INVESTIGATION OF ANTIDIABETIC ACTIVITY AND STRUCTURAL ELUCIDATION OF DIHYDROCHALCONE COMPOUND ISOLATED FROM MYANMAR TRADITIONAL MEDICINAL PLANT, *Grewia nervosa* (Lour.) Panigrahi

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Abstract

Grewia nervosa (Lour.) Panigrahi was distributed in Bangladesh, India, Sri Lanka, Cambodia, Laos, Thiland, Vietnam, Indonesia, Malaysia and Myanmar and it is locally called in Myanmar as Mya-yar. The roots of Mya-yar were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. Antidiabetic effect of methanol extract of root of *G. nervosa* was done at Department of Biotechnology, Mandalay Technological University, Pathein Gyi Township, Mandalay Region, Myanmar. In addition, dihydrochalcone compound was isolated from ethyl acetate extract of the selected sample by thin layer and column chromatography methods. The structure of this compound was elucidated by analysis of NMR spectroscopic and mass spectrometric data.

Keywords: thin layer and column chromatography, dihydrochalcone, Antidiabetic effect, *Grewia nervosa* (Lour.) Panigrahi, NMR

Introduction

Medicinal plants have played an essential role in the development of human culture. Plants are resources of traditional medicines and many of the modern medicines are produced in directly from plants. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries (Ahmadreza *et al*, 2015).

According to traditional beliefs in Myanmar, there are 96 diseases which afflict humans (Fame Pharmaceuticals, 2012). The belief in turning to nature to heal and cure is strong in nearly all Asian nations and in Myanmar. Traditional medicine treatments have been followed in Myanmar for generations and continue to be popular even today, though more in remote rural areas, not least due to non availability of western medicines. Traditional medicine uses plants and herbs to make powders, gels and tablets for treating disease and body disorders (Goldstein, 2000).

Diabetes mellitus refers to a group of diseases that affect how human body uses blood sugar (glucose). Glucose is vital to human's health because it is an important source of energy for the cells that make up muscles and tissues. It's also brain's main source of fuel. Chronic diabetes conditions include type 1 diabetes and type 2 diabetes. Diabetes symptoms vary depending on how much blood sugar is elevated (Dreschfeld, 1886).

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long- term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Knowler *et* al, 2002).

Type 1diabetes (insulin-dependent diabetes mellitus) is an autoimmune disease in which the pancreas is unable to produce enough of a hormone called insulin. This reduced insulin production results in a higher than normal level of glucose in the blood – a condition called hyperglycemia (Dillon *et al*, 1936).

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This form of diabetes, which accounts for nearly 90- 95 % of those with diabetes, previously referred to as non- insulin- dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative insulin deficiency (Meena *et al*, 2014).

G. nervosa, belonging to the family Malvaceae, is widely distributed along the Western Ghats of India. Although it has been commonly used in traditional medicine, the medicinal properties have not been scientifically evaluated. This plant contains phytochemical compounds and antioxidant activity. These results suggest that *G. nervosa* would be a potential source for treatment of diabetes and its associated complication such as oxidative stress and inflammation (Meena *et al*, 2014).

The aims of present research work are to evaluate antidiabetic activity and to elucidate the structure of isolated dihydrochalcone compound from root of Myanmar indigenous medicinal plant, *G. nervosa* (Figure 1).

Botanical Description

Figure 1 Leaves and roots of *Grewia nervosa* (Lour.) Panigrahi

Botanical name	: *Grewia nervosa* (Lour.) Panigrahi
Family	: Malvaceae
Myanmar name	: Mya- yar
Part used	: Roots
Distribution	: Bangladesh, India, Sri Lanka, Cambodia, Laos, Thiland, Vietnam, Indonesia, Malaysia and Myanmar (Ayeyarwady, Bago, Mangalay, Taninthayi, Yangon Regions and Mon State) (Panigrahi, 1985)
Medicinal uses	: Diabete mellitus, coughs, indigestion, eczema, itchy, small-pox, typhoid fever, dysentery and syphilitic ulceration of the mouth (Deepa *et al*, 2016)

Materials and Methods

Plant Material

The roots of *Grewia nervosa* (Lour.) Panigrahi, Myanmar named Mya-yar were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar, in June 2015.

