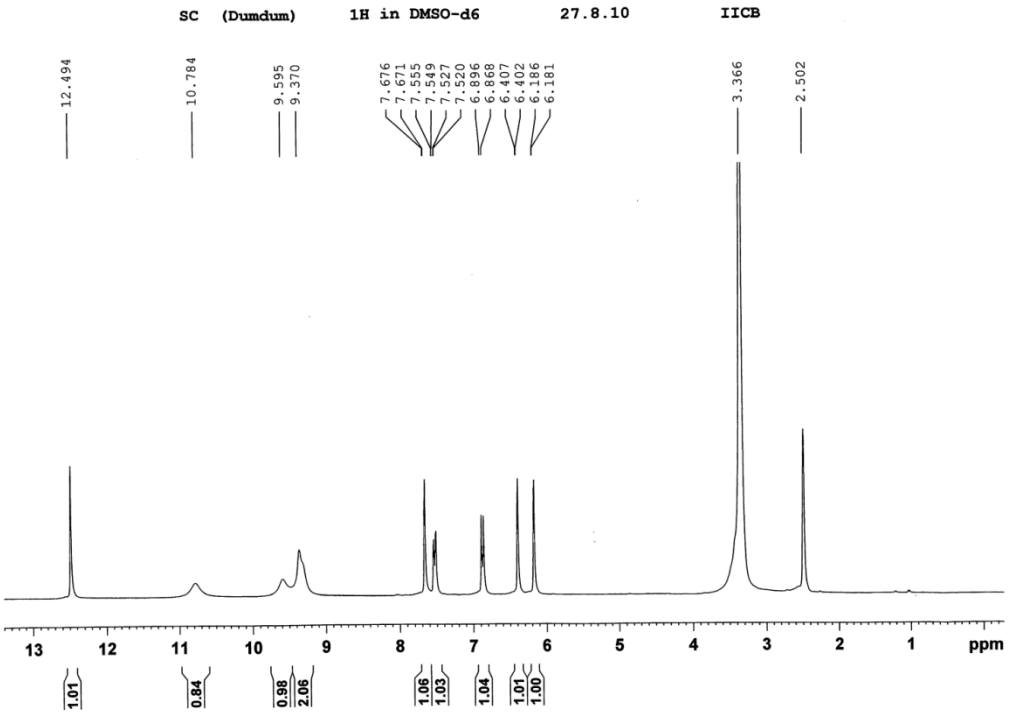

Figure 4 : The ^{1}H NMR spectrum of isolated compound

The ^{1}H NMR spectrum showed protons at aromatic regions from 6.1 – 7.6 ppm, and strong hydrogen bonding at 12.494 ppm. These suggest a 3′, 4′, 5, 7-tetra-hydroxy flavonol nucleus [7], The singlets at 10.784 and 9.595 ppm correspond to –OH protons at C-7 and C-3 [5]. The ^{1}H NMR spectrum is shown in Figure 4. ^{1}H NMR Data for isolated compound, DMSO d_6 as compared to standard sample from the literature data is tabulated in table 2.

Table 2:- 1_H of Data for isolated compound (CS) , DMSO d_6 as compared to standard sample from the literature data [7]

Position	1_H Data of isolated compound from *C. salignus*	1_H Data of standard compound from Literature
H-6	6.18	6.18
H-8	6.40	6.40
H-1′	7.67	7.67
H- 5′	6.89	6.89
H-6′	7.54	7.53
5-OH	12.49	12.42

The ^{13}C-NMR spectra revealed 15 carbon signals typical of flavonoid monoglycoside nucleus. The low field signal at 175.8 ppm was due to the carbonyl group at C-4. The NMR Spectral data values as reported in library reference are tabulated alongside the observed NMR Spectra of the isolated 3′, 4′, 5, 7-tetra-hydroxy flavonol. The spectra compares very well with that reported in literature [8]. The ^{13}C-NMR spectrum is shown in Figure 5. ^{13}C NMR Data for isolated compound as compared to the standard Sample from literature data is tabulated in table 3.

