A Study in Biological Activities from *Dregea volubilis* Benth. (Gway-tauk) Fruits

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Abstract

It was observed that methanol extract was exhibited the most potent antimicrobial activity against *Pseudomonas aeruginosa*. In the case of the antioxidant activity, the watery, ethanol extracts and two isolated compound were evaluated by DPPH assay method. The IC_{50} value, the radical scavenging activity of two isolated compounds were 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. Furthermore, the antitumor activity of 12.5 µg/disc of petroleum ether extract of *D. volubilis.* Benth. fruits was possessed that against on the tumor producing bacteria: *Agrobacterium tumefaciens*, which was isolated from gall tissues of leaves of *Sandoricium koetijape* Merr.(Thitto). Besides, from the separation of silica gel column chromatographic method, one terpenoid compound A: (0.25%, 288ºC, R_f = 0.43) and second terpenoid compound B: (0.13%, 261ºC, R_f = 0.32) were isolated from ethanol extract of *D. volubilis* Benth. The fruit of *D. volubilis.* Benth. plants planted in Loikaw City, Kayah district area is being reported for the first time and it can be used for development of new antioxidant and antitumor drug in Myanmar Traditional Medicine.

Keywords: : Dregea volubilis.,terpenoid, acute toxicity, antioxidant, antitumor,

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Introduction

***Dregea volubilis* Benth.**

A medicinal twinning perennial herb of *D. volubilis* Benth. is described as family Asclepiadaceae and commonly used in traditional treatment of various illnesses. It is a stout, smooth, hoary or mealy, woody vine (Karthika, 2012). The 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 pointed at the tip. The cymes are axillary or interpetiolar, and umbel-like. The flowers are also green, about 1 centimeter across. The fruits are usually double, broadly lanceolate, 7.5 to 10 centimeters long, turgid, longitudinally ribbed, and velvety until mature (Bharathamma, 2015). The roots and tender stalks are considered emetic and expectorant (Venkatesan, 2013). Some researchers have reported that the fruits are used for the treatment of sore throat, carbuncles, eczema, asthma, emetic, expectorant, febrifuge, eye-disease activity and antidote for poison (Purushoth, 2012). However, physiochemical analysis, elemental analysis, antimicrobial , total phenolic contents, antioxidant, acute toxicity of *D. volubilis* Benth. are rarely reported in Myanmar.

Therefore, in this study the phytochemical constituents, physiochemical analysis, elemental analysis of fruits of *D.volubilis* Benths. were studied. Moreover, antimicrobial, antioxidant, acute toxicity and antitumor activity of fruits from *D.volubilis* Benth. were also conducted by different methods. Moreover, some bioactive constituents of fruits of *D.volubilis* Benth. were also isolated by column chromatographic separation method.

Materials and Methods

Sample Collection and Phytochemical Investigation of *D.volubilis* Benth. Fruits

The fruits of *D.volubilis* Benth. 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 thoroughly with water and out into small pieces and then air-dried at the room temperature. The dried samples were ground to produce fine homogeneous powders by using electric blender. These powdered samples were stored in air-tight glass containers to prevent other contamination (Harbone,1984).

Besides, the phytochemical constituents of alkaloids, α-amino acids, carbohydrate, flavonoids, glycosides, saponins, steroids, phenolic compounds, tannins and terpenoids were carried out according to the appropriate reported method (M-Tin Wa,1972).

Determination of some Nutritional Values and Elemental Analysis of *D.volubilis* Benth.

The constituents of moisture, the ash, the fat, the protein by micro Kjeldahl method and the fiber content and the energy value were determined by AOAC method (AOAC,1990). Otherwise, the preparation of *D. volubilis* Benth. fruits sample solution was performed for analysis of mineral elements by AAS (Atomic Absorption Spectrometry).

Screening of Antimicrobial Activity of *D.volubilis* Benth.

Agar well diffusion method was employed for determining antimicrobial activity of the extracts (Balouiri, 2016). Firstly, nutrient agar (20-25) mL of the medium was boiled and poured into the test tube. It was plugged with cotton wool and sterilized at 121°C for 15 minutes in autoclave. After autoclaving, the tube was 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. They were allowed to set the agar for 2-3 hours. After setting the agar, 10 mm agar wells were made by the help of sterilized agar well cutter. After that, about 0.2 mL of sample namely: PE, EtOH, MeOH and H_2O solution of *D.volubilis* Benth. were introduced into each 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 (Cruickshank,1960).

