INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES AND ISOLATION OF PHYTOCONSTITUENTS FROM THE LEAVES OF *DREGEA VOLUBILIS* (L. F.) (GWAY-TAUK)

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Abstract

The study aims to investigate some biological activities and the isolation of phytoconstituents from the leaves of *Dregea volubilis* (L. f.). *Dregea volubilis* belongs to the Apocynaceae family, and it is locally known as "Gway-tauk" in Myanmar. In our experiments, six compounds (**1-6**) were isolated from the EtOAc extract of Gway-tauk leaves, and they were characterized by some physico-chemical tests, R_f values on TLC, and modern spectroscopic methods such as UV and FT IR. The antimicrobial activity of EtOH extract showed moderate activity with the inhibition zone diameter range between 10~15 mm against eight tested microorganisms. Then, EtOH and watery extracts were found to have no cytotoxic effect on brine shrimp up to the maximum dose of 1000 µg/mL. Moreover, the watery extract (bitterness value, 320) of Gway-tauk leaves was less bitter than standard quinine hydrochloride R (bitterness value, 2000). In the α-amylase inhibitory activity, the IC_{50} values of ethanol and watery extracts were observed to be 3.64 and 2.67 µg/mL. The antioxidant activity of ethanol (IC_{50}: 3.54 µg/mL) and watery (IC_{50}: 5.11 µg/mL) extracts of Gway-tauk leaves possessed potent antioxidant activity, as well as positive control ascorbic acid (IC_{50}: 2.60 µg/mL).

Keywords: *Dregea volubilis*, antimicrobial activity, cytotoxicity, bitterness value, α-amylase inhibitory activity, antioxidant activity

Introduction

Herbal medicines are traditionally used for the treatment of various illnesses. Hence, medicinal plants have been receiving great attention worldwide from researchers because of their safe utility. Medicinal plants that provide a large group of economically important plants provide the basic raw materials for indigenous pharmaceuticals (Natarajan *et al*., 2013). Myanmar is the second-largest country in Southeast Asia, and about half of the land area is covered with forest. Myanmar has been using herbal remedies for medicinal purposes due to the rich diversity of medicinal plants in various climate change zones. Approximately 11,800 species belonging to 273 families of plants have been recorded in the Myanmar flora. Several of these plant species are used in traditional cosmetics and/or folk medicine. However, most of the scientific evidence for the bioactivities of these medicinal plants and phytochemical constituents is still behind the scenes. The plant *D. volubilis* is a large, woody, twining perennial shrub of the Apocynaceae family. It is a stocky, smooth, frosted, woody vine (Karthika *et al*., 2012). The leaves are ovate, 7.5 to 15 cm long, 5 to 10 cm wide, rather leathery, rounded, or pointed at the base. The flowers are green in clusters, about 1 cm wide. The fruits are usually double, broadly lanceolate, 7.5 to 10 cm long, plump, longitudinally ribbed, and velvety until ripe (Barathamma *et al*., 2015). The plant is found in Southeast Asia, including India, Sri Lanka, Indonesia, Bangladesh, Cambodia, Vietnam, Malaysia, and the Philippines, Thailand, China, and Myanmar. It is widely used to treat eye infections, tumors, asthma, skin diseases, rheumatic pain, cough, fever, severe colds, dyspepsia, urinary tract infections, and hemorrhoids (Pandikumar *et al*., 2007; Biju, 2007; Rajadurai *et al*., 2009). Recently, numerous studies have suggested that the extract from *D. volubilis* leaf specimens has many biological activities. However, scientific evidence from this sample has not been reported yet in Myanmar. Therefore, in this present study, *D. volubilis* (Gway-tauk) leaves have been chosen to investigate some biological activities and isolate phytoconstituents.

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Materials and Methods

Plant Material

Dregea volubilis leaves were collected from Mawlamyine Township, Mon State, in November 2021. After collection, the sample was confirmed at the Department of Botany, University of Yangon. The fresh leaves were cleaned by washing them with water and air-drying at room temperature. The dried leaves were cut into small pieces and ground into powder by using a grinding machine. The powdered sample was stored in an airtight container to prevent contamination and kept for the isolation of organic compounds and screening biological activities.

