ISOLATION AND STRUCTURE ELUCIDATION OF ISOFLAVAN DERIVATIVE FROM THE BARK OF *ERYTHRINA CRISTA-GALLI* LINN.

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Abstract

Purification of ethyl acetate extract of *Erythrina crista-galli* Linn. led to the isolation of isolflavan derivative, namely, 4', 6', 7-trihydroxy-5'-prenyl isoflavan-4-ol. The structure of isolated compound was elucidated on the basis of 1D and 2D NMR spectral data and mass spectrometry. Moreover, antimicrobial and antioxidant properties of *Erythrina crista-galli* extract were also evaluated by agar-well diffusion method and DPPH radical scavenging assay respectively.

Keywords: isoflavan, 1D and 2D NMR spectral data, DPPH

Introduction

For thousands of years, plant has been used as medical treatments based on experience and folk remedies. In recent time, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems (Dahanukar *et al.*, 2000). According to the WHO, 80% of the World's population depends on plant derived medicines for their health care. Plants, one of the important sources of natural products have a long history in the treatment of various diseases (Dar *et al*., 2017). Natural products are organic compounds that are intermediates of primary and secondary metabolic pathways. They can be used as pharmacologically active compounds in treating various kinds of diseases (Chintoju *et al*., 2015). In Myanmar, there are many traditional medicinal plants which produce chemical compounds as part of their normal metabolic activities.

Erythrina crista-galli Linn. is widely distributed in tropical and subtropical regions of the American continent and is a popular ornamental plant in subtropical areas. It belongs to the family Fabaceae (Ayoub *et al*., 2017). It is also locally known as Thinbaw-kathit and distributed in Kayah state, Myanmar. The seed extracts of *Erythrina crista-galli* Linn. possess sedative, hypertensive and diuretic activities (Maier *et al*., 1999). The wood of this medicinal plant is used in infusions or decoctions as astringent, narcotic and sedative in Argentina. Antibacterial and anti-inflammatory activities have been reported for this plant (Weber *et al*., 2004). The bark of this plant is used for rheumatism, hepatitis, sedation, and hypnogenesis (Ayoub *et al*., 2017). The present study was conducted to isolate bioactive chemical constituent from the barks of *Erythrina crista-galli* Linn.

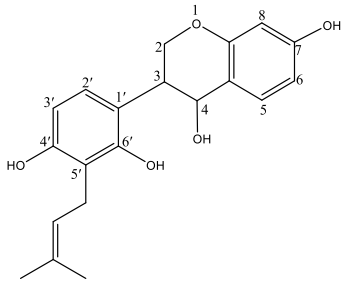

Figure 1 Structure of isolated compound (1)

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Figure 2 Plant and flowers of *Erythrina crista-galli* Linn.

Materials and Methods

General Experimental Procedures

NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. Column chromatography was carried out on MN silica gel 60, 0.05-0.2 mm; TLC was performed on Polygram SIL G/UV_{254}. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany. Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Freiburg, Germany, purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Commercial grade reagents and solvents were purchased from Super Shell Co. Ltd, Yangon. Common laboratory apparatus were used. PerkinElmer C93927 was used for FT-IR spectra measurement.

Plant Materials

The barks of *Erythrina crista-galli* were collected from Loikaw Township, Kayah State, Myanmar and identified by Dr Soe Myint Aye, Department of Botany, University of Mandalay. The plant materials were cut into small pieces and dried at room temperature for about two weeks.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of *Erythrina crista-galli* was determined using standard method of Harbone.

Antimicrobial Assay

Antimicrobial tests were performed at Pharmaceutical Research Department (PRD), Insein Township, Yangon Region. Antimicrobial activities of plant extracts were tested by agar-well diffusion method on six test microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillius pumilus*, *Candida albicans* and *Escherichia coli*.

Extraction and Isolation of Pure Compound

The air-dried samples of Erythrina crista-galli (1000 g) were percolated with methanol for two months. The methanol crude extracts were filtered and evaporated the solvent. The residue was extracted with ethyl acetate to attain 7.5 g of ethyl acetate crude extracts. The crude extracts were dissolved in a mixture of n-hexane/EtOAc and silica gel was added. The mixture was allowed to dryness under reduced pressure. The obtained crude extracts were subjected to silica gel by using various solvent systems of n-hexane and ethyl acetate. The obtained fraction was checked by TLC and iodine vapour for purity. Then, the same R_f value fractions were combined. Among them, pure compound (1) was isolated as colorless oil from selected fraction III after purification on Sephadex LH-20 using MeOH only.