Antioxidant, Acute Toxicity and Antitumor Activities of *D.volubilis* Benth.

Antioxidant activity of 95 % ethanol, watery extracts, isolated compound A and B were carried out by DPPH (1,1-Diphenyl, 2-Picryl Hydrazyl) radical scavenging assay using UV visible spectrophotometer (Halliwell, 2012). Then, IC_{50} (50 % oxidative inhibitory concentration) values were also calculated by linear regressive excel program (Kahlonene, 1999). The acute toxic class method provided that the information on the hazardous properties of a chemical limit test of OECD guideline 425 method were used. This method is reproducible, uses very few animals and able to rank substances in a similar manner to the other acute toxicity testing methods (OECD, 2000).

Besides, the tumor producing bacteria *Agobacterium tummefaciens* was isolated from gall tissues of leaves of *Sandoricium koetijape* Merr.(Thitto). The isolated bacteria was identified by its morphology, gram staining, spore staining, some biochemical test and compared with the references. The morphology of isolated bacteria was examined under Microscope (Cruickshank, 1960). In gram staining method, ammonium oxalate crystal violet solution (Hucker's solution), gram's modification of Lugol's solution and counter stain solution were used. In biochemical test, motility test, catalase test, starch hydrolysis test, gelatin test, nitrates reduction test, indole test and carbohydrate test were carried out. After that, 2 mL of broth culture of *Agrobacterium tumefaciens* and 0.5 mL of PE extracts of *D.volubilis* Benth. fruit were inoculated on each potato disc, spreading it over the disc surface. The plates were sealed with the tape to minimize moisture loss and incubated at room temperature for one week. After incubation, Lugol's solution (I_2=KI) was added and tumors were observed under the microscope and compared with control disc. The antitumor activity was detected with the result of tumor occurred or not (Ferrigni, 1982).

Isolation of Ethanol Extract by using Silica Gel Column Chromatographic Separation Method

The terpene compounds were isolated from ethanol extract by silica gel column chromatographic separation techniques. A total 60 fractions were collected. All the collected fractions were checked on TLC by spraying with 5 % H_2SO_4 followed by heating.

Characterization of Isolated Compounds

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

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

Then, these isolated compounds were characterized by some colour test such as 5 % H_2SO_4, Libermann Burchard, anisaldehyde/sulphuric acid, 5 % $FeCl_3$ and Mg/HCl. A chromatogram was prepared by developing in PE: EtOAc (9:1, 5:1, 2:1 v/v) solvent system. Among them, these chromatogram of compound A and B were 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. Moreover, the isolated compounds A was identified by modern spectroscopic techniques such as UV-visible and FT-IR spectroscopy (Markan, 1982).

Results and Discussion

Sample Collection and Phytochemical Investigation of *D.volubilis* Benth.

The fruits of *D.volubilis* Benth. were collected from Mine-Lone Quarter, Loikaw Township, Kayah State in the middle of December. The phytochemical constituents of alkaloids, α-amino acid, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were observed but, starch was not detected in collected sample, *D.volubilis* Benth.(Table 1).

Determination of Nutritional Values and Elemental Analysis of *D.volubilis* Benth.

In collected sample, the fiber (44.31%) was observed the highest amount. In addition, protein (9.75 %) and carbohydrate content (28.47 %) were also found higher than the other nutrient, moisture (7.74 %) and ash (7.01 %). The fat content (2.72 %) was possessed the lowest amount in fruits. The energy value was observed 179 kcal/100g in *D. volubilis* Benth.(Table 2). 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 greater amount than Fe, 14.34 ppm and Cd, Cu, Mn were not detected in it (Table 3).

Table 1 Results of Phytochemical Investigation of *D.volubilis* Benth.