Materials, Equipments, and Instruments

Ascorbic acid, acarbose (standard) and α-amylase enzyme (from human saliva),agar, caffeine , DPPH (2,2-diphenyl-1-picrylhydrazyl), distilled water, ethyl acetate, 95 % ethanol, glucose, peptone, yeast, 0.1 M hydrochloric acid, 1 mM iodine solution, petroleum ether PE (b.pt 60 –80 °C), 0.02 M phosphate buffer solution, potassium dichromate ($K_2Cr_2O_7$), purified drinking water, 0.5% starch solution, quinine hydrochloride R, sodium chloride (NaCl)

Air pump, automatic high-speed autoclave (Model S-90 N, Tomy Seiko Co., Ltd., chambers, clean bench (Hitachi Ltd., Japan), hot plate, hot oven (Modern, GM-10 E (DRWG, No.9 B-815051)), a refrigerator and incubator box (Sanyo Co., Ltd.), quartz cuvette (4 mL) and UV-visible spectrophotometer (GENESYS 10 S UV-VIS, China), water bath (Yamoto Model BT-18 No. 157)

Preparation of Crude Extracts

Air-dried powdered leaves of *Dregea volubilis* (L. f.) Benth ex Hook. f. (Gway-tauk) (400 g) was extracted with ethanol (2 L) by sonication (1 h × 6 times) and filtered. The filtrate was evaporated to get a crude extract. The crude ethanol extract was partitioned with PE (800 mL) and water (50 mL) by using a separatory funnel. When the solvent was removed, a PE extract was obtained. Then, the aqueous layer was partitioned with EtOAc (800 mL), resulting in EtOAc and H_2O extracts.

Isolation of Organic Compounds from Gway-tauk Leaves

Organic compounds were separated from the crude extract by the column chromatographic method. Silica gel (200 g) was used as an adsorbent and a PE: EtOAc mixture was used as eluent with different solvents in ratios from non-polar to polar. A total of 15 fractions were obtained. Each individual fraction was checked by TLC using 5 % H_2SO_4 as the visualization agent. The fractions with the same R_f values were combined, and nine combined fractions were obtained. Among them, Fraction F-2 was chromatographed by normal-phase silica gel open column chromatography with PE: EtOAc (19:1, v/v) as a solvent system, to obtain compound **1** (20 mg, 0.005%) and compound **2** (50 mg, 0.013%). The fractions F-3, F-5, and F-6 were purified by silica gel open column PE: EtOAc (9:1, 1:2, and 1:5, v/v) to obtain compound **3** (40 mg, 0.01%), compound **4** (15 mg, 0.004%) and compound **5** (16 mg, 0.004%). Compound **6** (60 mg, 0.015%) was obtained from the fraction by silica gel column chromatography, using EtOAc: MeOH (90:1, v/v) as a solvent system.

Antimicrobial Activity of Various Crude Extracts of *Dregea volubilis* (L. f.) Benth ex Hook. f. (Gway-tauk) Leaves by Agar Well Diffusion

The antimicrobial activity of PE, EtOAc, EtOH, and H_2O from leaves of Gway-tauk were determined against eight strains of microorganisms such as *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* by employing Agar well diffusion method. In this antibacterial activity assay, the nutrient agar (20-25 mL) was boiled, poured into the test tube, sealed with cotton, and autoclaved at 121 °C for 15 min (Finegold, 1978). After autoclaving, the tubes were cooled to 30-35 °C and poured into sterilized petri dishes, and 0.1-0.2 mL of test organisms were also added to them (Cruickshank, 1960). They were allowed to settle the agar for 2-3 h. After the agar was set, 8 mm agar wells were made using the agar well cutter. Thereafter, about 0.2 mL of the sample, namely the PE, EtOAc, EtOH, and H_2O solutions of Gway-tauk leaves, was added to each well and incubated at 37 °C for 24 h. The zone of inhibition that appeared around the agar well indicated the presence of antimicrobial activity.

Determination of Cytotoxicity of Ethanol and Watery Extracts of Gway-tauk Leaves

Crude extracts of Gway-tauk leaves were investigated by brine shrimp lethality bioassay according to the procedure described by Mayer *et al*. (1982). The brine shrimp was used in this study for cytotoxicity bioassay (Ali *et al*., 2013). Artemia cysts (0.1 g) were added to the 300 mL separating funnel of artificial sea water. Each extract and standard (5 mg) were dissolved in (5 mL) of distilled water to obtain a stock solution (1000 µg/mL) from that the concentrations of each solution (1000, 100, 10, and 1 µg/mL) were prepared bytenfold diluted with distilled water. After that, artificial seawater (9 mL) and (1 mL) of various concentrations of samples and standard solutions were added to each chamber. Live brine shrimp (10 nauplii) were then removed with a Pasteur pipette and added to each chamber. They were incubated at RT for about 24 hours. After 24 hours, the number of dead or surviving *Artemia* was counted, and the 50 % lethal dose (LD_{50}) was calculated by a linear regressive Excel programme (Sahgal *et al*., 2010). The control solution was prepared by using distilled water in place of the sample solution.