Measurement of DPPH Radical Scavenging Activity by UV Spectrophotometric Method

The control solution was prepared by mixing 1.5 mL of 20 µg/mL DPPH solution and 1.5 mL of 95 % ethanol. Similarly, the blank solution was prepared by mixing 1.5 mL of test sample solution and 1.5 mL of 95% ethanol. The test sample solution was also prepared by mixing 1.5 mL of 20 µg/mL DPPH solution and 1.5 mL of sample solution in various concentrations (0.78125, 1.5625, 3.125, 6.250, 12.5, 25, 50, 100, 200 and 400 mg/mL). The solutions were allowed to stand at room temperature for 30 min. After 30 min, measurements of absorbance at 517 nm were made by these solutions using UV-Vis spectrophotometer. The measured absorbance values were applied to calculate percent inhibition by the formula:

$$\%\,\text{inhibition} = \frac{\text{Abs}_{\text{DPPH}} - [\text{Abs}_{\text{sample}} - \text{Abs}_{\text{Blank}}]}{\text{Abs}_{\text{DPPH}}} \times 100\,,$$

where % inhibition = percent inhibition of test sample, Abs_{DPPH} = absorbance of control solution, $\text{Abs}_{\text{sample}}$ = absorbance of test sample solution, $\text{Abs}_{\text{blank}}$ = absorbance of blank solution.

The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as a reference compound in the same concentration range as the test compound.

Results and Discussion

Phytochemical Analysis

Preliminary phytochemical analysis was performed in order to know different types of organic compounds present in bark of *Erythrina crista-galli*. Analysis of the extract of bark sample revealed the presence of phytochemicals such as alkaloids, flavonoids, phenolic compounds, polyphenols, steroids, tannins, glycoside, and reducing sugars.

Antimicrobial Assay

The antimicrobial activities of plant extracts were tested by applying agar-well diffusion method on six test microorganisms. According to antimicrobial assay, the ethyl acetate extract responded medium activities on *Bacillus subitilis, Pseudomonas aeruginosa*, *Bacillus pumilus* and weak activities on *Staphylococus aureus*, *Candida albicans* and *Escherichia coli*. Ethanol extract showed medium activities on *Escherichia coli* and weak activities on other five test organisms. In addition, n-hexane extract exhibited weak activities on five test organisms. The results were tabulated in Table 1.

Table 1 Results of Antimicrobial Activities of *Erythrina crista-galli* Barks

Samples	Solvents	Inhibition Zone (mm)					
		I	**II**	**III**	**IV**	**V**	**VI**
Erythrina crista-galli	n-hexane	11	13	-	12	12	11
	EtOAc	15	13	18	15	14	13
	EtOH	14	13	12	13	13	15
Control	n-hexane	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-

Agar-well – 10 mm

10 mm ~14 mm (+) weak active
15 mm ~19 mm (++) medium active
20 mm above (+++) strong active

(I) *Bacillus subtilis*
(II) *Staphylococous aureus*
(III) *Pseudomonas aeruginosa*
(IV) *Bacillus pumilus*
(V) *Candia albicans*
(VI) *Escherichia coli*

Antioxidant Activity

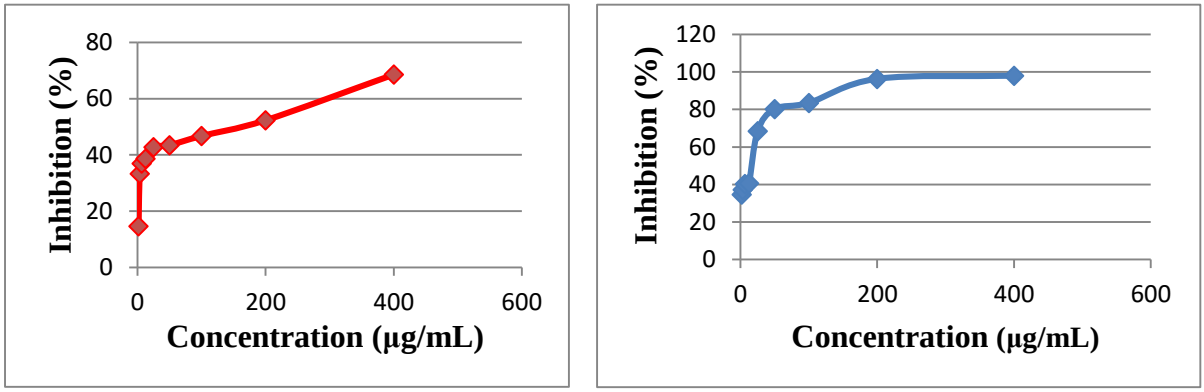
The antioxidant activity of methanolic extracts was analyzed by DPPH (2,2-diphenyl-1-picryl-hydrazyl) method. Mean absorbance values of methanolic extract of *Erythrina crista-galli* barks and ascorbic acid were shown in Table 2. The inhibition percentage of methanolic extracts of *Erythrina crista-galli* barks and standard ascorbic acid in various concentrations were described in Table 3.