No.	Types of compound	Extract	Test reagents	Observation	Remark
1.	Alkaloids	1% HCl	Mayer's	white ppt	+
			Dragendroff's	orange ppt	+
			Sodium picrate	yellow ppt	+
2.	α-Amino acid	H_2O	Ninhydrin	pink colour spot	+
3.	Carbohydrates	H_2O	10 % α naphthol, conc H_2SO_4	red ring	+
4.	Flavonoids	EtOH	conc: HCl and Mg ribbon	pink color	+
5.	Glycosides	H_2O	10 % lead acetate solution	white ppt	+
6.	Phenolic compounds	EtOH	1% $K_3Fe(CN)_6$, 1% $FeCl_3$	blue/green color	+
7.	Steroids	PE	Acetic Anhydride, conc:H_2SO_4	greenish blue	+
8.	Saponins	H_2O	shake	frothing	+
9.	Terpenoids	$CHCl_3$	acetic anhydride and conc: H_2SO_4	pink colour	+
10.	Tannins	H_2O	1 % gelatin	white ppt	+
11.	Starch	H_2O	I_2 solution	not change	-

(+) = Presence (-) = Absence

Table 2 Nutritional Values of *D. volubilis* Benth.

No.	Nutrient parameter	Percentage of content
1	moisture	7.74 %
2	ash	7.01 %
3	fat	2.72 %
4	protein	9.75 %
5	fiber	44.31 %
6	carbohydrate	28.47 %
7	energy values	179 kcal/100 g

Table 3 Elements Contents in *D. volubilis* Benth. by AAS

No.	Element	Contents (ppm)
1	Ca	112.18
2	Mg	104.27
3	Fe	14.34
4	Cd	ND
5	Cu	ND
6	Mn	ND

ND = not detected

Antimicrobial, Antioxidant, Acute Toxicity and Antitumor Activities of *D.volubilis* Benth.

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 4.

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 A and B were more potent antioxidant activity than 95% ethanol and watery extracts. The results of antioxidant activity are shown in Table 5 and Figures 1 and 2. In acute toxicity test, there is no lethality at the dose of 5000 mg/kg b.w of the extracts and LD_{50} was supposed to be more than 5000 mg/kg b.w It can be concluded that the ethanoic and watery extracts of *D.volubilis* Benth. were performed practically nontoxic. The results of acute toxicity activity are shown in Table 6, Figure 3, and Figure 4.

In the case of antitumor activity, the PE extract of *D.volubilis* Benth. fruits was detected by Potato Crown Gall test with the isolated bacterium *Agobacterium tummefaciens*. These isolated bacteria were also identified by morphology, gram-staining method, staining procedure and some biochemical test such as nitrate reduction test, gelatin test, starch hydrolysis test, indole test, motility and carbohydrate test were described (Figure 5). The broth cultures containing $5x10^9$ 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 incubation for one week. 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 μg/disc. The results of antitumor activity are shown in Figure 6.

Table 4. 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_2O
1	*Bacillus subtilis*	13	14	13	12
2	*Staphylococcus aureus*	13	13	13	12
3	*Pseudomonas aeruginosa*	13	13	15	12
4	*Bacillus pumilus*	13	14	13	13
5	*Candida albicans*	13	13	13	13
6	*Escherichia coli*	13	14	13	11

Table 5. Oxidative Percent Inhibitions and IC_{50} Values of Crude Extracts and Isolated Compounds 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	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	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
Compound A	20.748 ± 0.601	39.532 ± 0.601	52.262 ± 1.202	21.428 ± 0.601	25.17 ± 0.601	25.489 ± 0.601	24.499 ± 0.601	25.987 ± 0.531	7.68
Compound B	24.45 ± 0.651	34.45 ± 0.681	54.45 ± 1.452	44.45 ± 0.651	34.45 ± 1.621	24.45 ± 0.635	39.45 ± 0.671	46.45 ± 0.697	9.25
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

Table 6. Results of Acute Toxicity Study of 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

