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PHYTOCHEMICAL STUDIES ON *DELONIX ELATA* BARK

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ABSTRACT: *Delonix elata* bark was collected, authenticated, dried, coarse-finely powdered and extracted using 70% alcohol and subjected for preliminary phytochemical screening. Total phenolic and flavonoidal contents were determined using UV/ visible spectrophotometer. In addition the silica column chromatographic separation was carried out to separate the phytochemicals of *D. elata* bark extract. Preliminary phytochemical screening showed positive tests for saponins, flavonoids, tannins and carbohydrates in the alcoholic extract of *Delonix elata* bark. When the extract was analyzed quantitatively by UV/ visible spectrophotometer, 75 mg/gm of gallic acid equivalent total phenolics and 57.4 mg/gm of rutin equivalent flavonoids were observed. Two phenolic compounds, caffeic acid and apigenin, were isolated and characterized by spectral analysis from the alcoholic extract of the *Delonix elata* bark. Moreover, HPLC method was employed to analyze the isolated phytoconstituents i.e. caffeic acid and apigenin present in the fraction of alcoholic extract of *D. elata* bark which showed the similar R_t of separated constituents. The present study reveals the presence of flavonoids in *D. elata* bark as evidenced by the phytochemical screening, and identification of caffeic acid and apigenin.

INTRODUCTION: *Delonix elata* is commonly known as white gulmohur belonging to the family of *Fabaceae* and subfamily *Caesalpinoidae* [1]. *Delonix elata* is not a classical Ayurvedic drug, but found included in Shodhala Nighantu under the Sanskrit name of '-Siddeshwara-' during 12th century AD [2].

The medical usefulness of the tree is acknowledged by people living in the villages who take a decoction of the leaves and barks to get relief from rheumatic problems like pain and stiffness of the joints, especially affecting the knees [3, 4].

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It was observed that local people and Siddha practitioners in Tamil Nadu, India use the *Delonix elata* bark and leaves for treating inflammation and arthritic conditions. The benefits may be attributed to the chemical constituents like β-sitosterol, quercetin, lupelol, lysine, alanine, valine, tyrosine and rhamnose are which reported from *Delonix regia*. Quercetin 3-O-rhamnoglucoside and Quercetin-3O-galactoside are also reported [3].

Extensive pharmacological studies on *Delonix elata* exhibited anti-inflammatory [4, 5, 6, 7], anti-arthritic [4, 5], immune modifying potentials [5] and anti-oxidant activities [8] were studied. Earlier studies in our investigations found promising anti-arthritic and immune modifying potentials in the alcoholic extract of *Delonix elata* bark [4, 5]. Therefore, an attempt was made to carry out phytochemical studies on *D. elata* bark 70 % v/v alcoholic extract using advanced separation techniques.

MATERIALS AND METHODS:

Plant Material: The Barks of the plant *Delonix elata* were obtained from the outskirts of Tiruchengode, Namakkal Dt. Tamilnadu, India. It was identified and authenticated by Prof. K. P. Sreenath (Taxonomist) Bangalore University, Bangalore. A voucher specimen has been prepared and deposited at the Department of Pharmacognosy, PES College of Pharmacy, Bangalore. The collected bark was dried in shade, crushed to coarse-fine powder and used for the studies.

Chemicals: All the solvents and chemicals used for the studies are laboratory grade procured from SD fine chemicals. Gallic acid and Rutin were procured as gift samples from Himalaya drug company, Bangalore. Folin- Ciocalteu reagent was obtained from Merck (India).

Preparation of Extract: The dried powdered bark (1000g) was subjected to continuous hot extraction using 70% v/v methanol in distilled water for 8 h. The extract was filtered, concentrated and the solvent was removed by rotary flash evaporator to get 98g extract. The extract was dried over a desiccator and used for the study.

Phytochemical analysis: The extract was subjected to preliminary qualitative tests to identify the various phytoconstituents i.e. saponins, flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids and carbohydrates by standard procedure [9].