Table 2 Absorbance of Standard Ascorbic Acid and Methanolic Extracts of the Barks of *Erythrina crista-galli* at 517 nm by UV Spectrophotometer

No.	Concentration (µg/mL)	Ascorbic acid		*Erythrina crista-galli*	
		Abs_{sample}	Abs_{blank}	Abs_{sample}	Abs_{blank}
1	1.5625	0.283	0.002	0.370	0.003
2	3.125	0.272	0.002	0.289	0.002
3	6.250	0.260	0.003	0.273	0.002
4	12.5	0.259	0.004	0.265	0.001
5	25	0.141	0.005	0.250	0.004
6	50	0.092	0.007	0.248	0.005
7	100	0.072	0.001	0.235	0.006
8	200	0.025	0.009	0.213	0.008
9	400	0.014	0.005	0.139	0.004

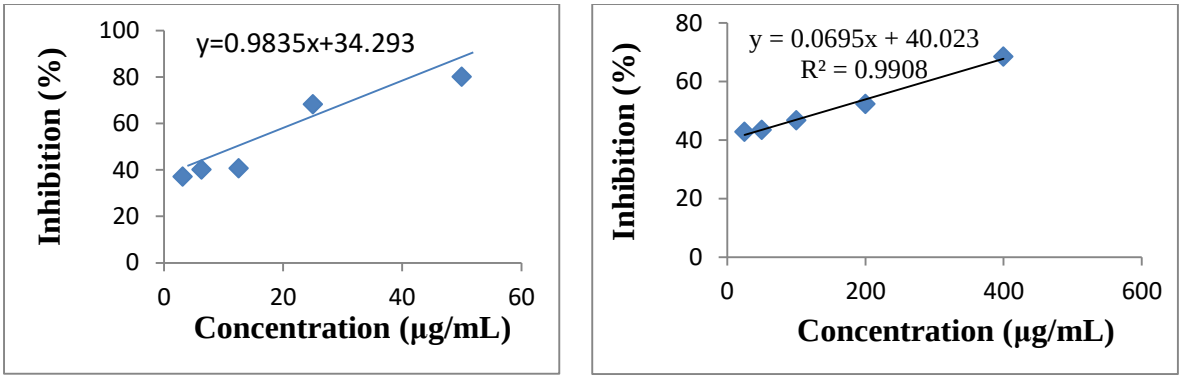
Table 3 Percent Inhibition of Methanolic Extracts of *Erythrina crista-galli* Barks and Standard Ascorbic Acid in Various Concentrations

No	Concentration (µg/mL)	% Inhibition	
		Ascorbic acid (Standard)	*Erythrina crista-galli*
1	1.562	34.65	14.65
2	3.125	37.21	33.26
3	6.25	40.23	36.98
4	12.5	40.70	38.64
5	25.0	68.37	42.79
6	50.0	80.23	43.49
7	100	83.49	46.74
8	200	96.28	52.35
9	400	97.91	68.60



(a) (b)

Figure 3 Percent inhibition of (a) methanolic extracts of *Erythrina crista-galli* barks and (b) standard ascorbic acid in various concentrations



(a) (b)

Figure 4 Linear regression equation for IC_{50} value of (a) *Erythrina crista-galli* bark and (b) standard ascorbic acid

Table 4 The Linear Regression Equations and IC_{50} Values of *Erythrina crista-galli* Bark and Standard Ascorbic Acid

No.	Test Solution	Regression Equations	IC_{50} (ppm)
1	Ascorbic acid	y = 0.9835x + 34.293	15.97
2	*Erythrina crista-galli*	y = 0.0684x + 39.729	143.6