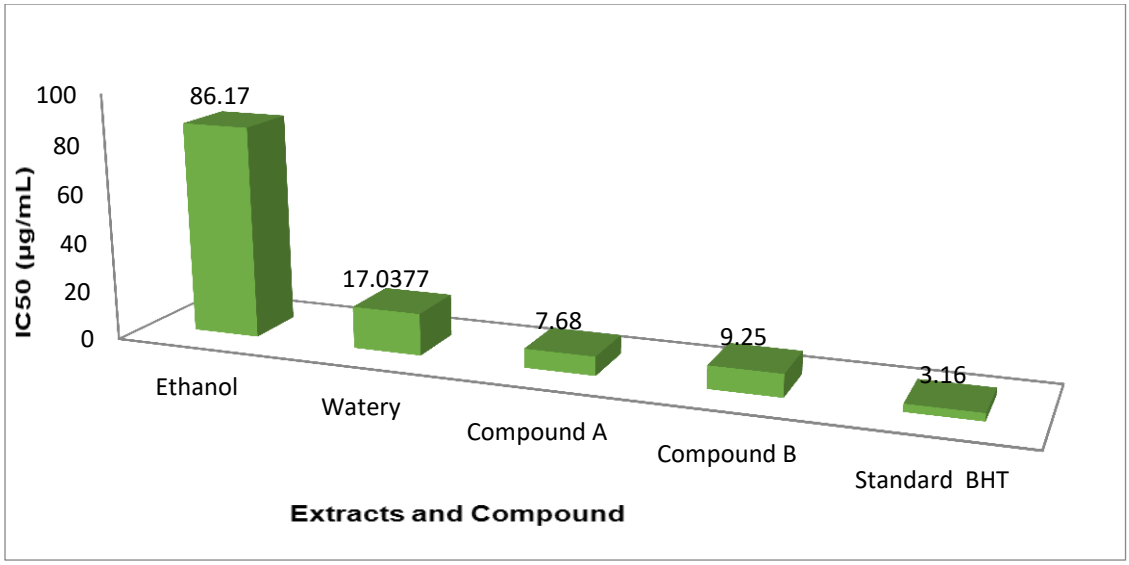


Figure 1. A bar graph of IC_{50} (µg/ml) values of different concentration of watery, EtOH extracts, isolated compound A and B from *D. volubilis* Benth.

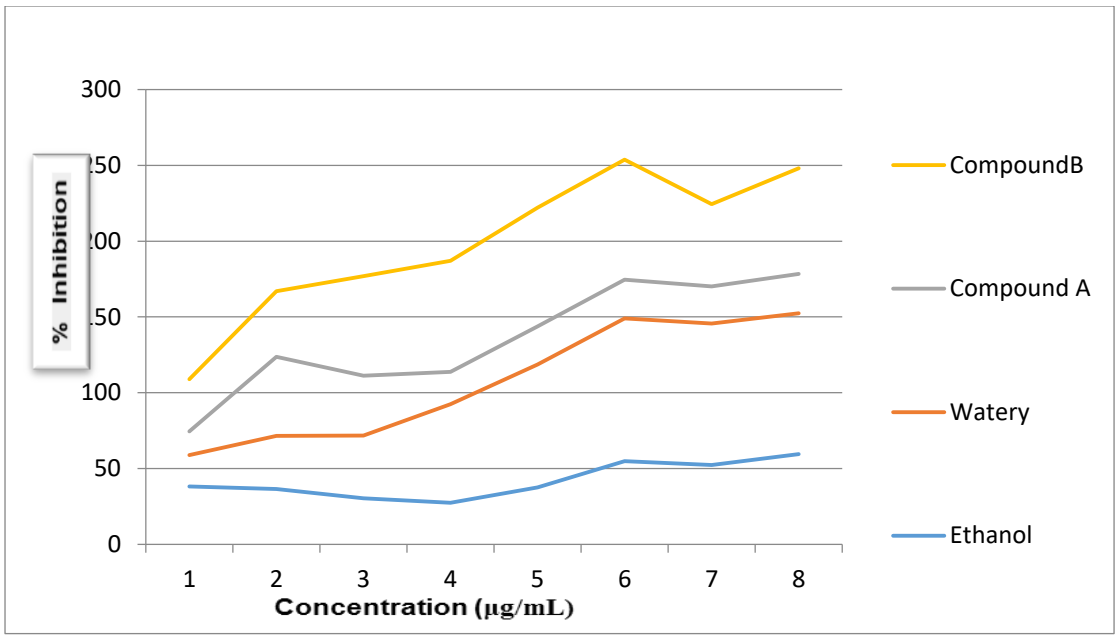
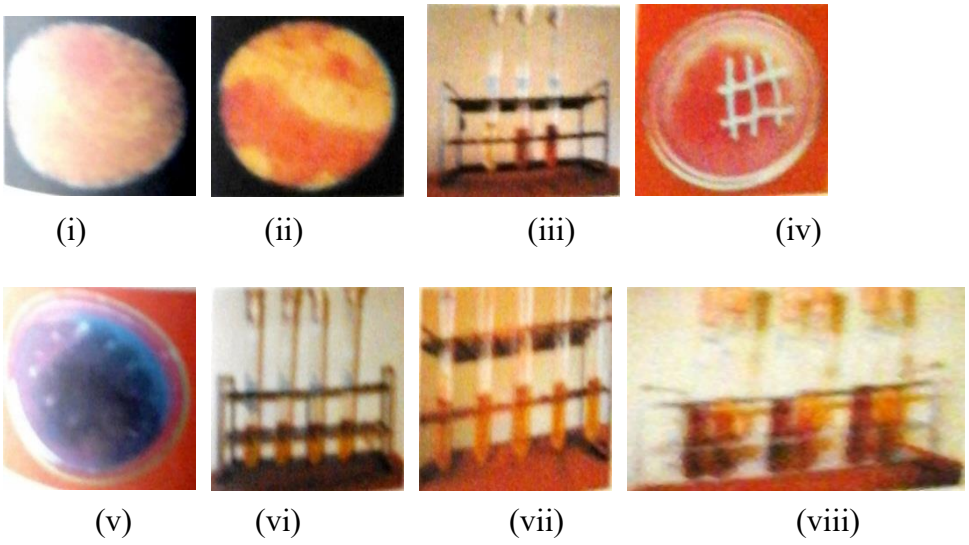