Determination of Bitterness Values

Bitters are medicinal plant materials that have a strong bitter taste. The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of materials with that of dilute solution of quinine hydrochloride R. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1 g of quinine hydrochloride R in 2000 mL (WHO, 1998).

$$\text{Bitterness value} = \frac{2000 \times C}{A \times B}$$

Where,

A = concentration of stock solution (C_s) mg/mL

B = volume of (V_s) mL tube with threshold bitterness concentration

C = quantity of quinine hydrochloride (in mg) tube with threshold bitter Concentration

Determination of *α*-Amylase Inhibition Potency

Alpha amylase is an enzyme that hydrolyzes the alpha-bonds of large alpha linked polysaccharides such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed and later employed by others for determination of amylase activity in plant extracts with some modifications (Yang *et al*., 2012). In the *α*-amylase assay, the starch-iodine method was used. The percent inhibition of each sample solution was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ = the absorbance of the control solution

A_{sample} = the absorbance of sample solution

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The antioxidant activity of 70 % ethanol and watery extracts was measured by the DPPH Free Radical Scavenging Assay (Lee *et al*., 2002). The active free radical scavenging of DPPH (2,2-diphenyl,1-picrylhydrazyl) was determined by a spectrophotometric method. The following equation was used to calculate the percentage inhibition of each plant material: IC_{50} values (half-maximal inhibitory concentration) were calculated using the linear regressive Excel programme.

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ = the absorbance of control solution

A_{sample} = the absorbance of tested sample solution

Results and Discussion

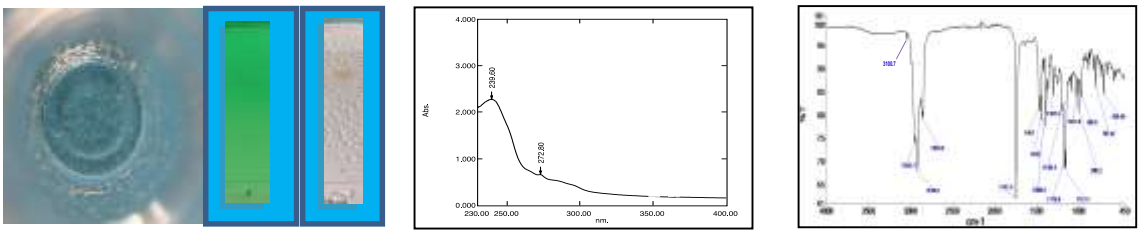
Identification of Isolated Compounds from Gway-tauk Leaves

The six isolated compounds (1-6) from the EtOAc extract of the leaves of Gway-tauk were identified by chemical tests, TLC, UV and FT IR spectroscopy.

Identification of isolated compound 1

Compound **1** (0.005 % yield) was isolated as a colourless needle crystal from an EtOAc extract of the leaves of Gway-tauk. It is soluble in PE, $CHCl_3$, EtOAc, and acetone, but MeOH, EtOH, and H_2O are insoluble. It is UV active and the R_f value was found to be 0.71 in PE: EtOAc (19:1 v/v). Compound **1** was classified as steroid compound. Because the reaction with the Libermann-Burchard test produced green coloration, compound 1 was classified as a steroid compound. The presence of C=C resulted a yellow spot on the TLC chromatogram with iodine vapour as shown in (Figure 1(a)). It also provided a yellow colour on TLC when treated with 5 % H_2SO_4 followed by heating. The UV spectrum of compound **1** in MeOH is shown in (Figure 1 (b)). According to the UV spectrum, the main absorption bands were found at 239 and 272 nm. Therefore, compound **1** contained a double bond conjugation. The functional groups present in compound **1** were also examined by FT IR spectroscopy. The presence of =C-H stretching and bending of the alkene group appears at 3100 cm^{-1} and 995 cm^{-1}. The bands at 1742 cm^{-1} indicated

the stretching vibration of C=O in the carbonyl group. The characteristic bands at 2918 and 2850, 1442 and 1388, 1170, and 1152 cm^{-1} also indicated the presence of C-H stretching of asym and sym CH_2 and CH_3 groups, C-H bending of methylene and methyl groups, and C-O stretching of the cyclic O group (Figure 1(c)). All of the above results, such as R_f value, chemical properties, and UV and FT IR spectral data of compound **1,** may be considered as steroid compounds containing ester groups.