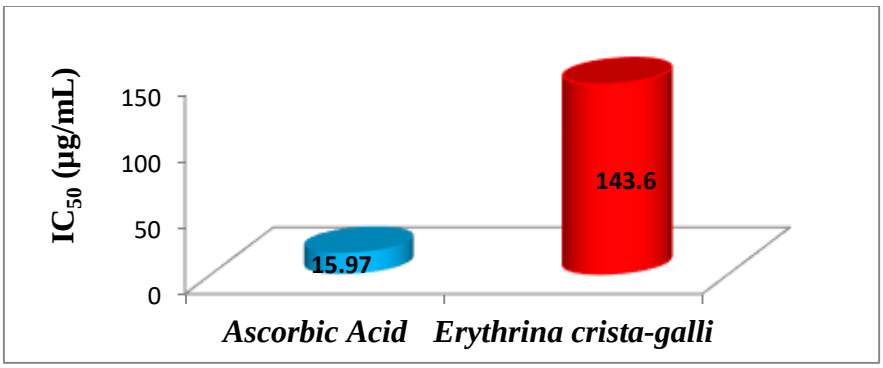


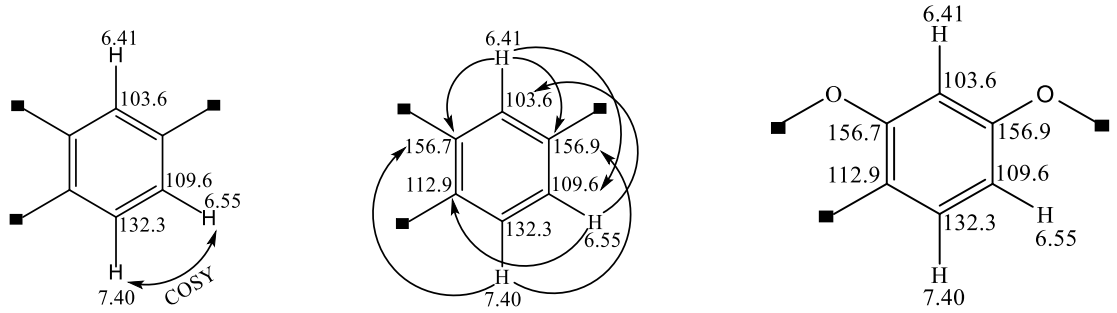
Figure 5 Comparison of IC_{50} values of methanolic extract of *Erythrina crista-galli* and standard ascorbic acid

According to DPPH assay, IC_{50} value of the sample extracts was found to be 143.6 µg/mL. Based on the obtained results, the sample extracts exhibited low antioxidant activity which is comparison with IC_{50} value of standard ascorbic acid (15.97 µg/mL).

Structure Elucidation of Pure Compound

In the aromatic region of the ^{1}H NMR spectrum (Figure 15.b), one doublet of doublet methine proton at δ 6.55 ppm displayed ortho coupling with one doublet methine proton at δ 7.40 ppm (J = 8.35 Hz) and meta coupling with another doublet methine proton at δ 6.41 ppm (J = 2.50 Hz). Therefore, 1, 2, 4-trisubstituted benzene ring (fragment a) could be drawn. Moreover, in the DQF COSY spectrum (Figure 15.f), the methine proton at δ 6.55 ppm showed correlation with another methine proton at δ 7.40 ppm as expected. In the HMBC spectrum (Figure 15.g), the methine proton at δ 6.55 ppm (δ_c 109.6) showed β-correlations with one sp^2 methine carbon at δ 103.6 ppm and one sp^2 quaternary carbon at δ 112.9 ppm. Moreover, one methine proton at δ 7.40 ppm (δ_c 132.3) revealed β-correlations with two sp^2 quaternary carbons at δ 156.7 and

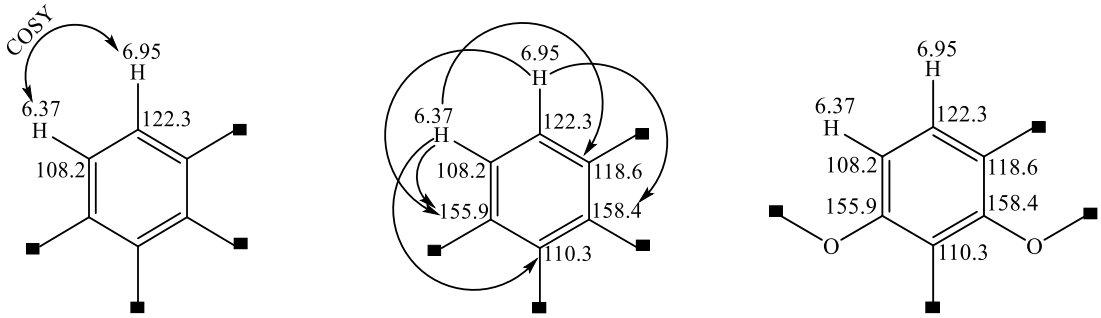
156.9 ppm. Furthermore, the HMBC spectrum (Figure 15.g) showed the observation of β-correlation of the methine proton at δ 6.41 ppm (δ_c 103.6) with one sp^2 methine carbon at δ 109.6 ppm and α-correlations with two sp^2 quaternary carbons at δ 156.7 and 156.9 ppm. According to these correlations, the carbon atoms in the benzene ring could be assigned. Moreover, two sp^2 quaternary carbons at δ 156.7 and 156.9 ppm could be connected to oxygen due to their downfield chemical shifts. Thus, the partial structure (I) could be elucidated.