Figure 2. DPPH radical scavenging activity different concentration of watery, EtOH extracts, isolated compound A and B from *D. volubilis* Benth.

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

Figure 4. The observation of practically nontoxic after 14 days with with watery extract of *D.volubilis* Benth.

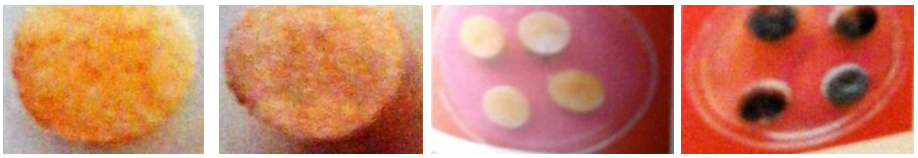


(i) (ii) (iii) (iv)

(v) (vi) (vii) (viii)

Figure 5. Results of identification of tumor producing bacteria *Agrobacterium tumefaciens*

(i) Gram staining
(ii) Spore staining
(iii) Nitrate reduction test
(iv) Gelatin test
(v) Starch hydrolysis
(vi) Indole test
(vii) Motility test
(viii) Carbohydrate test



(a) (b) (c) (d)

Figure 6. 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

Isolation of some Organic Constituents from Ethanol Extract of *D.volubilis* Benth.

The ethanol extract of (2 g) was separated by silica gel column chromatogyphic separation method and two terpene compounds were performed. The solvent system in the ratio of petroleum and ethyl acetate (9:1), (5:1) and (2:1) v/v were successively used to elute the isolated compound. In this separation a total of 60 fractions ($3cm^3$/fractions) were collected. Fractions F_{28-34} showed the similar TLC behavior provided the compound A, flat shaped yellow crystal (0.25%), from fractions F_{40-45} compound B was occurred as an amorphous yellow crystal (0.13%) and from fractions F_{53-60} compound C was observed properly not clear on TLC. The isolated compound A and B were recrystallized by acetone.

Classification Isolated Compounds from Ethanol Extract of *D.volubilis* Benth.by Colour Reaction Test

The isolated compounds A and B were observed that brown color on TLC by spraying with 5% H_2SO_4 and heating and pink colour were observed testing with vanillin sulphuric acid and heated and violet colour were found treated with anisaldehyde sulphuric acid and heated on TLC. Compounds A and B from ethanol extract of *D. volubilis* Benth. were classified as terpene compounds due to pink colouration occurred when it was treated with Libermann Burchard reagent in test tube. There was no colouration on TLC by spraying with 5 % $FeCl_3$ followed by heating and Mg/HCl in test tube. In addition the isolated compounds A and B were

observed as a yellow color with bromothylmol blue solution test in test tube. Therefore, these above observation were confirmed that these isolated compound A and B from ethanol extract of *D. volubilis* Benth. as a terpene compound as shown in Table 7,8,9,and Figure 7,8,9,10.