(a) **(b)** **(c)**

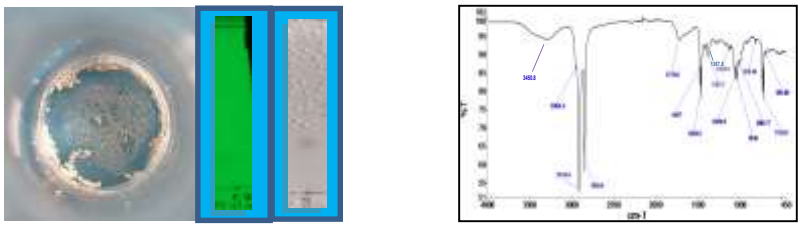
Figure 1. (a) TLC of isolated compound 1

(b) UV spectrum of isolated compound 1 in MeOH

(c) FT IR spectrum of isolated compound 1

Identification of isolated compound 2

Compound 2 (0.013 %) was isolated from an EtOAc extract of Gway-tauk leaves as a white, amorphous substance. It is soluble in PE, $CHCl_3$, and EtOAc but insoluble in acetone, MeOH, EtOH, and H_2O. It is UV inactive, and the R_f value was found to be 0.36 in PE: EtOAc (19:1 v/v). Compound **2** was classified as an organic acid since the reaction with bromocresol green gave a yellow coloration (Figure 2(a)). It also provided a pink colour to TLC when treated with 5 % H_2SO_4 followed by heating. The functional groups present in compound **2** were also studied by FT IR spectroscopy as shown in Figure 2 (b). The presence of O-H stretching in the alcoholic group was also confirmed by the appearance of a peak at 3450 cm^{-1}. The bands at 1713 cm^{-1} showed stretching vibrations of C=O in the carbonyl group. The characteristic bands at 2918 and 2850, 1463, 1377, 1367, 1059 and 1038 cm^{-1} also showed the presence of C-H stretching and bending of CH_2 and CH_3 groups, C-H bending of the methylene and methyl groups, O-H bending of the alcoholic group and C-O stretching of the cyclic-O group. All of the above-mentioned results obtained from R_f value, physico-chemical characterization, and modern spectroscopic techniques such as FT IR spectral data, indicate that the compound **2** may be considered an organic acid compound.



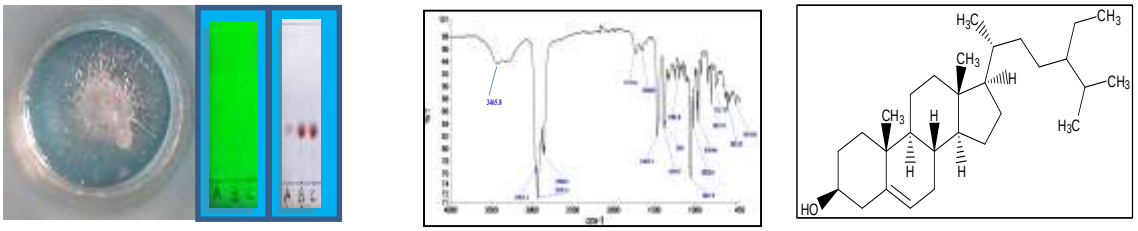
(a) **(b)**

Figure 2. (a) TLC of isolated compound 2

(b) FT IR spectrum of isolated compound 2

Identification of isolated compound 3

Compound 3 was isolated as a colourless needle crystal from an EtOAc extract of Gway-tauk leaves (0.01 % yield). The melting point is 138-140 ºC. The FT IR spectral data of compound **3** were found to be 3645, 2932, 2850, 1645, 1463, 1377, 1367, 1052 and 959 cm^{-1} (Patra *et al*., 2010). The compound **3** was found to be similar to those of reported *β*–sitosterol. So, the compound **3** was assigned as *β*-sitosterol and its chemical structure is shown in Figures 3 (a, b, c).

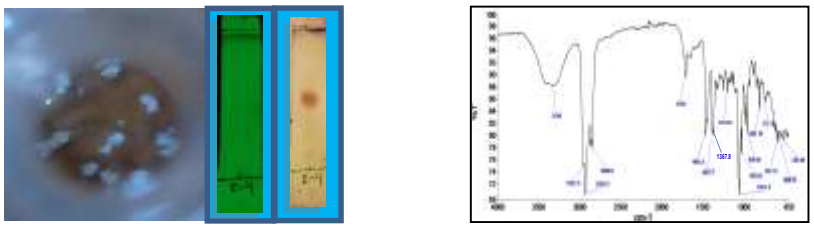


(a) **(b)** **(c)**

Figure 3. (a) Image of morphology and TLC of isolated compound 3
(b) FT IR spectrum of isolated compound 3
(c) Chemical structure *β*-sitosterol ($C_{29}H_{50}O$)