Evaluation of Antidiabetic Activity

The selected *Mus musculus* mice were randomly divided into three groups such as positive control group, tested plant extract group and negative control group. Each group contains three animals. These mice were prepared to cause hyperglycemia by using adrenaline injection (Figure 2). For giving adrenaline injection, the selected mice were fasted overnight. The

animals were given intraperitoneally with adrenaline 0.2 mL/kg body weight in distilled water. They were starved for 4 hr after injection and then they were given 0.5 mL of glucose solution orally at hourly interval to prevent hypoglycemic shock. They were offered unlimited amounts of standard laboratory diet food and water. Mice were fasted for 18 hours prior to drug administration allowing access only to water (Figure 3). Before the drugs and vehicle administration, base line fasting blood sugar levels were measured Ominitest Glucometer in all groups.

In this experiment, the dosage of methanol extract of root of *G. nervosa* was calculated on the body weight basic for each animal. Before giving the extract to the animals, the methanol extract was dissolved in distilled water. After that, the following procedures were done.

1. Blood glucose levels of all mice were measured by Glucometer and test strips.
2. Positive control group was treated with standard drug Glibenclamide 0.5 mg/kg.
3. Sample control group was treated with 0.5 mg/kg of methanol extract sample solution.
4. Negative control group was treated with 0.5 mg/kg of distilled water.

During the experimental procedure, three observations were performed at five times (225 min) of 45 min interval after orally administration of the plant extract, Glibenclamide and distilled water by using Glucometer and test strips. The results were collected from each group for the data analysis.

(a) (b)

Figure 2 (a) Both sexes of 30 g *Mus musculus* mouse
(b) Injected adrenaline subcutaneously

(a) (b)

Figure 3 (a) Oral administration of plant extract
(b) Measured blood glucose level with Omniltest plus glucometer

Extraction and Isolation

The air-dried roots of Mya-yar were percolated with ethanol at room temperature for one month. The ethanol extract was concentrated and the residue (28.1 g) was extracted with ethyl acetate. The ethyl acetate extract was concentrated to produce a residue (2.88 g). The extract was fractionated on a silica gel column using *n*- hexane and ethyl acetate gradient to afford fifteen fractions (frs 1- 12). Fraction 9 was recrystallized by 25 % ethyl acetate in *n*- hexane to yield dihydrochalcone compound (21.5 mg).

Structural Elucidation of Dihydrochalcone Compound

The spectra of isolated pure compound were measured by ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, HMQC, DQF-COSY, HMBC and EI-MS at Meijo University, Nagoya, Japan.

Results and Discussion

Antidiabetic Activity of Methanol Plant Extract

The mean blood glucose levels of adrenaline induced hyperglycemic mice in methanol plant extract treated group, positive control group and negative control group were shown in Table 1.

Table 1 Antidiabetic Effect of Methanol Extract of Root of Mya-yar on Hyperglycemic Mouse Models

Group	Blood Glucose Level Mean ± SD (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
Tested plant extract	119±2.12	252±2.00	162±2.00	126±2.00	134±3.08	123±6.92
Positive control	123±9.33	265±3.24	185±2.35	127±1.58	128±5.79	104±2.35
Negative control	118±2.65	272±2.55	239±2.00	244±1.58	228±1.58	174±2.55

SD = Standard Deviation, Tested plant extract = MeOH extract,
Positive control = Glibenclamide drug, Negative control = Distilled water

The mean blood glucose levels of tested plant extract group, positive control group and negative control group were calculated and the changes in blood glucose levels of all groups are illustrated in Figure 4.