(i) (ii) (iii)

Figure 6 (i) (↔) DQF COSY correlation, (ii) (→) HMBC correlations in fragment (a) and (iii) partial structures (I)

According to the ^{1}H NMR spectrum (Figure 15.b), one doublet methine proton at δ 6.37 ppm (δ_c 108.2) showed ortho coupling with another doublet methine proton at δ 6.95 ppm (δ_c 122.3) with the coupling constant of 7.98 Hz. Therefore, 1, 2, 3, 4-tetrasubstituted benzene ring could be drawn. In the DQF COSY spectrum (Figure 15.f), these two methine protons showed correlation as expected and fragment (b) could be confirmed. The HMBC spectrum (Figure 15.g) revealed β-correlations of methine proton at δ 6.37 ppm (δ_c 108.2) with two sp^2 quaternary carbons at δ 110.3 and 118.6 ppm and α-correlation with one sp^2 quaternary carbon at δ 155.9 ppm. Moreover, in the HMBC spectrum (Figure 15. g), one doublet methine proton at δ 6.95 ppm (δ_c 122.3) gave β-correlations with two sp^2 quaternary carbons at δ 155.9 and 158.4 ppm. According to these correlations, the carbon atoms in the benzene ring could be assigned. According to the downfield chemical shifts of two sp^2 quaternary carbons at δ 155.9 and 158.4 ppm, these carbons could be connected to oxygen. Thus, the partial structure (II) could be assigned.



(i) (ii) (iii)

Figure 7 (i) (↔) DQF COSY correlation, (ii) (→) HMBC correlations in fragment (b) and (iii) partial structures (II)

In the DQF COSY spectrum (Figure 15.f), the methine proton at δ 3.51 ppm which is attached to carbon at δ 40.1 ppm showed correlations with the sp^3 methine proton at δ 5.46 ppm (δ_c 78.1) and one of the diastereotopic methylene protons at δ 3.63 ppm (δ_c 66.6). Furthermore, the two diastereotopic methylene protons at δ 3.36 and 4.20 ppm showed correlation with each other.

According to these correlations, the fragment (c) could be assigned. In the HMBC spectrum (Figure 15.g), the doublet methine proton at δ 5.46 ppm (δ_c 78.1) showed β-correlation with the sp^3 methylene carbon at δ 66.6 ppm. Moreover, the two diastereotopic methylene protons at δ 3.63 and 4.20 ppm showed HMBC correlations to the two sp^3 methine carbons at δ 40.1 and 78.1 ppm. Thus, the fragment (c) could be confirmed.

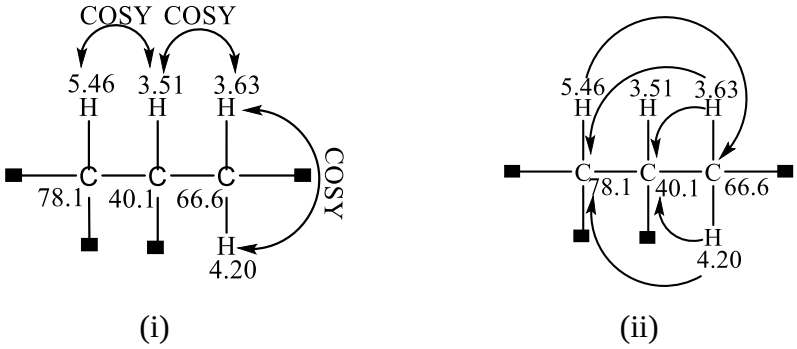


Figure 8 (i) (↔) DQF COSY correlation and (ii) (→) HMBC correlations in fragment (c)

According to the HMBC spectrum (Figure 15.g), the methine proton at δ 5.46 ppm (δ_c 78.1 ppm) showed correlations to two sp^2 quaternary carbons at δ 112.9 and 156.7 ppm and one sp^2 methine carbon at δ 132.2 ppm. Moreover, the methine proton at δ 7.40 ppm (δ_c 132.3) also showed the HMBC correlation to the sp^3 methine carbon at δ 78.1 ppm. Thus, the fragment (c) could be connected to the partial structure (I) as shown in partial structure (III). Furthermore, one of the diastereotopic methylene protons at δ 4.20 ppm which is attached to carbon at δ 66.6 ppm showed the HMBC correlation to the sp^2 quaternary carbon at δ 156.7 ppm.