Table 7. Results of Colour Tests on TLC of Isolated Compounds from Ethanol Extract of *D. volubilis* Benth.

No.	Spraying reagents	Observation on the test of isolated compounds	
		A	B
1	5 % H_2SO_4, Δ	brown	brown
2	Vanillin + H_2SO_4, Δ	pink	pink
3	Anisaldehyde+ H_2SO_4, Δ	violet	violet
4	10 % $FeCl_3$	ND	ND
5	I_2vapour	yellow	yellow

Table 8. Classification of Isolated Compounds from Ethanol Extract of *D. volubilis* Benth.

No.	Reagent tested					Remarks
	Acetic anhydride and H_2SO_4in $CHCl_3$	Mg and Conc: HCl in EtOH	1 % $FeCl_3$ in EtOH	10 % lead acetate in EtOH	Bromo thylmol blue	
A	Pink	—	—	—	Yellow	terpenoid
B	Pink	—	—	—	Yellow	terpenoid

Table 9. Yield Per cent, R_f Values and Melting Points of Isolated Compound A and B from Ethanol Extract of *D.volubilis* Benth.

Isolated Compound	Yield (%)	R_f	mpt°C	Appearance
A	0.25	0.43 (PE:EtOAc, 9:1)	226-228 (PE/EtOAc)	flat shaped yellow crystal
B	0.13	0.32 (PE:EtOAc, 5:1)	261-262 (PE/EtOAc)	amorphous yellow crystal

Figure 7. Appearance of crystal A from ethanol extract of *D. volubilis* Benth.

(a) (b)

Figure 8. TLC chromatogram of isolated compound A from ethanol 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

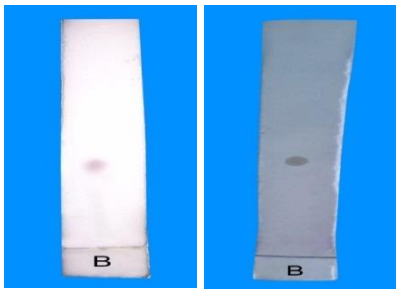


Figure 9. Appearance of crystal B from ethanol extract of *D.volubilis* Benth.

Figure 10. TLC chromatogram of isolated compound B from ethanol 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) = 5:1, R_f = 0.32

Identification of Isolated Compound A from Ethanol Extract of *D. volubilis* Benth.

The UV spectrum of compound 'A' in MeOH is shown in Table 10 and Figure11. According to UV spectrum the major absorption bands were found to be 214 and 226 nm. This information pointed out that compound A contained double bond conjugation (Markham, 1982).

The functional groups present in compound "A" were also studied by FTIR spectroscopy as shown in Table 11 and Figure12. 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}. 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 physical properties of compound A is described in Table 12.

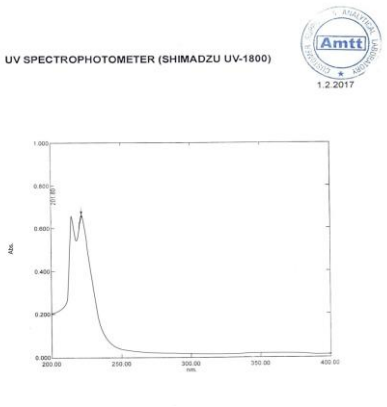


Figure 11. UV-visible spectrum of isolated compound A from ethanol extract of *D. volubilis* Benth. in methanol

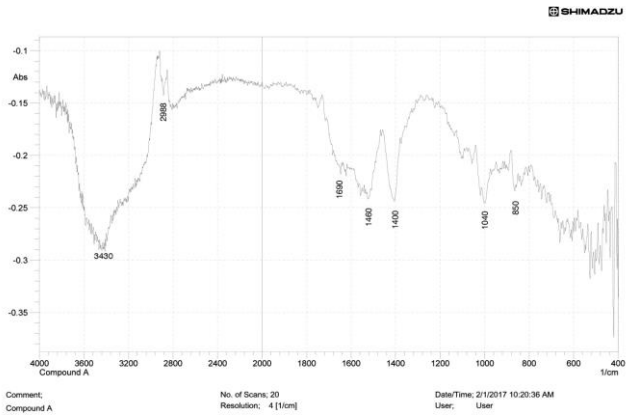


Figure 12. FT IR spectrum of isolated compound A from ethanol extract of *D. volubilis* Benth.