Identification of isolated compound 4

Compound **4** (0.004% yield) was isolated as a colourless crystal from an EtOAc extract of the leaves of Gway-tauk. It is soluble in PE, $CHCl_3$, EtOAc, and acetone but insoluble in MeOH, EtOH, and H_2O. It is UV inactive, and the R_f value was found to be 0.51 in PE: EtOAc (1:2 v/v). Compound **4** was classified as an organic acid compound since the reaction with bromocresol green gave yellow coloration. It also provided a cherry red colour on TLC when treated with 5 % H_2SO_4, followed by heating (Figure 4(a)). From the spectrum, the presence of O-H stretching of the alcoholic group could also be confirmed, with the peak appearing at 3319 cm^{-1}. The bands at 1703 cm^{-1} indicated the stretching vibration of C=O in the carbonyl group. The characteristic bands at 2932 and 2850, 1463 and 1377, and 1052 cm^{-1} also indicated the presence of C-H stretching of asym and sym CH_2 and CH_3 groups, C-H bending of methylene and methyl groups, and C-O stretching of the cyclic O group. With all of the above data obtained from the R_f value, physico-chemical data, and FT IR spectral data, compound **4** may be considered an organic acid compound (Figure 4(b)).

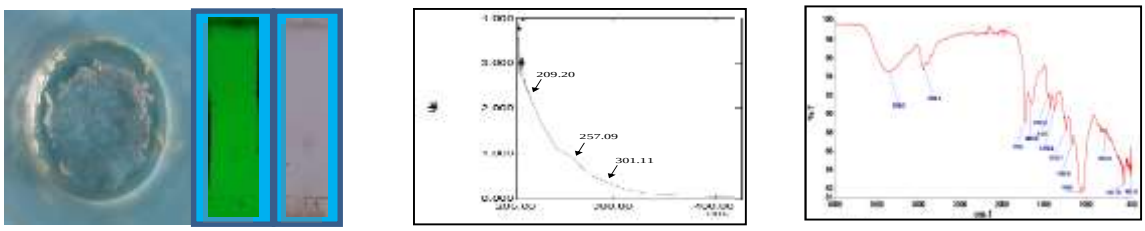


(a) **(b)**

Figure 4. (a) TLC of isolated compound 4
(b) FT IR spectrum of isolated compound 4

Identification of isolated compound 5

Compound **5** (0.004 % yield) was isolated as a white amorphous compound from an EtOAc extract of the leaves of Gway-tauk. It is soluble in $CHCl_3$, EtOAc, acetone, MeOH, and EtOH but insoluble in H_2O. It is partially dissolved in PE. It is UV active, and the R_f value was found to be 0.27 in PE: EtOAc (1:9 v/v). Compound **5** was classified as an organic acid compound since the reaction with bromocresol green gave it a green coloration. It was classified as terpenoids compound since the reaction with Libermann-Burchard test gave red colouration. It also provided a pink colour to TLC when treated with 5 % H_2SO_4 followed by heating. And then, it was given a blue colour on TLC when treated with vanillin followed by heating (Figure 5(a)). The UV spectrum (Figure 5 (b)), of compound **5** revealed the absorption maxima (λ_{max}) at 216 nm and 257 nm in MeOH indicating the presence of a conjugated double bond group due to $\pi \rightarrow \pi^*$ transition. Compound **5** is illustrated in (Figure 5 (c)). The broad band ranging between 3550~2500 cm^{-1} indicated a carboxylic acid –COOH group and a –OH stretching vibration. The absorption band which appeared at 3358 cm^{-1} showed the presence of an alcoholic group. The band at 1728 cm^{-1} referred to α, β-unsaturated carbonyl group and at 1645 cm^{-1} and 1452 cm^{-1} indicated the presence of C=C group of aromatic rings. In addition, the absorption bands at 1370 cm^{-1} are appeared due to O-H bending vibration and the absorption bands at 1234 cm^{-1}, 1166 cm^{-1} and 1056 cm^{-1} due to C-O stretching vibration of the alcoholic group. All of the above data obtained from R_f value, physico-chemical and UV and FT IR spectral data, compound **5** may be considered as terpenoid compound containing acid group.