Determination of total poly phenol content: Qualitative chemical tests revealed that the alcoholic extract of *D. elata* showed prominent results for phenolics and tannins. Hence, estimation of polyphenol content was performed. Total phenolic content of the extract was determined using Folin-Ciocalteu reagent method with Gallic acid as a standard. The alcoholic extract (1000 µg/ ml) was taken in a 50 ml volumetric flask with 45 ml distilled water and 1 ml Folin-Ciocalteu reagent and the flask was shaken thoroughly. After 3 min, 3 ml solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm using UV/ visible spectrophotometer.

The procedure was repeated for all the standard Gallic acid concentrations (20, 40, 60, 80 and 100 µg) and standard curve derived to obtain linear regression equation [10, 11].

Total flavonoids assay: The total flavonoid content of *Delonix elata* bark extract was measured with Aluminum chloride assay. An aliquot of 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard Rutin and 2.5 ml of samples were separately transferred to a 10 ml volumetric flask and 4 ml of distilled water was added. All the flasks were added with 0.3 ml of 5% $NaNO_2$ and allowed to stand for 5 min. A 0.3 ml of $AlCl_3$ was added followed by 2 ml of 1 M NaOH and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against a reagent blank at 510 nm with UV/ visible spectrophotometer [11].

Column Chromatography -1 (CC-1): The alcoholic extract (65 g) of *D. elata* bark loaded onto silica (60-120 mesh size) column prepared in chloroform. The fractions were eluted with chloroform- ethyl acetate (100: 0, 90: 10, 80: 20, 70: 30, 50: 50, 75: 25, 0: 100, v/v). All the elutes were analyzed by TLC method and pooled as master fraction (MF) which yielded seven fractions with yield of 140, 200, 220, 200, 340, 470 and 710 mg (MF 1 to MF7).

Later the silica column was eluted with ethyl acetate-methanol (90: 10, 80: 20, 70: 30, 60: 40, 40: 60, 20: 80 and 0: 100, v/v). The master fractions were yielded as 1.63, 2.26, 1.9, 2.7, 3.2, 2.93 and 5.8 g (MF 8 to MF14).