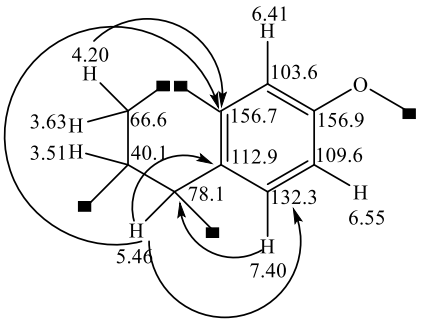


Figure 9 (→) HMBC correlations in partial structure (III)

According to the downfield chemical shifts of sp^3 methylene carbon at δ 66.6 ppm, the sp^3 methine carbon at δ 78.1 ppm and the sp^2 quaternary carbon at δ 156.7 ppm, these carbons could be connected to oxygen and the partial structure (IV) could be elucidated.

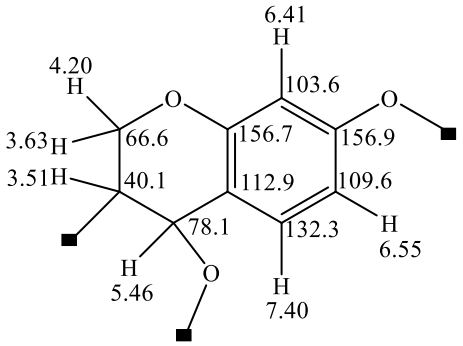


Figure 10 Partial structure (IV)

Furthermore, in the HMBC spectrum (Figure 15.g), the methine proton at δ 3.51 ppm (δ_c 40.1) gave α-correlation with the sp^2 quaternary carbon at δ 118.6 ppm from partial structure (IV). According to this correlation, the partial structure (II) and partial structure (IV) could be connected as shown in partial structure (V).

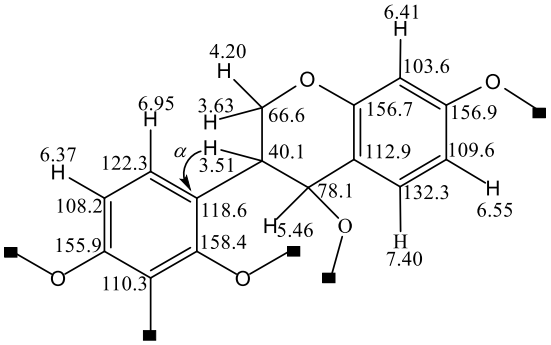
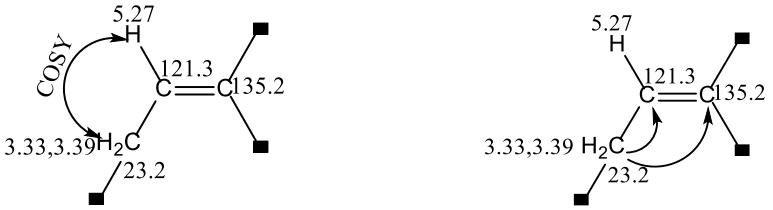


Figure 11 (→) HMBC correlations in partial structure (V)

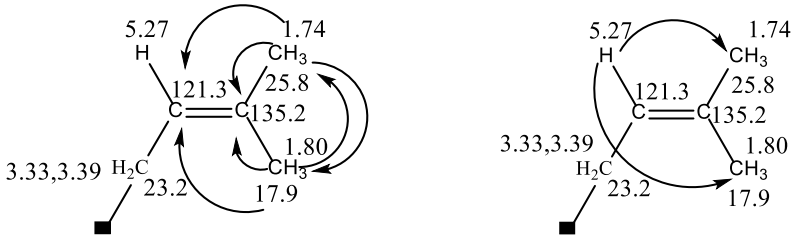
In the DQS COSY spectrum (Figure 15.f), the sp^2methine proton at δ 5.27 ppm which is attached to carbon at δ 121.3 ppm showed correlation with the doublet methylene protons at δ 3.33 and 3.39 ppm (δ_c 23.2). According to this correlation, fragment (d) could be assigned. Moreover, the HMBC spectrum (Figure 15. g) revealed the observation of β correlation of the methylene protons at δ 3.33 and 3.39 ppm (δ_c 23.2) with the sp^2 quaternary carbon at δ 135.2 ppm and α correlation with the sp^2 methine carbon at δ 121.3 ppm.



(i) (ii)

Figure 12 (i) (↔) DQF COSY correlation and (ii) (→) HMBC correlations in fragment (d)

In the HMBC spectrum (Figure 15.g), two methyl singlets at δ 1.74 ppm (δ_c 25.8) and 1.80 ppm (δ_c 17.9) showed correlation with each other. Moreover, these two methyl groups showed correlations with one sp^2 methine carbon at δ 121.3 ppm and one sp^2 quaternary carbon at δ 135.2 ppm. Furthermore, the sp^2 methine proton at δ 5.27 ppm (δ_c 121.3) showed HMBC correlation with two methyl carbons at δ 17.9 and 25.8 ppm. Therefore, the fragment (e) could be confirmed.