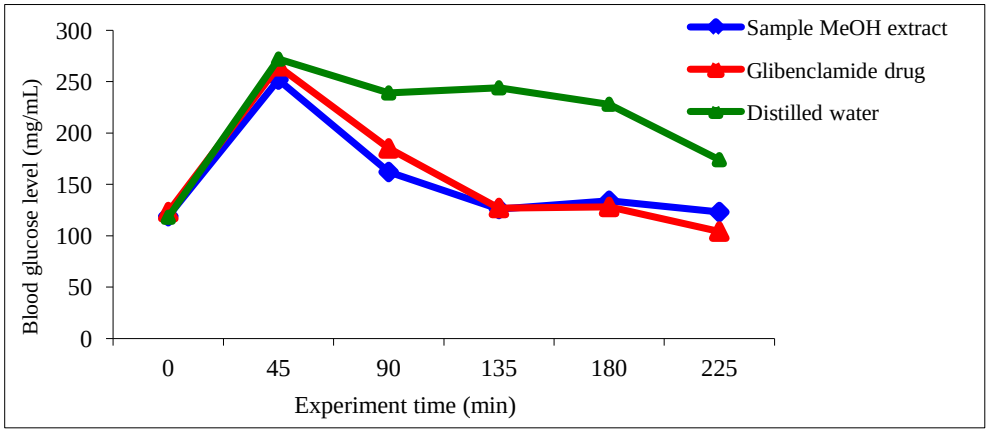


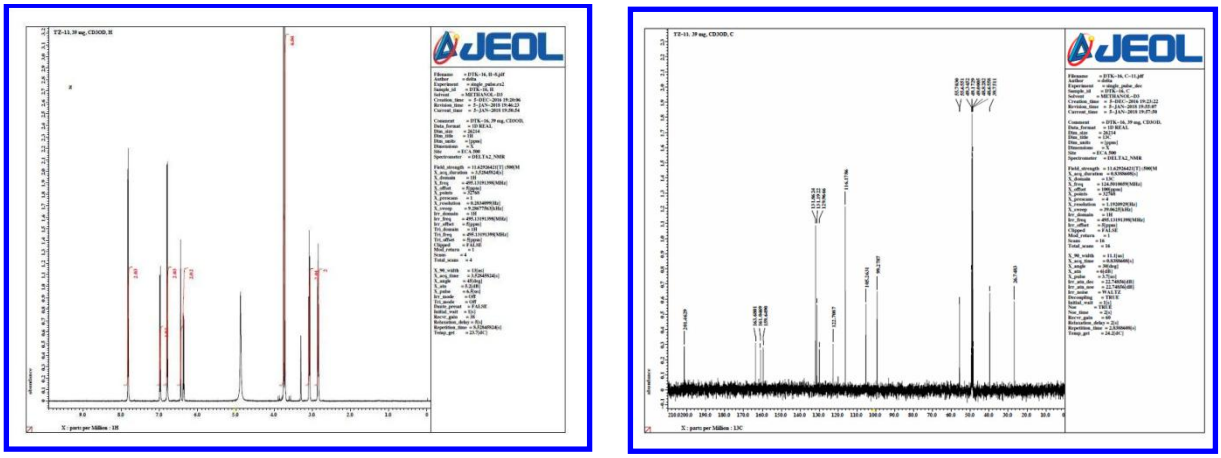
Figure 4 Changes of mean blood glucose levels of test group and control groups

The mean blood sugar levels of tested plant extract group showed hypoglycemic activity against the hyperglycemia response to adrenaline. There was significant reduction of fasting blood sugar level of test group compared with positive control group in this current study at 45, 90, 135, 180 and 225 min.

Determination of Structure of Dihydrochalcone Compound

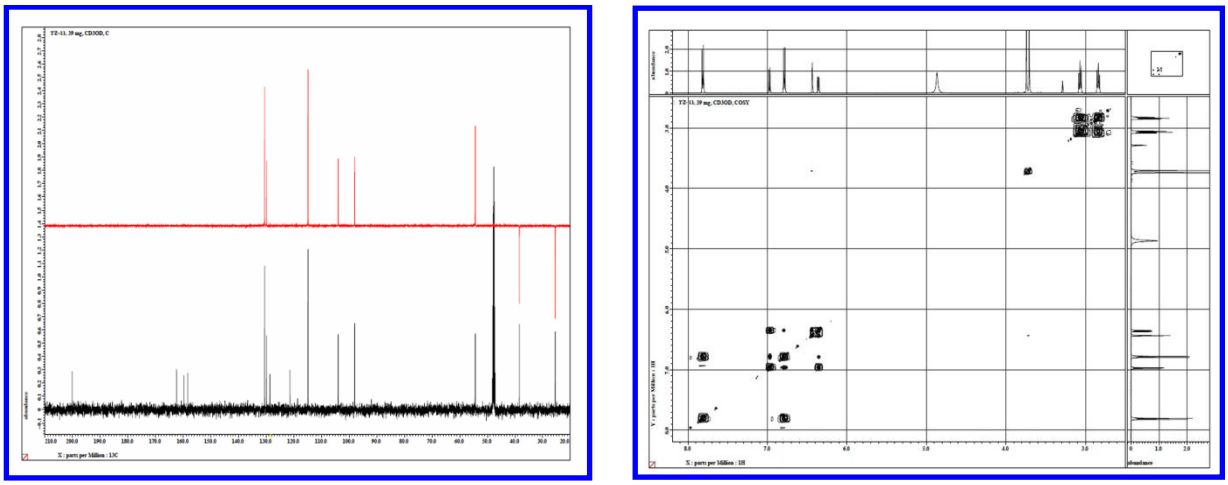
The structure of isolated pure compound was elucidated by ^{1}H NMR (500 MHz), ^{13}C NMR(125 MHz), DEPT, HMQC, DQF-COSY, HMBC and EI-MS spectra (Figure 5, 6, 7 and 8).

