

Some Biological Activities and Isolation of Bioactive Organic Constituents from *Dregea volubilis* Benth. (Gway-tauk) Fruits

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Abstract

The physicochemical analysis such as 7.74 % of moisture, 7.01 % of ash, 9.75 % of protein, 44.31 % of fiber, 2.72 % of fat, 28.47 % of carbohydrate and 179 kcal/100 g of energy value were found in fruit of *D. volubilis*. Benth. by AOAC method. The collected sample was occurred Ca (112.18 ppm), Mg (104.27 ppm), Fe (14.34 ppm) determined by atomic absorption spectrophotometry method. Other toxic elements: Cd, Cu, Mn, As and Pb were not detected in this sample. It was possessed that methanol extract was exhibited the most potent antimicrobial activity against *Pseudomonas aeruginosa*. Antioxidant activity of the watery, ethanol extracts and one isolated compound were evaluated by DPPH assay method. The IC₅₀ value, the radical scavenging activity of isolated compound was found more potent activity than that of ethanol extract and watery extract. By the acute toxicity test *in vivo* there was no toxic effect in *D. volubilis*. Benth. fruits. Moreover, the antitumor activity of 12.5µg/disc of petroleum ether extract of *D. volubilis*. Benth. fruits was observed in the prevention of tumor formation by potato crown gall method. Furthermore, from the separation of silica gel column chromatographic method, one terpenoid compound: (0.25%, 288°C, R_f = 0.43) was isolated from petroleum ether extract of *D. volubilis* Benth. The isolated compound, was identified by UV visible and FTIR spectroscopy. The fruit of *D. volubilis*. Benth. plants grown in Loikaw City, Kayah State area is being reported for the first time and it can be used for development of new antioxidant and antitumor drug in Myanmar herbal plants.

Keywords: : *Dregea volubilis*. , terpenoid, UV, FTIR, acute toxicity, antioxidant, antitumor,

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Introduction

***Dregea volubilis* Benth. (Gway-tauk) Fruits**

D. volubilis Benth. belongs to the family Asclepiadaceae and is a medicinal twinning glabrous perennial herb with densely lenticulate branches (Moulisha *etal*, 2009). It is a stout, smooth, hoary or mealy, woody vine and commonly used in traditional treatment of various illnesses (Karthika, 2012). Leaves are ovate or somewhat rounded, 7.5 to 15 centimeters long, 5 to 10 centimeters wide, rather leathery, rounded or pointed at the base, and at the tip. The fruits are usually double, broadly lanceolate, 7.5 to 10 centimeters long, turgid, longitudinally ribbed, and velvety until mature (Bharathamma, 2015). It has been reported that the leaves are much employed as an application to boils and abscesses. The roots and tender stalks are considered emetic and expectorant (Venkatesan, 2013). The young roots are cut and the exuding juice is inserted into the nose to cause sneezing. Subsequently, (Purushoth,2012) has been reported that the fruits of *D. volubilis* Benth. are treatment of sore throat, carbuncles, eczema, asthma, emetic, expectorant, febrifuge, eye-disease activity and antidote for poison.

Therefore, in this study the phytochemical constituents, physicochemical analysis, elemental analysis of fruits of *D.volubilis* Benth. were conducted. Moreover, antimicrobial, antioxidant, acute toxicity and antitumor activity of fruits from *D.volubilis* Benth. were determined by different methods. Then, some chemical contents of fruits of *D.volubilis* Benth. were isolated by column chromatographic method.

Materials and Methods

Sample Collection

The fruits of *D.volubilis* Benth. (Gway-tauk) samples were collected from Mine-Lone Quarter, Kayah State, Loikaw City. The sample was identified at the Department of Botany, University of Yangon.

The collected samples were cleaned by washing with water and cut into small pieces and air-dried and ground to produce fine homogeneous powders using electric blender. After that, these powdered samples were stored in air-tight glass containers to prevent other contaminations.

Preliminary Phytochemical Investigation of *D.volubilis* Benth. (Gway-tauk) Fruits

In order to find out types of phytoorganic constituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, saponins, steroids, phenolic compounds, tannins and terpenoids were present in the *D.volubilis* Benth.(M-Tin Wa,1972). The preliminary phytochemical tests were carried out according to the appropriate reported as test tube method (Harbone,1984).