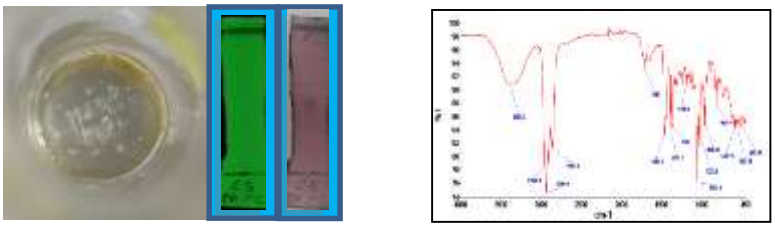


(a) **(b)** **(c)**

Figure 5. (a) TLC of isolated compound 5
(b) UV spectrum of isolated compound 5 in MeOH
(c) FT IR spectrum of isolated compound 5

Identification of isolated compound 6

Compound **6** (0.015 % yield) was isolated as a colorless crystal from an EtOAc extract of the leaves of Gway-tauk. It is soluble in EtOAc, $CHCl_3$, and MeOH but insoluble in PE, acetone, and EtOH. It is partially dissolved in H_2O. It is UV inactive and the R_f value was found to be 0.48 in EtOAc: MeOH (90:1 v/v). Compound **6** was classified as a glycoside compound since the reaction with 10 % lead acetate solution gave white precipitation. It also provided a blue colour on TLC when treated with vanillin followed by heating (Figure 6(a)). The functional groups present in compound **6** were also studied by FT IR spectroscopy. FT IR spectrum (KBr, ν_{max} cm^{-1}) of isolated compound **6** is illustrated in Figure 6 (b). The presence of O-H stretching and bending in the alcoholic group could be confirmed by with the peaks appearing at 3397 and 1377 cm^{-1}. The bands at 1703 cm^{-1}, suggested the stretching vibration of C=O in carbonyl group. The characteristic bands at 2932, 2850, 1463, 1191, 1052, and 1023 cm^{-1} also showed the presence of C-H stretching and bending in the CH_2 group and C-O stretching of the alcoholic group. All of the above results obtained from R_f value, physico-chemical characterization, and modern spectroscopic techniques such as FT IR spectral data suggest that the compound **6** may be considered a glycoside compound containing an ester group.



(a) **(b)**

Figure 6. (a) TLC of isolated compound 6
(b) FT IR spectrum of isolated compound 6

Antimicrobial Activity of Various Crude Extracts of *Dregea volubilis*

The antimicrobial activities of various crude extracts of *D. volubilis* (Gway-tauk) leaves such as PE, EtOAc, EtOH, and H_2O were investigated by the agar well diffusion method. From the results, it was found that EtOH extract possessed moderate activity with the inhibition zone 10-15 mm against all tested microorganisms. EtOAc and H_2O extracts exhibited the inhibition zone diameters in the range of 10-14 mm against the six tested microorganisms. Except for the other six microorganisms, PE extract showed antimicrobial activity with inhibition zone diameters of 9 mm against two microorganisms, *B. subtilis* and *C. albicans*. The observed data are summarized in Table 1.

Table 1. Inhibition Zone Diameter of Various Crude Extracts from Leaves of Gway-tauk by Agar Well Diffusion Method

No.	Microorganisms	Diameter of inhibition zone (mm) in various crude extracts				
		PE	EtOAc	EtOH	H_2O	STD
1	*A. tumefaciens*	-	14	10	-	18
2	*B. pumilus*	-	12	10	12	17
3	*B. subtilis*	9	12	13	11	17
4	*C. albicans*	9	12	14	10	15
5	*E. coli*	-	12	15	10	18
6	*M. luteus*	-	-	10	11	17
7	*P. fluorescens*	-	13	12	-	19
8	*S. aureus*	-	-	10	11	17

Diameter of agar well = 8 mm 15 mm - 19 mm activity = (++) Medium
10 mm - 14 mm activity = (+) Low 20 mm above activity = (+++) High

Cytotoxicity of Ethanol and Watery Extracts of Gway-tauk Leaves

According to the results, the cytotoxicity of ethanol and watery extracts from *D. volubilis* (Gway-tauk) leaves was evaluated by brine shrimp lethality bioassay. The ten of *Artemia salina* are used in each chamber. The LD_{50} values of both ethanol and watery extracts were found to be > 1000 g/mL. It means that the Gway-tauk leaves have no cytotoxic activity. Standard caffeine showed no cytotoxicity up to a concentration of100 g/mL, whereas the cytotoxicity of standard $K_2Cr_2O_7$ was LD_{50} - 43.75 g/mL. The results are shown in Table 2.