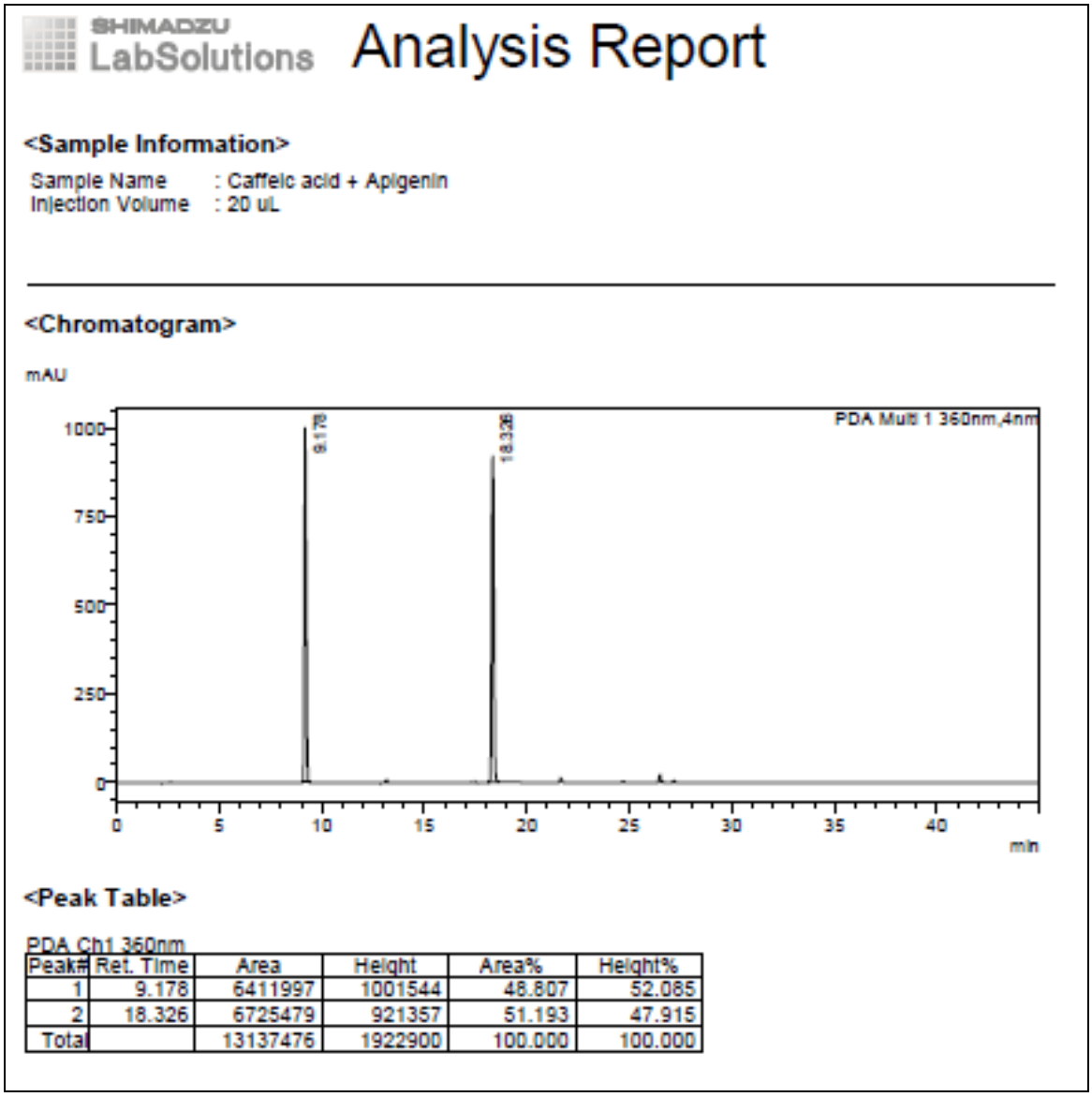
Based on their TLC profiles of CC-1 fractions which eluted with 70: 30, 60: 40, 40: 60 v/v of ethyl acetate-methanol were combined together (7.80 g) and subjected further for silica column chromatography-2 (CC-2) for purification. Similarly fractions obtained using 20: 80 and 0: 100, v/v ethyl acetate-methanol were combined together (8.73g) and further subjected for chromatographic purification i.e. column chromatography-3 (CC-3).

Column chromatography-2 (CC-2): The collective fraction (7.80g) was subjected for silica column chromatography.

Silica gel (100-200 mesh size) was used as adsorbent and the column was packed with ethyl acetate as initial solvent. Elution was carried out by increasing the polarity with methanol, which yielded a yellow colored compound (40 mg) with the elution ratio of 65:35, v/v ethyl acetate in methanol, named as DECP1.

Column chromatography-3 (CC-3): Similarly, the collective fraction (8.73g), as described above, was subjected using silica (100-200 mesh size) column chromatography with ethyl acetate as initial mobile phase. Elution was carried out by increasing the polarity with methanol, which yielded a yellow colored compound (35 mg) with the elution ratio of 10: 90, v/v of ethyl acetate in methanol, named as DECP2.

HPLC analysis: Isolated compounds i.e. caffeic acid (DECP1) and apigenin (DECP2) and the silica column chromatography fractions i.e. CC-2 and CC-3 were analyzed on a Shimadzu HPLC system using C18 column using standard Apigenin and caffeic acid authentic markers. HPLC column was 5 micron with the dimension of 4.6 x 250 mm length. Mobile phase A was Buffer (consisting of 0.14g KH2PO4 with 0.5 ml H_3PO_4in HPLC grade water QS to 1000 ml) and Mobile phase B was Acetonitrile. Gradient elution was performed with 95: 5, v/v to 20: 80 v/v of A: B. in 30 min, later 95: 5, v/v till 40 min. Samples in the volume of 20 µl was injected and analyzed at 360 nm wavelength. The chromatograms are shown in **Fig. 1-5**.