Determination of some Nutritional Values of *D.volubilis* Benth. (Gway-tauk) Fruits

The moisture content, the ash content, the fat content, the protein content by micro Kjeldahl method and the fiber content and the energy value were determined by AOAC method (AOAC,1990).

Quantitative Elemental Analysis by Atomic Absorption Spectrometry (AAS)

The preparation of *D.volubilis* Benth. fruits sample solution was ready for analysis of mineral elements by AAS.

Screening of Antimicrobial Activity of the Samples

Agar well diffusion method was employed for determining antimicrobial activity of the extracts. Firstly, nutrient agar (20-25 ml) was boiled and poured into the test tube and plugged with cotton wool and sterilized at 121°C for 15 minutes in autoclave (Finegold, 1978). After autoclaving, the tubes were cooled down to 30-35°C and poured into sterilized petridish and 0.1-0.2 mL of test organisms were also added into dishes (Cruickshank, 1960). They were allowed to set the agar for 2-3 hours. After the agar was set, 10 mm agar well are made by the help of sterilized agar well cutter. After that, about 0.2 mL of sample namely PE, EtOH MeOH and H₂O solution of *D.volubilis* Benth. were introduced into the agar well and incubated at 37°C for 24 hours. The inhibition zone appeared around the agar well indicated that the presence of antimicrobial activity.

Screening of Antioxidant Activity of *D.volubilis* Benth. (Gway-tauk) Fruits.

Antioxidant activities of 95 % ethanol, watery extracts and isolated compound were carried out by DPPH (1, 1-Diphenyl, 2-Picryl Hydrazyl) radical scavenging assay using UV visible spectrophotometer (Halliwell, 2012). Then, IC₅₀ (50 % oxidative inhibitory concentration) values were also calculated by linear regressive excel program (Kahlonene, 1999).

Screening of Acute Toxicity of *D.volubilis* Benth. by OECD Method

The information on the hazardous properties of a chemical limited test of OECD guideline 425 was used for acute toxicity test. Because this procedure was utilized few animals than the other acute toxicity testing methods (OECD, 2000).

Screening Antitumor Activity of *D.volubilis* Benth. by Potato Crown Gall Method

The tumor producing bacteria *Agrobacterium tumefaciens* was isolated from gall tissues of leaves of *Sandoricium koetijape* Merr.(Thitto). It was cultured for the Potato Crown Gall (PCG) test and used petroleum ether extract of *D.volubilis* Benth. for the antitumor activity test (Ferrigni, 1982).

Isolation of PE Extract by using Silica Gel Column Chromatographic Method

The PE extract of *D.volubilis* Benth was separated by silica gel column chromatographic techniques. A total 60 fractions of various solvent system (compound A, compound B, compound C) were collected. All the collected fractions were checked on TLC by spraying with 5 % H₂SO₄ followed by heating. Among them, the fraction of (F₂₈₋₃₄), compound A, (PE:EtOAc,30:1), the fraction of (F₄₀₋₄₅) compound B,(PE:EtOAc,25:1), were observed not clear on TLC under UV shorter and longer wavelength. Moreover, the fractions of F₅₃₋₅₇ (PE:EtOAc,9:1), showed one spot on TLC and provided as the compound C.

Characterization of Isolated Compounds

(i) Determination of R_f Value

The R_f values of the isolated compounds were calculated according to the following equation:

$$R_f = \frac{\text{Distance moved by sample}}{\text{Distance moved by solvent}}$$

(ii) Classification of Isolated Compounds by Physicochemical Test

The isolated compound was characterized by some colour test such as 5 % H_2SO_4 , Liebermann Burchard, anisaldehyde/sulphuric acid, 5 % $FeCl_3$ and Mg/HCl . A chromatogram was prepared by developing in PE: EtOAc (30:1, 25:1, 9:1v/v) solvent system. Then the chromatogram was sprayed with 5 % H_2SO_4 followed by heating, Liebermann Burchard followed by heating, anisaldehyde / sulphuric acid followed by heating, 5 % $FeCl_3$ and treated with Mg/HCl in test tube method. The observed colouration were denoted.