Dihydrochalcone Compound: FT IR (KBr) : (cm^{-1}) 3341.1 (-OH), 3072.6, 3000.6 (=CH), 2931.4, 2834.5 (Sat-H/C), 1657.9 (C=O), 1649.6, 1605.3, 1575.81, 1504.72 (ArH), 1364.4, 1336.7 (C-OH), 1206.73 (C-C-O), 1167.9, 1154.02, 1122.23 (C-O-C): ^{1}H NMR (500 MHz, CD_3OD) δ: 2.86 (2H, t, J= 7.23, H-2), 3.10 (2H, t, J= 7.23, H-3), 6.46 (1H, d, J= 2.42, H-2′), 7.00 (1H, d, J= 8.22, H-5′), 6.39 (1H, dd, J= 2.42, 8.22, H-6′), 6.82 (1H, d, J= 6.80, H-5″), 7.88 (1H, d, J= 6.80, H-6″), ^{13}C NMR (125 MHz, CD_3OD) δ: 201.46 (C-1), 26.75 (C-2), 39.75 (C-3), 122.71 (C-1′), 99.28 (C-2′), 161.05 (C-3′), 159.65 (C-4′), 131.19 (C-5′), 105.26 (C-6′), 129.97 (C-1″), 131.86 (C-2″, 6″), 116.18(C-3″, 5″), 163.68 (C-4″), EI-MS *m/z* (rel.int.): 286 [M^{+}] ($C_{17}H_{18}O_4$).



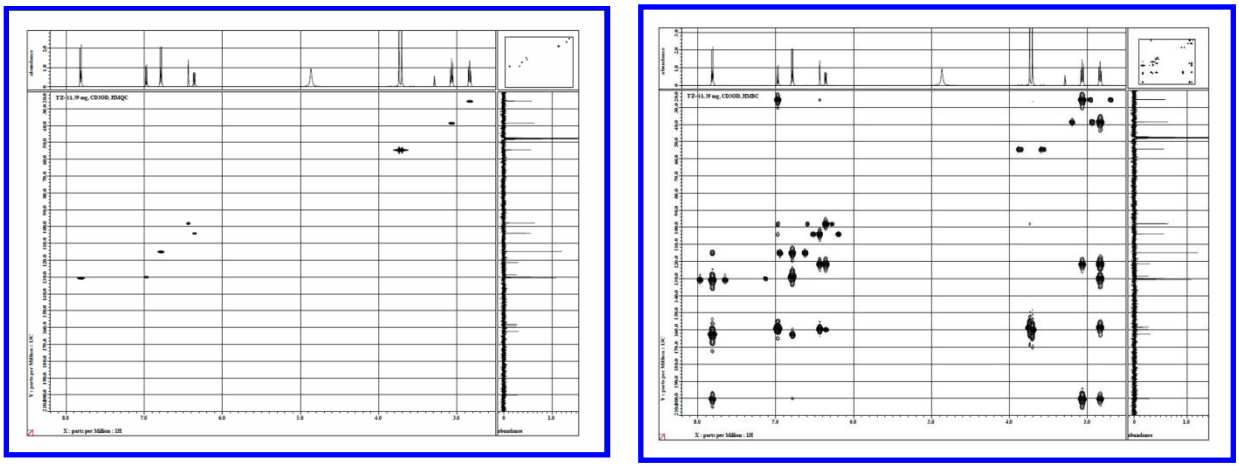
(a) (b)

Figure 5 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of dihydrochalcone compound