(i) (ii)

Figure 13 (i) and (ii) (→) HMBC correlations in fragments (e)

In the HMBC spectrum (Figure 15.g), the sp^3 methylene protons at δ 3.33 and 3.39 ppm (δ_c 23.2) showed correlations to the sp^2 quaternary carbons at δ 110.3, 155.9 and 158.4 ppm. According to these correlations, fragment (e) could be connected to partial structure (V) as shown in partial structure (VI).

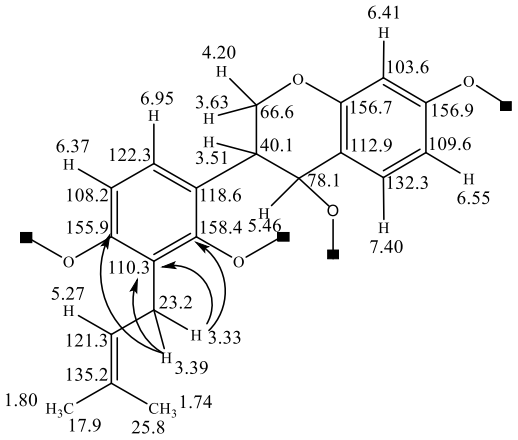
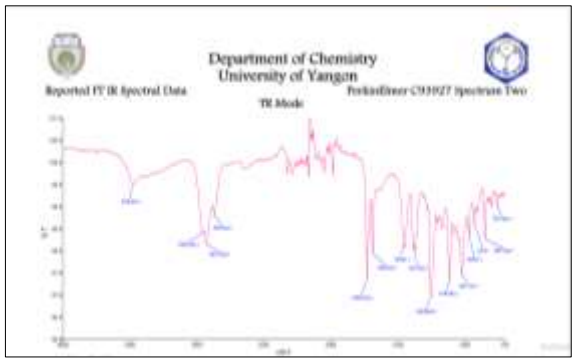


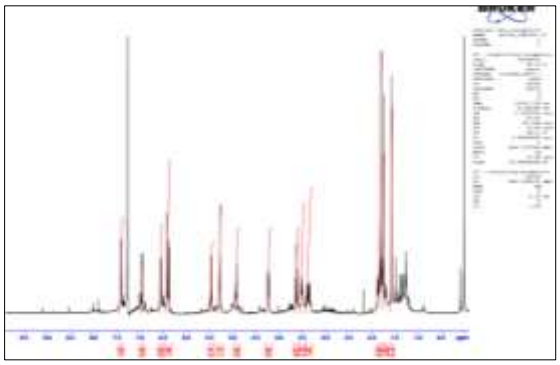
Figure 14 (→) HMBC correlations in partial structure (VI)

The partial molecular formula of compound was $C_{20}H_{18}O_5$ and molecular mass was 338. The (+)-DART mass spectrum (Figure 15.h) showed the peak, $[M+H-H_2O]^+$ at 325. Therefore, the molecular mass was deduced as 342 with the molecular formula of $C_{20}H_{22}O_5$. Thus, the remaining molecular mass was four and it was responsible for four hydrogen atoms. The structure of compound was elucidated 4', 6', 7-trihydroxy-5'-prenyl isoflavan-4-ol (1). Furthermore, the FT IR (Figure 15.a) spectrum of pure compound (1) revealed the presence of -OH group.

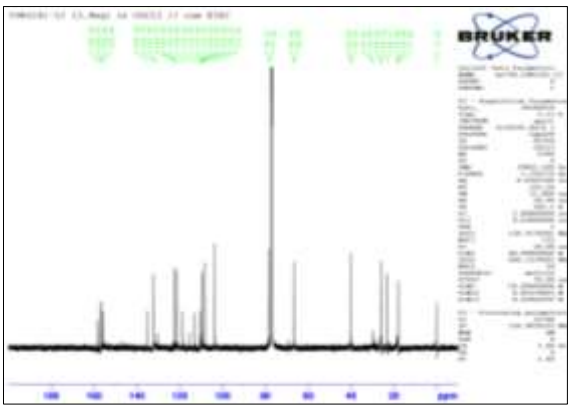
(a)