(iii) Identification of Isolated Compounds from *D.volubilis* Benth.

(Gway-tauk) Fruits

The isolated compound C was identified by modern spectroscopic techniques such as UV-visible and FT IR spectroscopy (Markan, 1982).

Results and Discussion

Phytochemical Constituents of *D.volubilis* Benth. (Gway-tauk) Fruits

The fruits of *D.volubilis* Benth. (Gway-tauk) were collected from Mine-Lone Quarter, Loikaw Township, Kayah State in the middle of December. According to these experiments, glycosides, flavonoids, alkaloids, α -amino acid, carbohydrates, phenolic compounds, saponins, steroids, tannins and terpenoids were found in *D.volubilis* Benth. Whereas, starch was not detected in collected sample.

Nnutritional Values and Elemental Constituents of *D.volubilis* Benth. (Gway-tauk) Fruits

In this sample, the fiber (44.31%) was observed the highest amount. In addition, protein (9.75 %) and carbohydrate content (28.47 %) were

higher than other nutrient, moisture (7.74 %) and ash (7.01 %). The fat content (2.72 %) was the lowest amount in *D.volubilis* Benth. fruits. The energy value was observed 179 kcal/100g from *D.volubilis* Benth.

Mineral elements present in dried powder of fruits from *D.volubilis* Benth. was determined by Atomic Absorption Spectrometer (AAS). Ca, 112.18 ppm and Mg, 104.27 ppm were found as major amounts but Fe, 14.34 ppm as trace elements and Cd, Cu, Mn were not detected in it.

Screening on Antimicrobial Activity of *D.volubilis* Benth. (Gway-tauk) Fruits by Agar Well Diffusion Method

PE and watery extracts were not exhibited antimicrobial activity against six microorganisms. But MeOH extract (inhibition zone diameter 15 mm) was exhibited the most potent antimicrobial activity against *Pseudomonas aeruginosa*. The remaining extracts were showed antimicrobial activity against on six strains of microorganisms. The results of inhibition zone diameters are described in Table 1.

Table 1. Inhibition Zone Diameter of Various Extracts of *D.volubilis* Benth. Against Six Microorganism by Agar Well Diffusion Method

No.	Microorganisms	Inhibition Zone Diameter (mm)			
		PE	EtOH	MeOH	H ₂ O
1	<i>Bacillus subtilis</i>	13	14	13	12
2	<i>Staphylococcus aureus</i>	13	13	13	12
3	<i>Pseudomonas aeruginosa</i>	13	13	15	12
4	<i>Bacillus pumilus</i>	13	14	13	13
5	<i>Candida albicans</i>	13	13	13	13
6	<i>Escherichia coli</i>	13	14	13	11

Determination of Antioxidant Activity of *D.volubilis* Benth. (Gway-tauk) Fruits

The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC_{50}). The lower the IC_{50} values, the higher the antioxidant activity of the sample. By using DPPH free radical scavenging assay, the compound C was found the most potent antioxidant activity than 95% ethanol and watery extracts. The results of antioxidant activity are shown in Table 2 and Figure 1 and Figure 2.

Results of Acute Toxicity Study of Aqueous and Ethanoic Extracts of *D.volubilis* Benth.(Gway-tauk) Fruits by OECD Test

In acute toxicity, there is no lethality at the dose of 5000 mg/kg body weight of the extracts. It can be concluded that the ethanoic and watery extracts of *D.volubilis* Benth were supposed to be practically nontoxic. The results of acute toxicity activity are shown in Table 3, Figure 3, and Figure 4 (OECD,2000).

Results of Antitumor Activity of Petroleum Ether Extract of *D.volubilis* Benth. (Gway-tauk) Fruits by Potato Crown Gall Test

In antitumor activity of PE extract of *D.volubilis* Benth. fruits was detected by Potato Crown Gall test with the isolated bacterium *Agrobacterium tumefaciens*. The broth cultures containing 5×10^9 cells /mL of the potato disc were inoculated for 48 hour. The test sample of PE extract was dissolved in DMSO, diluted and mixed with the bacterial culture for inoculation. After that, the tumors were appeared on potato discs and checked by staining the knob with Lugol's ($I_2=KI$) solution. From this observation, PE extract of *D.volubilis* Benth. was possessed the prevention of tumor formation with the doses of 12.5 μ g/disc. The results of antitumor activity are shown in Figure 5.