Peak#	Ret. Time	Area	Height	Area%	Height%
1	9.178	6411997	1001544	48.807	52.085
2	18.326	6725479	921357	51.193	47.915
Total		13137476	1922900	100.000	100.000

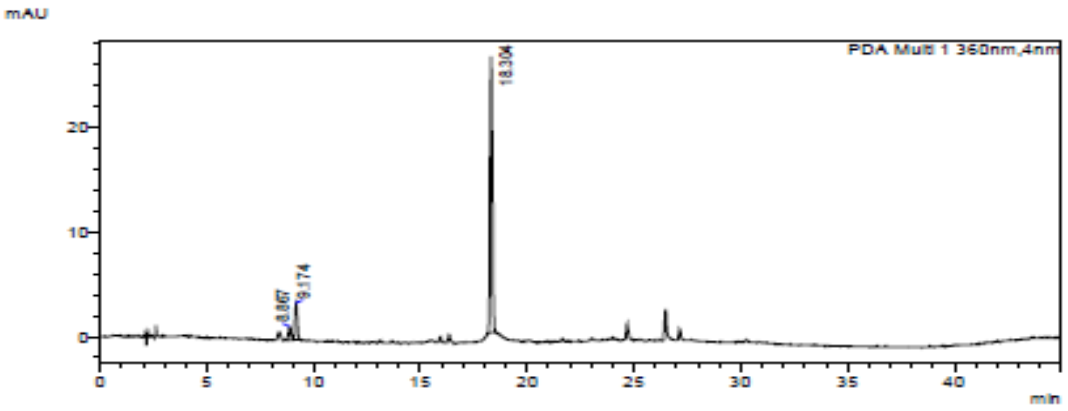
FIG. 1: HPLC CHROMATOGRAMS OF STANDARD APIGENIN AND CAFFIC ACID

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name : CC2
Injection Volume : 20 uL

<Chromatogram>



<Peak Table>

PDA Ch1 360nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	8.867	10254	1334	4.533	4.276
2	9.174	25274	3524	11.174	11.297
3	18.304	190664	26335	84.293	84.426
Total		226192	31193	100.000	100.000

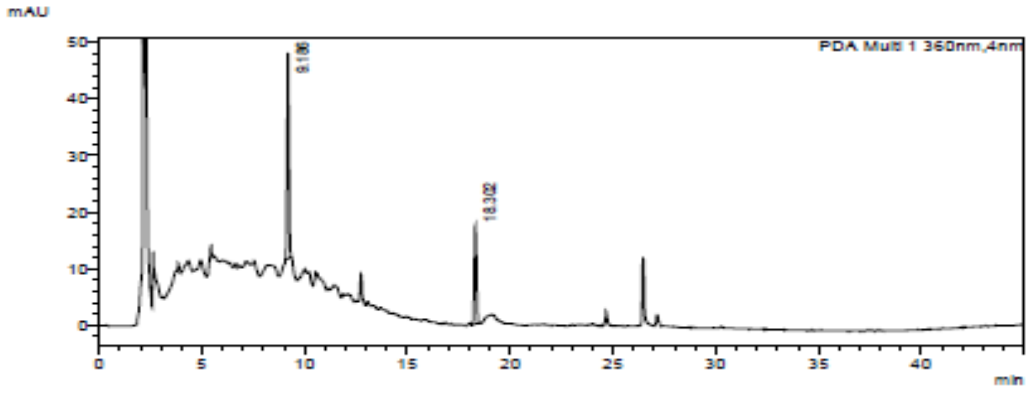
FIG. 2: HPLC CHROMATOGRAM OF CC2 FRACTION

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name : CC3
Injection Volume : 20 uL

<Chromatogram>



<Peak Table>

PDA Ch1 360nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	9.186	227749	36354	64.869	67.704
2	18.302	123340	17341	35.131	32.296
Total		351090	53696	100.000	100.000