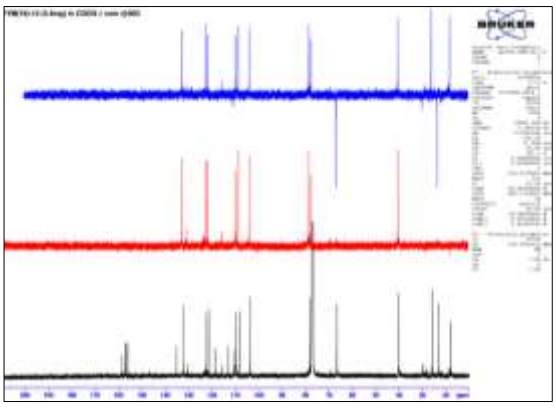
(b)



(c)



(d)



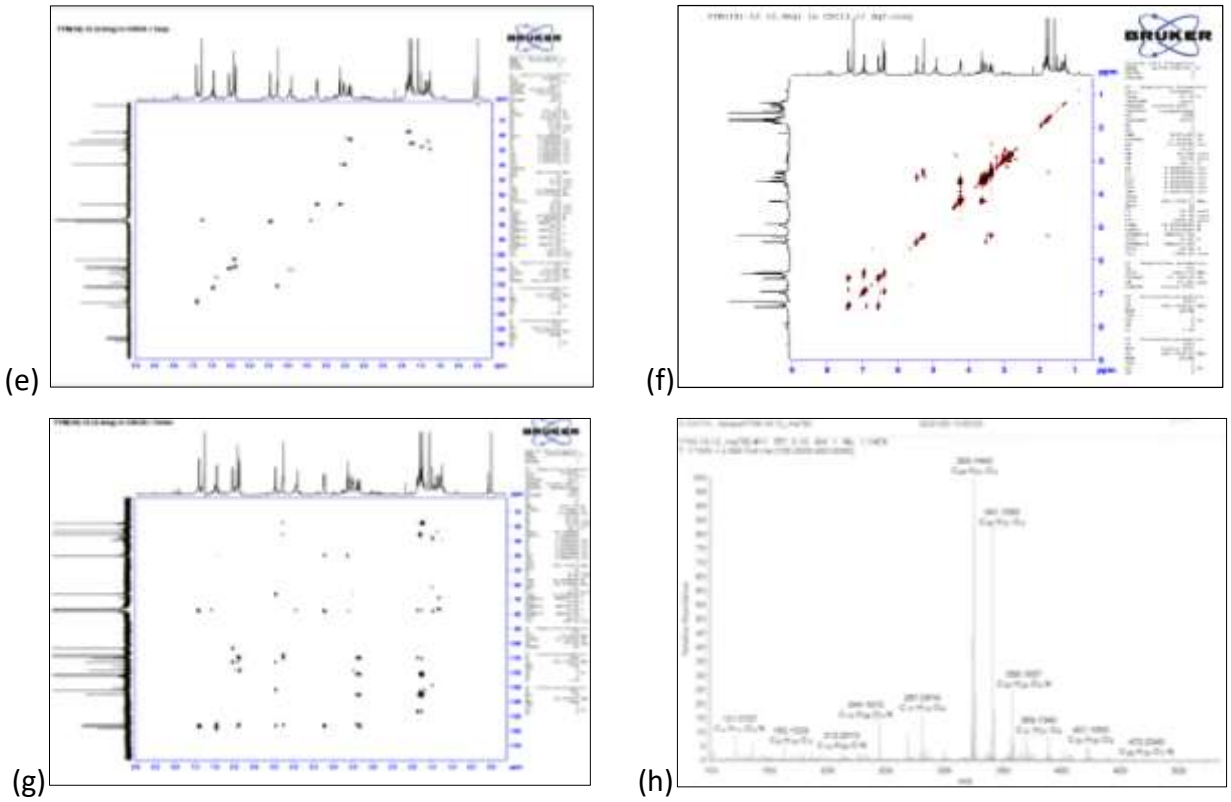


Figure 15 (a) FT IR, (b) ^{1}H NMR (c) ^{13}C NMR (d) DEPT (e) HSQC (f) DQF COSY (g) HMBC and (h) (+) DART mass spectra of 4',6',7-trihydroxy-5'-prenyl isoflavan-4-ol

Conclusion

In this research work, medicinal plant *Erythrina crista-galli* Linn. was selected for chemical screening due to its interesting medicinal uses. From ethyl acetate extract, isoflavan derivative, namely, 4',6', 7-trihydroxy-5'-prenyl isoflavan-4-ol was isolated and characterized. According to DPPH assay, IC_{50} value of methanolic extract was found to be 143.6 μg/mL. Therefore, methanolic extract showed antioxidant activity but less than ascorbic acid (IC_{50} 15.97 μg/mL), standard antioxidant.

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