Table 2. Oxidative Percent Inhibitions and IC₅₀ Values of Crude Extracts of *D. volubilis* Benth. and Standard BHT

Sample	% Inhibitions (Mean ± SD) in various Concentrations (µg/ml)								IC ₅₀ (µg/ml)
	3.125	6.25	12.5	25	50	100	200	400	
95 % EtOH extract	38.095 ± 3.863	36.395 ± 2.567	30.272 ± 7.167	27.347 ± 18.395	37.551 ± 4.081	54.762 ± 4.248	52.381 ± 5.802	59.372 ± 15.587	86.17
Watery extract	20.748 ± 0.601	35.034 ± 0.601	41.497 ± 1.202	64.966 ± 0.601	80.952 ± 0.601	94.217 ± 1.202	93.197 ± 0.601	93.06 ± 1.323	17.04
Isolated Compound C	20.748 ± 0.601	52.262 ± 0.601	39.532 ± 1.202	21.428 ± 0.601	25.17 ± 0.601	25.489 ± 0.601	24.499 ± 0.601	25.987 ± 0.531	7.68
Standard BHT	43.301 ± 1.40	53.582 ± 2.49	65.53 ± 1.132	74.82 ± 0.621	83.321 ± 0.782	87.412 ± 2.372	91.516 ± 1.113	94.702 ± 0.692	3.16

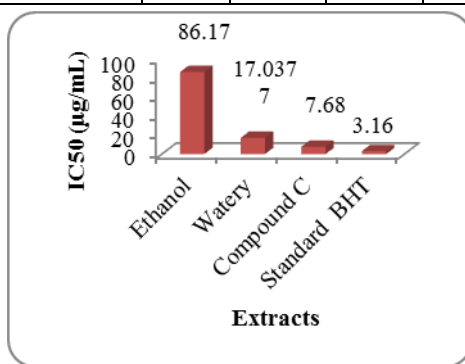


Figure 1. A bar graph of IC₅₀ (µg/ml) Values of different concentration of watery, EtOH extracts and isolated compound C from *D. volubilis* Benth.

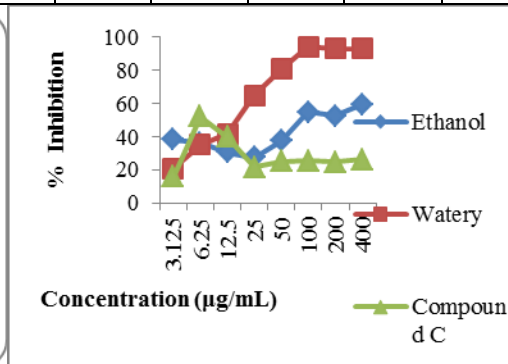


Figure 2. DPPH radical scavenging activity of different concentration of watery, EtOH extracts and isolated compound C from *D. volubilis* Benth.

Table 3. Results of Acute Toxicity Study of Both Aqueous and Ethanoic Extracts of *D.volubilis* Benth by OECD Test Guideline 425

Animal ID	Dose(mg/kg)	Short Term Result	Long Term Result
1	175	0	0
2	550	0	0
3	1750	0	0
4	5000	0	0
5	5000	0	0
6	5000	0	0



Figure 3. The observation practically nontoxic after 14 days with ethanol extract of *D. volubilis* Benth.



Figure 4. The observation of practically nontoxic after 14 days with watery extract of

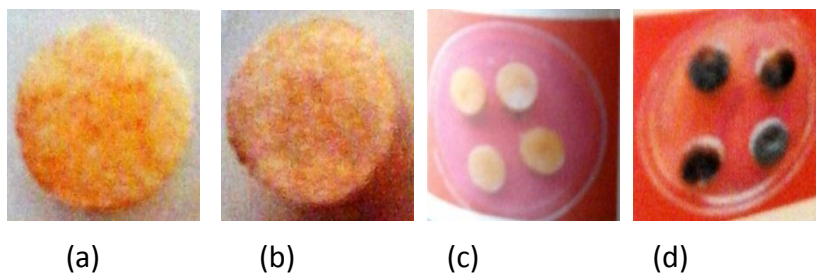


Figure 5. Antitumor activity of *D. volubilis* Benth. by Potato Crown Gall Test

- (a) Control potato disc without test sample
- (b) Potato disc containing test sample
- (c) Before treating with Lugol's solution
- (d) After treating with Lugol's solution

Some Organic Constituents from Petroleum Ether Extract of *D. volubilis* Benth.