bTable 10. UV-Visible Spectral Data of isolated compound A from Ethanol Extract of *D. volubilis* Benth. in MeOH

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

Table 11. FT IR Spectral Data of Isolated Compounds A from Ethanol Extract of *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 12. Some Physical Properties of Isolated Compound A from Ethanol Extract of *D. volubilis* Benth.

Experiment	**Observation**	**Remark**
Melting point/ºC	226-228	recrystallized from acetone
R_f	0.43	PE:EtOAc (9:1/v/v) on TLC
UV	active	conjugated double bond
5% H_2SO_4, Δ	brown on TLC	terpenoid
Libermann Burchard, Δ	pink on TLC	terpenoid
Vanillin, H_2SO_4, Δ	pink on TLC	terpenoid
Anisaldehyde, H_2SO_4, Δ	violet on TLC	terpenoid
I_2vapour	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 Burchard	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 acid, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were present in *D. volubilis* Benth. 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 IC_{50} values of *D. volubilis* Benth. fruit was determined by linear regressive excel program. By using DPPH free radical scavenging assay, compound A (7.68μg/mL) and B (9.25μg/mL) were found the most potent antioxidant activity than watery extract 17.04 μg/mL and 95% ethanol extract 86.17μg/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 μg/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. Two terpene compound: A, (0.25%, R_f=0.43, mp=226-228 ºC) and B (0.13%, R_f=0.32, mp=261-262 ºC) were isolated from ethanol extract *D. volubilis* Benth. fruit by column chromatographic technique. This compound A 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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References

AOAC. (1990). *Official Methods of Analysis*, 5th Ed., Washington, DC

Bharathamma, G. and G. Sudarsanam. (2015). "Phytochemical Investigation of Aqueous Fruit Extracts of *Dregea volubilis* Benth", *Indian Journal of Plant Sciences*, **9** (1), 11-15

Balouiri,M., Sadiki, M., and Koraichi, I., (2016), Methods for *in vitro* evaluating antimicrobial activity, *J of Pharmaceutical Analysi*, **6**, 71-79

Cruickshank, R. (1960). *Medical Microbiology*, 1st Ed., E.E. Livingstone Ltd, London, 81-95

Ferrigni, N.R, J.E. Putnan and B.Jacobsen. (1982). "Modification and Evaluation of the Potato Disc Assay and Antitumor Screening of Euphobiaceae Seeds", *Journal of Natural Products*, **45** (6),679-686

Halliwell, B. (2012). "Free Radicals and Antioxidants, Updating a Personal View", Journal of Nutrition Reviews, **70** (5), 65-257

Harborne, J.B. (1984). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Chapman and Hall, London 76-78

Kahlonene, P. (1999). "Antioxidant Activity of Plant Extracts Containing Phenolic Compounds", *Journal of Agriculture., Food Chemistry*., **47** (3), 3954-3962

Karthika, K.S. and K.S. Sanjaya.(2012). "A Pharmacognostic Evaluation on *Dregea Volubilis* Benth." International Journal of Pharmacy., **7** (3),319-336

Markham, K.R., (1982), *Technique of Flavonoids Identification*, Academic Press, London

M-Tin Wa, (1972). "Phytochemical Screening Method and Procedures", *Phytochemical Bulletin of Botanical Society of America Inc.* **5** (3), 4-10

OECD, (2000), "Guidance Document on Acute Oral Toxicity", Environmental Health and Safety Monograph Series on Testing and Assessment No 24.

Purushoth, P. and M. Deepth. (2012). "Preliminary Phytochemical and Standardization of the Plant *Dregea Volubilis Benth*", *Journal of Research gate*, 195

Venleateson, N., and S. Anton. (2013). "Phytochemical Composition and in Vitro Antimicrobial, Antioxidant Activities of Ethanolic Extract of *Dregea volubilis* Benth.", *J of Advances in Biological Research*, **7** (3), 81-88