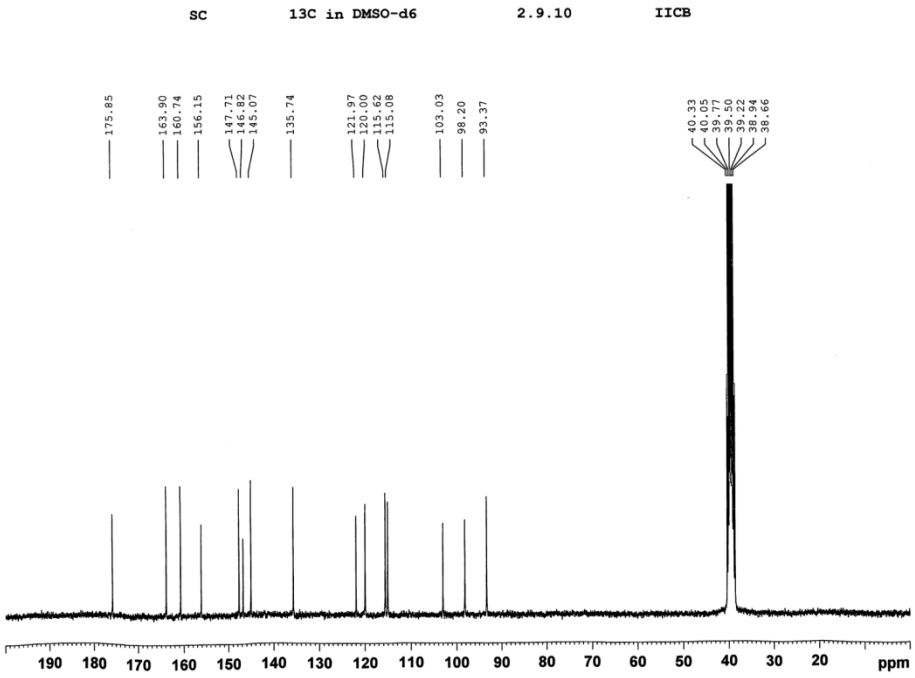

Figure 5 : The ^{13}C-NMR spectra of isolated compound

Table 3: ^{13}C NMR Data for isolated compound (CS) as compared to the standard Sample from literature data: [6]

Position	^{13}C Data of isolated compound from *C. salignus*	^{13}C Data of Standard compound from Literature
2	147.71	146.8
3	135.74	135.6
4	175.85	175.7
5	160.74	160.6
6	98.20	98.1
7	163.9	163.8
8	93.37	93.3
9	156.15	156.1
10	103.03	103.0
1′	121.97	121.9
2′	115.08	115.1
3′	145.07	145.0
4′	147.71	147.6
5′	115.62	115.5
6′	120.00	119.9

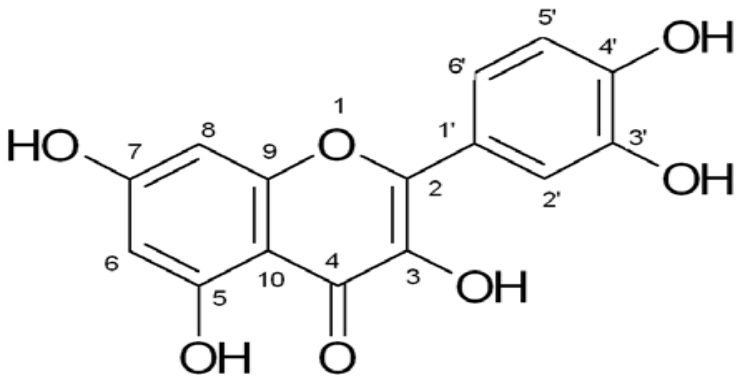

Figure 6: 3′, 4′, 5, 7 Tetra Hydroxy Flavonol

CONCLUSION

On the basis of this chemical and spectral evidence and upon comparison with the literature data, the isolated compound is identified as 3′, 4′, 5, 7-tetra-hydroxy flavonol (Figure 6). The compound was isolated first time from this plant extract. The structure of the compound is as below

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