The petroleum ether crude extract (2 g) was separated by silica gel column chromatography, the solvent system in the ratio of petroleum and ethyl acetate (30:1), (25:1) and (9:1) v/v were successively used to elute the isolated compound. In this separation a total of 60 fractions (3cm³/fractions) were collected. The fraction of (F₂₈₋₃₄), (PE:EtOAc,30:1), compound A, the fraction of (F₄₀₋₄₅), (PE:EtOAc,25:1), compound B, were observed not clear on TLC check under shorter and longer wavelength. However, the fractions of F₅₃₋₅₇ (PE:EtOAc,9:1), showed the similar TLC behavior provided the compound C, yellow colour crystal (0.25 %). The isolated compound C was recrystallized by acetone (Figure 6).

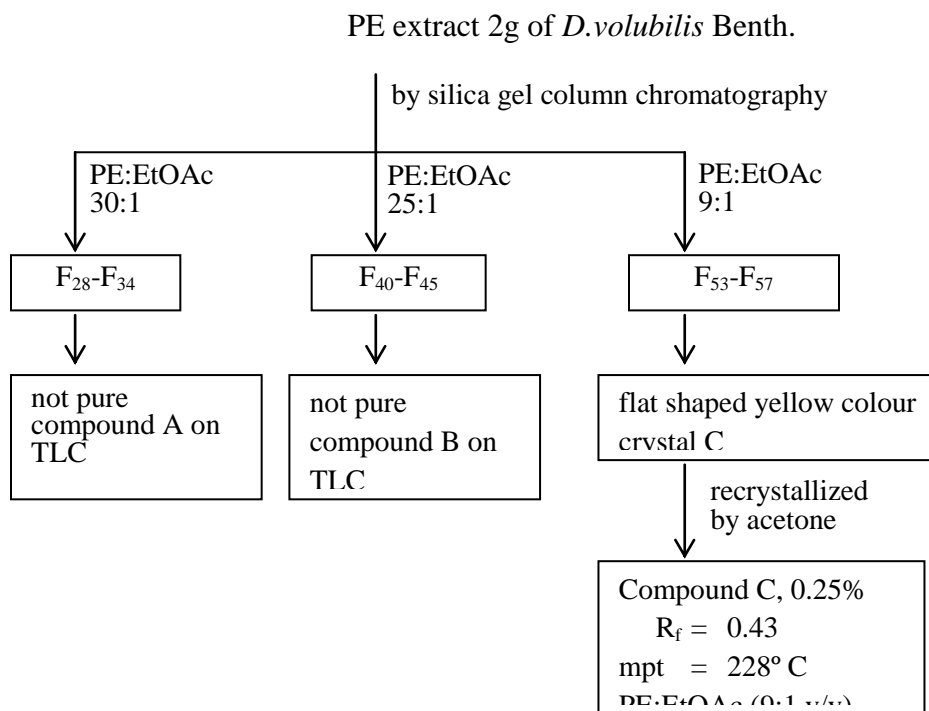


Figure 6. Isolation of Organic Compound from Petroleum Ether Extract of *D. volubilis* Benth.

Classification of Isolated Compound C from *D.volubilis* Benth. by Colour Reaction Test

The isolated compound C, was observed that brown color on TLC by spraying with 5 % H₂SO₄ and heating. After that, the pink colour were observed testing with vanillin sulphuric acid and heating. Moreover, the violet colour was observed anisaldehyde sulphuric acid and heated on TLC. Compounds C, from *D. volubilis* Benth. was classified as terpenoid compounds due to pink colouration occurred when it treated with Libermann Burchard reagent in test tube. There was no colouration on TLC by spraying with 5 % FeCl₃ followed by heating and Mg/HCl in test tube. In addition the isolated compound C was observed as a yellow color with bromothymol blue solution test in test tube. The above results confirmed

that, the isolated compound C, from *D. volubilis* Benth. as a terpenoid compound as shown in Table 4, Table 5 Table 6 and Figure 7 and 8.