Table 2. Cytotoxicity of Ethanol and Watery Extracts of Leaves of Gway-tauk and Standard on *Artemia salina* (Brine Shrimp)

No.	Tested samples	Dead % by using different concentration (µg/mL) of samples (Mean ± SEM)				LD_{50} (µg/mL)
		1	10	100	1000	
1	Watery extract	17 ± 0.99	23 ± 0.38	30 ± 0.65	40 ± 0.00	>1000
2	EtOH extract	25 ± 1.00	40 ± 0.00	43 ± 1.15	47 ± 0.58	>1000
3	*Caffeine	0 ± 0.00	23 ± 0.00	30 ± 0.00	40 ± 0.00	>1000
4	*$K_2Cr_2O_7$	7 ± 1.00	20 ± 0.00	100 ± 0.00	100 ± 0.00	43.75

*Used as cytotoxic standard

The Bitterness Values of Gway-tauk Leaves

The bitter properties of plant substances are determined by comparing the threshold bitter concentration of an extract with that of standard standard quinine hydrochloride. The watery extract of Gway-tauk gives a bitter sensation (320). The tested sample was found to be less bitter than standard quinine hydrochloride (2000).

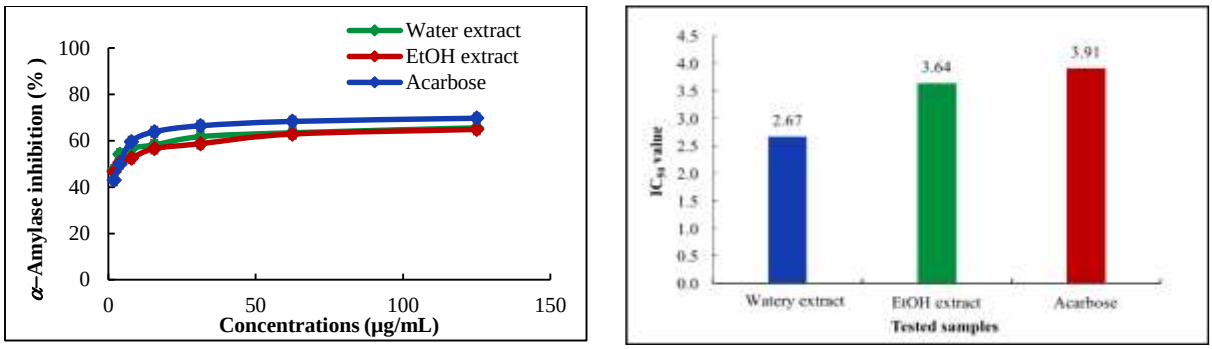
α-Amylase Enzyme Inhibition Activity of Ethanol and Watery Extracts of Gway-tauk Leaves

The *α*-amylase inhibitory activities of ethanol and watery extracts of Gway-tauk leaves were investigated by the starch- iodine method. The percentage inhibition of ethanol and watery extracts was studied at concentrations of (125, 62.5, 31.25, 15.62, 7.81, 3.92 and 1.95 µg/mL), respectively. The percent inhibition of *α*-amylase activity of watery extract (IC_{50} - 2.67 µg/mL) is more potent than that of ethanol extract (IC_{50}- 3.64 µg/mL). These two extracts exhibited higher activity than standard acarbose (IC_{50} = 3.91 µg/mL). These observations are detailed in Figure 7 and Table 3.

Table 3. *α*-Amylase Inhibition % and IC_{50} of the Crude Extracts of Gway-tauk Leaves and Standard (Acarbose)

No.	Tested samples	% Inhibition in different concentrations (µg/mL)							IC_{50} (µg/mL)
		1.95	3.91	7.81	15.62	31.25	62.5	125	
1	Watery extract	47.4 ± 0.48	54.28 ± 0.71	56.83 ± 0.48	58.19 ± 0.22	61.73 ± 0.12	63.42 ± 0.13	65.54 ± 0.32	2.67
2	EtOH extract	46.69 ± 0.34	50.57 ± 0.19	52.57 ± 0.46	56.66 ± 0.22	58.79 ± 0.13	62.97 ± 0.17	64.97 ± 0.54	3.64
3	*Acarbose	42.99 ± 0.19	49.99 ± 0.25	59.58 ± 0.66	63.91 ± 0.20	66.51 ± 0.37	68.40 ± 0.21	69.78 ± 0.39	3.91

*Used as standard



(a) **(b)**

Figure 7. (a) *α*-Amylase inhibition % of Gway-tauk leaves

(b) A bar graph of IC_{50} value of antidiabetic activity of crude extracts of Gway-tayk leaves