FIG. 3: HPLC CHROMATOGRAM FOR CC3 FRACTION

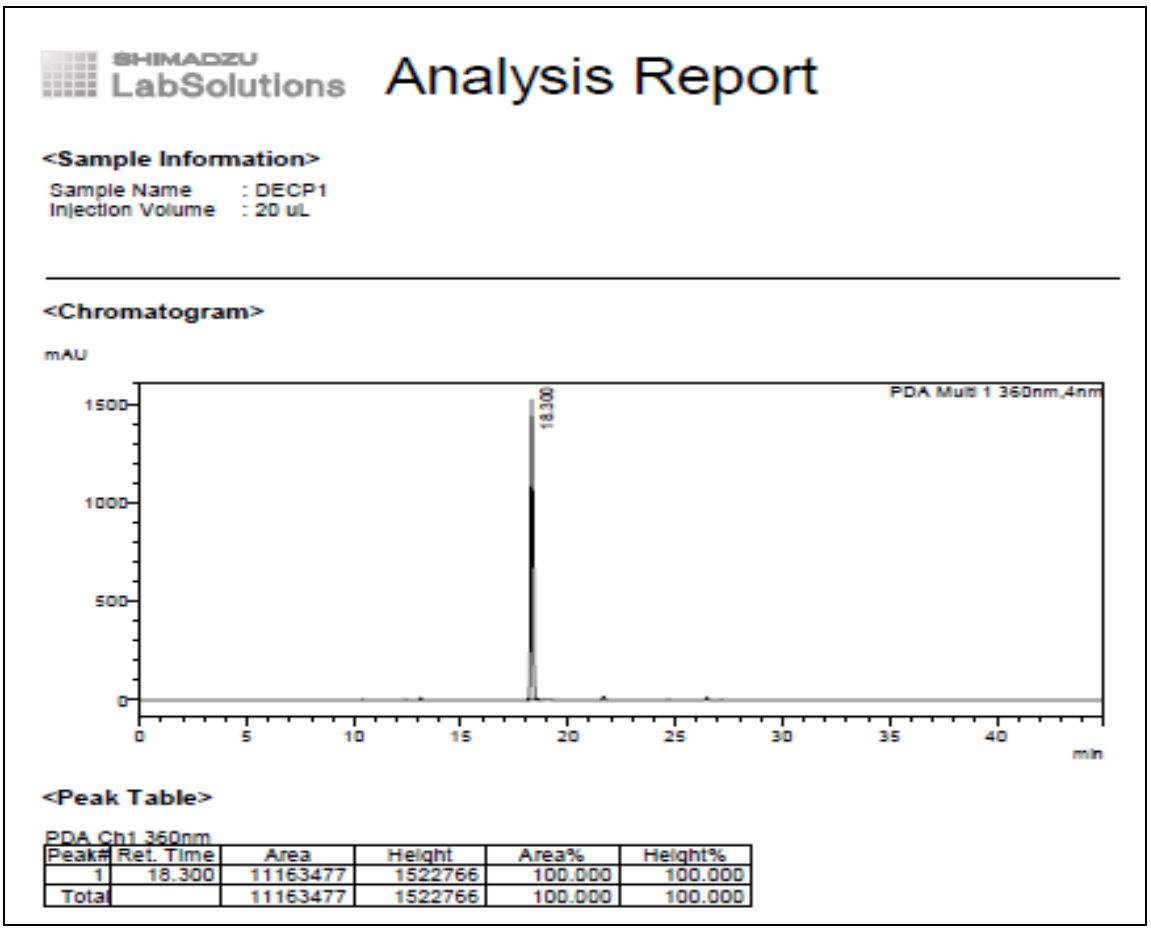


FIG. 4: HPLC CHROMATOGRAM FOR DECP1

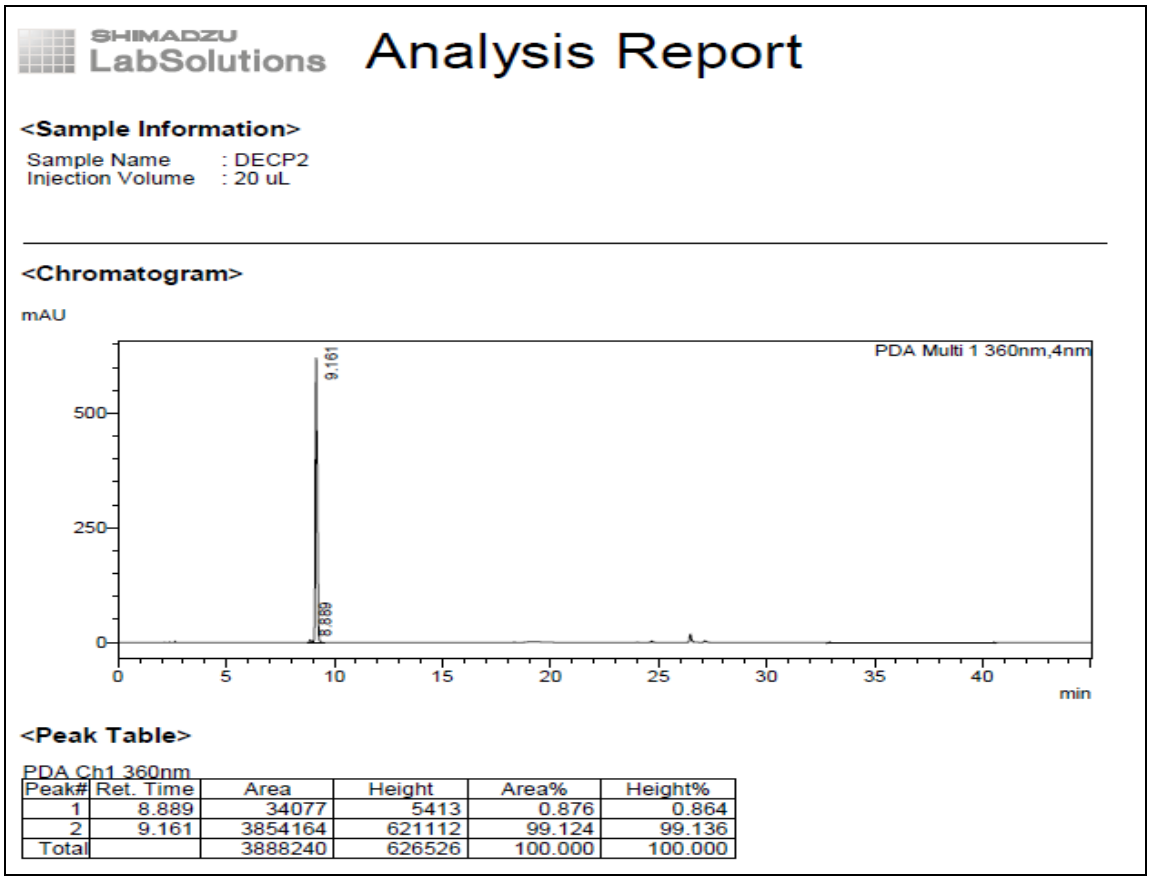


FIG. 5: HPLC CHROMATOGRAM OF DECP2

RESULTS AND DISCUSSION:

Phytochemical analysis: The alcoholic extract of *D. elata* showed the presence of flavonoids, tannins, terpenoids, steroids and carbohydrates in qualitative chemical analysis. UV/visible spectrophotometric determination revealed the presence of 75mg/gm polyphenol content equivalent to 100 g Gallic acid and the flavonoid content as 57.4 mg/gm equivalent to Rutin.

Column Chromatography: During CC-2 silica column chromatographic separation, a yellow colored compound (40 mg) was obtained with the elution ratio of 65:35, v/v ethyl acetate-methanol. After spectral characterization, it was identified as caffeic acid. Similarly, during CC-3 Silica column chromatographic separation, a dull yellowish white colored compound (35 mg) was isolated with ethyl acetate-methanol (10: 90, v/v). After spectral characterization, it was identified as Apigenin, a flavonoid.