Table 4. Results of Colour Tests on TLC of Isolated Compound C from *D.volubilis* Benth.

No.	Spraying reagent	Observation on the test of isolated compound
1	5% H ₂ SO ₄ , Δ	brown color on TLC
2	Vanillin+H ₂ SO ₄ , Δ	pink color on TLC
3	Anisaldehy de+H ₂ SO ₄ , Δ	violet color on TLC
4	1% FeCl ₃	no color change
5	I ₂ vapour	pink color on TLC

Table 5. Classification of Isolated Compound C from of *D. volubilis* Benth.

No.	Reagent tested					Remarks
	Acetic anhydride and H ₂ SO ₄ in CHCl ₃	Mg and conc:HCl in EtOH	1% FeCl ₃ in EtOH	10% lead acetate in EtOH	Bromothylmol blue	
C	Pink	-	-	-	Yellow	terpenoid

Table 6. Yield Percent, R_f Values and Melting Points of Isolated Compound C from of *D. volubilis* Benth.

Isolated Compound	Yield (%)	R_f	mpt°C	Appearance
C	0.25	0.43 (PE:EtOAc, 9:1)	226-228 (PE/EtOAc)	flat shaped yellow crystal



Figure 7. Appearance of crystal C from *D.volubilis* Benth.

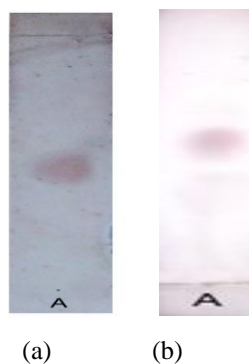


Figure 8. TLC chromatogram of isolated compound C from PE extract of *D. volubilis* Benth.
 (a) Spraying with 5 % H_2SO_4 on heating
 (b) Spraying with vanillin
 H_2SO_4 , PE:EtOAc(v/v)= 9:1, $R_f = 0.43$,
 UV active = shorter wavelength (254 nm)
 UV active = longer wavelength (365 nm)

Identification of Isolated Compound C from Petether Extract of *D.volubilis* Benth.

The UV spectrum of compound 'C' in MeOH is shown in Table 7 and Figure 9. According to UV spectrum the major absorption bands were found to be 214 and 226 nm. This information pointed out that compound C contained double bond conjugation system in it.(Markham, 1982).

The functional groups present in compound "C" were also studied by FTIR spectroscopy as shown in Table 8 and Figure 10. The present of O-H stretching of alcoholic and carboxylic O-H group could also be confirmed with the peak appeared at 3430 cm^{-1} and the bands at 1690 cm^{-1} , suggested the stretching vibration of C=O in carboxylic acid.

The characteristic bands at 2988, 1400, 1460 and 1040 cm^{-1} also showed the presence of C-H stretching and bending of CH_2 , CH_3 group, C=C stretching of alkene and C-O stretching of alcohol and carboxylic group. In addition the appearance of crystal C, TLC chromatogram and physical properties are described in Table 9.

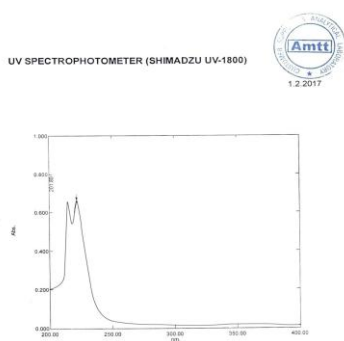


Figure 9. UV-visible spectrum of isolated compound C from *D.volubilis* Benth. in MeOH

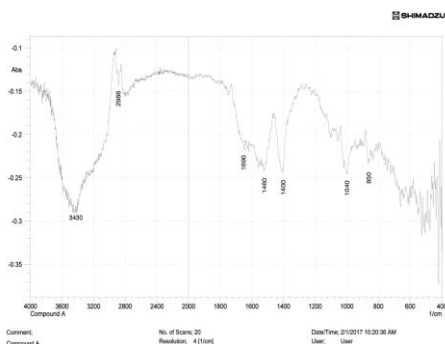


Figure 10. FT IR spectrum of isolated compound C from PE extract of *D.volubilis* Benth.