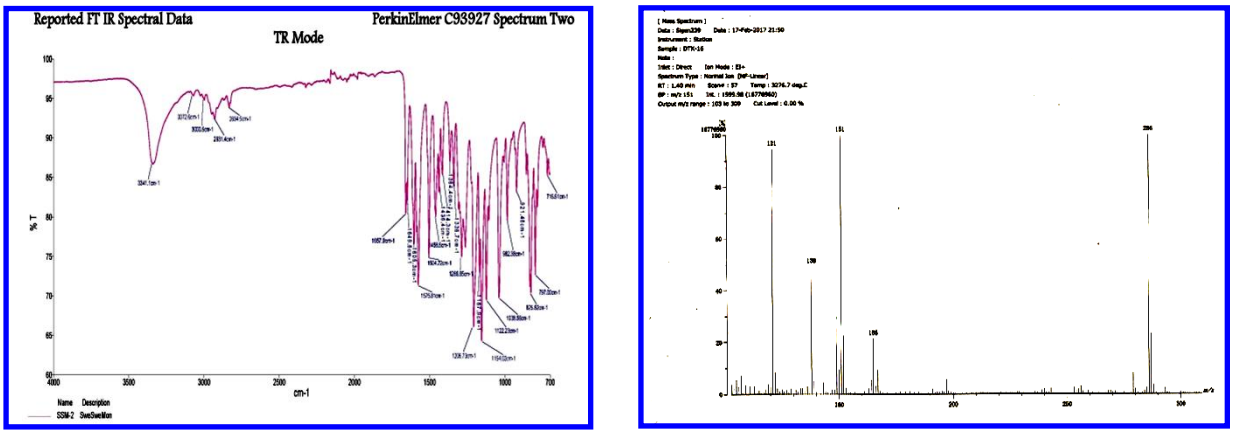
(a) (b)

Figure 6 (a) DEPT and (b) DQF-COSY spectra of dihydrochalcone compound



(a) (b)

Figure 7 (a) HMQC and (b) HMBC spectra of dihydrochalcone compound



(a) (b)

Figure 8 (a) FT IR and (b) EI-MS spectra of dihydrochalcone compound

According to these spectral data, the molecular formula of this dihydrochalcone compound, yellow needle shape crystal was found to be ($C_{17}H_{18}O_4$) from the observation of a molecular ion peak at *m/z* 286 [M^+] on EI- mass spectrometry. Elucidated structure of isolated compound could be identified with the previous reported data of dihydrochalcone compound.

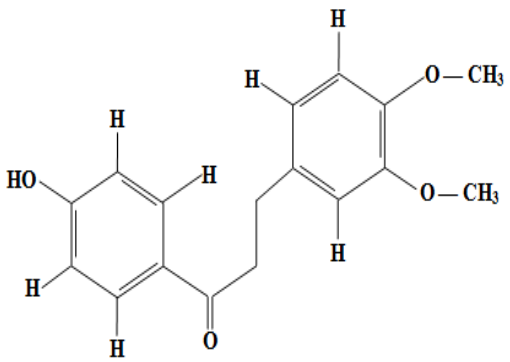


Figure 9 Structure of dihydrochalcone compound

Table 2 ^{13}C, ^{1}H NMR Spectral Data of Dihydrochalcone Compound and ^{1}H -^{13}C, ^{1}H-^{1}H Correlation Exhibited in NMR Spectra in CD_3OD

Carbon.	^{13}C(DEPT)	1H(J,Hz)	HMBC correlation	COSY correlation
1	26.75	2.86	C-1, C-1′	H-3
2	39.75	3.10	C-1, C-1′	H-2
3	55.65	3.73	CH_3, C-3′	-
4	55.70	3.76	CH_3, C-4′	-
5	99.28	6.46	-	H-6′
6	105.26	6.39	C-1′	H-5′, H-2′
7	116.18	6.82	C-1″	H-6″
8	122.71	-	-	-
9	129.97	-	-	-
10	131.19	7.00	C-4′, C-3′	H-6′
11	131.86	7.88	C-4″, C-1	H-5″
12	159.65	-	-	-
13	161.05	-	-	-
14	163.68	-	-	-
15	201.46	-	-	-

Conclusion

In the present investigation, antidiabetic activity of methanol extract of root of Mya-yar was tested by comparing control groups. At the end of the experiment, methanol extract of plant sample was significantly observed to possess the anti-diabetic effect as the standard drug. These results suggest that *Grewia nervosa* (Lour.) Panigrahi would be a potential source for the treatment of diabetes. Moreover, the isolation of dihydrochalcone compound was described. Further studies are required and are in progress here.

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