HPLC Analysis: HPLC studies showed that standard caffeic acid and apigenin were eluted at retention time (Rt) of 18.32 min and 9.17 min respectively. The isolated compounds i.e. DECP 1 (caffeic acid) showed Rt of 18.30 while DECP2 (apigenin) eluted out at Rt 9.16. The CC-2 loaded fraction exhibited prominent major peaks at Rt 18.30 with some other minor impurities. Similarly CC-3 loaded fraction showed peak at both Rt 9.16 and 18.30 along with some impurities (Figure 2-6). The present work, therefore, suggests that the caffeic acid and apigenin are present in sufficient concentrations to be determined by HPLC method described above.

Spectral data of DECP 1: *^{1}H-NMR (DMSO-d_6):* δ in ppm as 6.1 (1H, d, H-2'), 6.7 (1H, d, H6), 6.9 (1H, d, H-1'), 7.0 (1H, s, H2), 7.4 (1H, d, H5) (aromatic protons); 9.5 (s) and 9.1 (s) (phenolic protons) and 12 (s) (carboxylic acid proton).

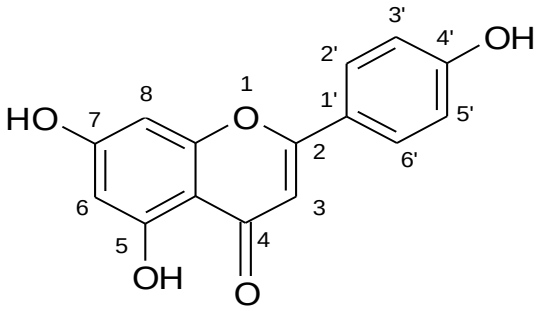
EI-MS: m/z = 179 (corresponds to molecular formula $C_9H_8O_4$ of the compound caffeic acid).

FTIR (KBr): 3427 cm^{-1} (-OH of carboxylic acid); 3234 cm^{-1} (Phenolic –OH); 3022 cm^{-1} (aromatic -C-H stretch); 1647 cm^{-1} (carbonyl peak of carboxylic acid); 1282 cm^{-1} (vinyl stretching) and 1610 cm^{-1} (aromatic -C=C-).

Spectral data of DECP2: *^{1}H-NMR (DMSO-d_6)*; δ values in ppm as 6.1 (1H, d, H3), 6.4 (1H, d, H8) 6.7 (1H, s, H6), 6.9 (2H, m, H2' and H6'), 7.9 (2H, m, H3' and H5') (corresponding to aromatic protons); 10.3 and 10.8 (phenolic protons at 5th and 7th C-atom) and 13 (phenolic proton at C-4' position).

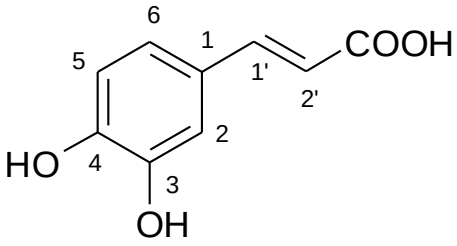
EI-MS: a molecular ion peak at m/z = 271 which corresponds to molecular formula $C_{15}H_{10}O_5$ of apigenin.

FTIR (KBr): 3280 cm^{-1} (phenolic –OH group); 3016 cm^{-1} (aromatic C-H stretch); 1656 cm^{-1} (carbonyl group); 1606 cm^{-1} (aromatic C=C peak) and 1242 cm^{-1} (-C-O-C stretching of the flavone ring).



Apigenin

A



Caffeic acid

B

FIGURE 1: CHEMICAL STRUCTURES OF A. APIGENIN AND B. CAFFEIC ACID.

CONCLUSION: An equivalent 75 mg/gm of the total polyphenol and 57.4 mg/gm of the flavonoid content was found in the alcoholic extract of the *D. elata* bark extract. Apigenin and caffeic acid were isolated and characterized for the first time from *D. elata* bark. These constituents are found in *Delonix elata* bark where comparable to other plant-based sources.

Our work hence, confirms and quantitates the reliable sources of Apigenin and Caffeic acids from *Delonix elata* bark which will be helpful for future studies in the extraction of these pharmacologically active compounds.

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