Table 7. UV-Visible Spectral Data of isolated compound C from *D. volubilis* Benth. in MeOH

Reagent	Observed λ_{max} (nm)	Remark
MeOH	214, 226 (π - π^*)	double bond conjugation

Table 8. FT IR Spectral Data of Isolated Compound C from *D. volubilis* Benth.

Wave Number (cm^{-1})	Band Assignment
3430	O-H stretching of alcoholic and carboxylic O-H group
2988	C-H stretching of CH_2 and CH_3 group
1690	C=O stretching of frequency of unsaturated carboxylic acid
1460	C=C stretching of alkene
1400	C-H bending of methyl group
1040	C-O stretching of alcohol and carboxylic group
850	out-of-plane bending of C-H group

Table 9. Some Physical Properties of Isolated Compound C from *D. volubilis* Benth.

Experiment	Observation	Remark
Melting point/°C	226-228	recrystallized from acetone
R _f	0.43	PE:EtOAc (9:1/v/v) on TLC
UV	active	conjugated double bond
5% H ₂ SO ₄ , Δ	brown on TLC	terpenoid
Libermann Buchard, Δ	pink on TLC	terpenoid
Vanillin, H ₂ SO ₄ , Δ	pink on TLC	terpenoid
Anisaldehyde, H ₂ SO ₄ , Δ	violet on TLC	terpenoid
I ₂ vapour	yellow on TLC	terpenoid
10% KMnO ₄ solution test	decolourized	C=C present
1% FeCl ₃ solution test	no colour change	phenolic OH absent
Libermann Buchard	pink in CHCl ₃	terpenoid
Bromothymol blue solution test	Yellow colouration	carboxylic acid

Conclusion

In concluding the overall assessments of the research work, the preliminary phytochemical investigation indicated that alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were present in *D. volubilis* Benth. (Gway-tauk) fruit. The nutritional values as 28.47% of carbohydrates, 9.75% of protein, 44.31 % of fiber, moisture 7.74 % and ash 7.01 % were observed in it. The fat content (2.72%) was found the lowest amount in *D. volubilis* Benth. fruit. The energy value was observed to be 179 kcal/100g in collected sample. According to qualitative elemental analysis carried out by AAS spectrometry, Ca and Mg were occurred as major components and Fe as trace elements in *D. volubilis* Benth. In *in vitro* antibacterial activity by using agar well diffusion method, MeOH extract of *D. volubilis* Benth. fruit was found the most potent antimicrobial activity 15 mm against on the microorganism *Pseudomonas aeruginosa*. The remaining extracts were showed antimicrobial activity against six microorganisms. The IC₅₀ value of *D. volubilis* Benth. fruit was determined by linear regressive excel program. By using DPPH free radical scavenging assay, compound C (7.68 $\mu\text{g/mL}$) was found the most potent antioxidant activity than watery extract 17.04 $\mu\text{g/mL}$ and 95% ethanol extract 86.17 $\mu\text{g/mL}$. In acute toxicity, there is no lethality at the dose of 5000 mg/kg of the both watery and ethanol extracts. From the determination of antitumor activity, *D. volubilis* Benth was possessed the prevention of tumor formation with the doses of 12.5 $\mu\text{g/disc}$ of petroleum ether extract by Potato Crown Gall Test. In order to find out some organic constituents from petroleum ether extract of *D. volubilis* Benth. fruit, silica gel column chromatographic technique using PE/EtOAc solvent system with various ratios was carried out. One terpenoid compound C: (0.25%, R_f=0.43, mp=228 °C) was isolated from PE extract *D. volubilis* Benth. fruit by column chromatographic technique. This compound C was identified by modern spectroscopic methods: UV and FTIR spectroscopy. Consequently, it can be deduced that *D. volubilis* Benth. fruit may be used as antioxidant in reducing of oxidative stress and some aged related orders. In addition, it may also be contributed in the areas of diseases related to bacterial infection, new antioxidant and antitumor drug in Myanmar Traditional medicine.

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