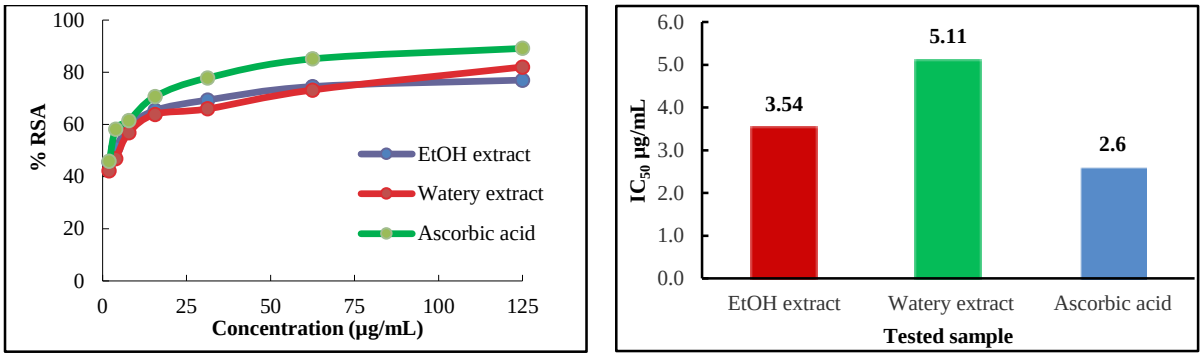
Antioxidant Activity of Ethanol and Watery Extracts of Gway-tauk Leaves

The antioxidant activity of ethanol and watery extracts of *D. volubilis* (Gway-tauk) leaves was evaluated by a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenger test. Ethanol extract (IC_{50} - 3.54 µg/mL) was found to be more potent than watery extract (IC_{50} - 5.11 µg/mL). Their antioxidant activity was compared with that of standard ascorbic acid (IC_{50} = 2.60 µg/mL). Since the lower the µg/mL values, the higher the antioxidant activity of the sample, an ethanol extract of Gway-tauk leaves possessed higher antioxidant activity than a watery extract. Ethanol extract of Gway-tauk leaves was found to be less effective than standard ascorbic acid (IC_{50} = 2.60 µg/mL). The results are shown in Table 4 and Figure 8.

Table 4. %RSA and IC_{50} Values of Crude Extracts of Gway-tauk Leaves and Standard (Ascorbic acid)

No.	Tested samples	% RSA ± SD of different concentrations (µg/mL)							IC_{50} (µg/mL)
		1.95	3.91	7.81	15.63	31.25	62.5	125	
1	Watery extract	42.22 ± 0.19	46.93 ± 0.33	56.90 ± 0.38	63.91 ± 0.31	66.00 ± 0.47	73.22 ± 0.57	82.02 ± 0.38	5.11
2	EtOH extract	45.52 ± 0.14	51.02 ± 0.69	59.20 ± 0.70	65.46 ± 0.45	69.38 ± 0.58	74.55 ± 0.62	77.01 ± 0.69	3.54
3	*Ascorbic acid	45.89 ± 0.31	58.20 ± 0.51	61.58 ± 0.25	70.76 ± 0.26	77.89 ± 0.51	85.19 ± 0.07	89.19 ± 0.07	2.60

*Used as standard



(a) **(b)**

Figure 8. (a) Radical scavenging activity of different concentrations of crude extracts of Gway-tauk leaves

(b) A bar graph of IC_{50} values of crude extracts of Gway-tauk leaves and standard ascorbic acid

Conclusion

The column chromatographic method was used to isolate chemical constituents from an EtOAc extract of Gway-tauk leaves, which were then characterised by physio-chemical property tests and modern spectroscopic techniques such as UV and FT IR. Compound **1** was a steroid compound (0.005%, colourless needle crystal), compound **2** was an organic acid compound (0.013%, white amorphous), compound **3** was *β*-sitosterol (0.01%, colourless needle crystal), compound **4** was an organic acid compound (0.004%, colourless crystal), compound **5** was a terpenic acid compound (0.004%, white amorphous), and compound **6** was a glycoside compound (0.015%, colourless crystal). From the results of antimicrobial activity, it was found that EtOH extract showed the highest level of inhibition zone diameters in the range of 10-15 mm against all tested microorganisms. According to the observed cytotoxicity results, ethanol and watery extracts had no cytotoxic effect on the brine shrimp at a 1000 µg/mL concentration. The bitterness value of the watery extract of Gway-tauk leaves was lower. The *α*-amylase inhibitory activity of ethanol and watery extracts of Gway-tauk leaves was evaluated by the starch-iodine method. The IC_{50} values of ethanol and watery extracts were observed to be 3.64 and 2.67 µg/mL. Both extracts were more potent than standard acarbose (3.91 µg/mL). Furthermore, an ethanol extract (IC_{50} =3.54µg/mL) of Gway-tauk leaves was more potent than a watery extract (IC_{50} = 5.11 µg/mL) in antioxidant activity.

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