ANTIMICROBIAL, HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF THE STEM OF *DRACAENA ANGUSTIFOLIA* (MEDIK.)ROXB.

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Abstract

In this research work the stem of *Dracaena angustifolia* (Medik.) Roxb., Myanmar named Nant thar ku was selected for determination of antimicrobial, hypoglycemic and antioxidant activities. The stem of the selected plant was collected from Loikaw Township, Kayah State, in January, 2019. The aim of this research is to investigate the stem of *Dracaena angustifolia* (Medik.) Roxb. chemically and pharmaceutically. Preliminary phytochemical tests were performed by test tube method which gave positive for flavonoid, alkaloid, phenolic compound, polyphenol, glycoside, saponin, terpene, reducing sugar and tannin compounds. Antimicrobial activity of various solvent (*n*-hexane, ethyl acetate, acetone, ethanol, methanol) extracts was determined by agar well diffusion method and tested on six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli*. Ethanol, acetone and ethyl acetate extracts responded high activity on all tested organisms. Moreover, a potent hypoglycemic activity of the stem of this plant was examined by adrenaline induced diabetic mice model method. Glibenclamide was used as standard drug. This plant showed remarkable inhibitory activity. Antioxidant activity of the stem of selected plant was measured by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay method. IC_{50} value of selected plant was 3.17μg/mL by comparing with standard ascorbic acid 0.90μg/mL.

Keywords: *Dracaena angustifolia* (Medik.) Roxb., antimicrobial activity, hypoglycemic activity, antioxidant activity

Introduction

Dracaena angustifolia (Medik.) Roxb., family Asparagaceae is a herbaceous plant, forming large climbing shrubs with few ascending branches or small Slender tree with weak pendulous twigs, and 1-3 m tall (Flora of China,2000). The stems of this plant are simple or few branches, greyish and smooth. Leaves of this plant are born in dense rosettes, followed by yellow flowers in terminal racemes (Gupta, 2008). *Dracaena angustifolia* (Medik.) Roxb., Myanmar named Nant thar ku is one of the medicinal plants in Myanmar traditional medicine. It is one of Dragon blood trees. Dragon blood is a deep red resin, which has been used as a famous medicine in ancient times. Local people in Loikaw Township, Kayah State, Myanmar use the stem of Nant thar ku for the treatment of hypertension, diabetic, dysentery, anemia and diarrhea. And then this plant is used as tonic and diuretic. Hence, the stem of *Dracaena angustifolia* (Medik.) Roxb. is selected for chemical and pharmaceutical investigation.

In this research work, antimicrobial activity of various solvent (*n*-hexane, ethyl acetate, acetone, ethanol and methanol) extracts, hypoglycemic and antioxidant activities of ethanol extract from the stem of *Dracaena angustifolia* (Medik.) Roxb. were evaluated. Antimicrobial activity of various solvent extracts was determined by agar well diffusion method and tested on six microorganisms. Hypoglycemic activity of the stem of this plant was determined by adrenaline induced diabetic mice model method. Glibenclamide was used as standard drug. Antioxidant activity of the selected sample was measured by DPPH assay method and ascorbic acid was used as standard antioxidant.

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Botanical description (Flora of China, 2000)

Genus	:	*Dracaena*
Species	:	*D. angustifolia*
Botanical name	:	*Dracaena angustifolia* (Medik.) Roxb.
Myanmar name	:	Nant thar ku
Part used	:	Stem
Medicinal uses	:	Hypertension, antibacterial, antifungal, diuretic, anti-diabetes, dysentery, diarrhea, purgative, anemia (Local people in Loikaw Township, Myanmar)

Figure 1 Flowers and plant of *Dracaena angustifolia* (Medik.) Roxb.

Materials and Methods

A. Sample Collection and Preparation

The stem of the *Dracaena angustifolia* (Medik.) Roxb. was collected from Loikaw Township, Kayah State, Myanmar in January, 2019. The plant sample was verified at the Botany Department of the University of Mandalay. They were cut into small pieces and air dried at room temperature for about one month. Then, the air-dried sample was stored in a well stoppered bottle, and used throughout the experiment.

Extraction:

The stems of the air-dried plant were percolated with ethanol. After two weeks, the mixture solution was filtered; filtrate was evaporated with rotary evaporator. Ethanol extract was obtained. This ethanol extract was used for the determination of antioxidant and hypoglycemic activities.

B. Preliminary Phytochemical Screening

Phytochemical evaluation for major phytochemicals was done using standard qualitative methods (Harbone, 1984). Tests for presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, and polyphenols, reducing sugars, saponins, tannins, steroids and terpenes were carried out.

C. Determination of Antimicrobial Activity

Antimicrobial activity of various solvent extracts from the stem of *Dracaena angustifolia* (Medik.) Roxb. was determined by agar well diffusion method and tested on six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* respectively. This determination was performed at Myanmar Pharmaceutical Industrial Enterprise, Ministry of Industry, Yangon.

1. Samples: The crude extracts of the sample were prepared by extracting the sample with different solvents like *n*-hexane, ethyl acetate, acetone, ethanol and methanol by

percolating method. The extracts (1g each) were introduced into sterile petri-dishes and 1mL of their respective solvent.

2. Procedure: The antimicrobial activity of the crude extracts from the selected sample was determined against six strain microorganisms by the agar well diffusion method. The extract 1g was introduced into sterile petri-dish and dissolved in 1mL or with least amount of its respective solvent till it was dissolved. The bacteria suspension from trypticase soy broth was done evenly onto the surface of the trypticase soy agar slants immediately after hardening of the agar-well were made with a 10 mm sterile cork borer from each extract's agar. After inoculums had dried for 5 minutes, the agar discs were removed and the wells were filled with sample to be tested. And then, the plates were incubated at 37°C. After overnight incubation at 37°C the diameter of inhibition zone including 10mm wells was measured. This method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organism used. The extracts from sample were tested with six microorganisms. The observation was done the inhibition zone diameters and the measurements were recorded (Fransworth, 2005; Cowan, 1999).

D. Determination of Hypoglycemic Activity

The hypoglycemic activity of the ethanol extract from the stem of the selected plant was determined by adrenaline induced diabetic mice model method (Balssells *et.al*, 2015; Cryper, 1981; Pimenta, 2006; Kitabchi *et.al*, 2009; Verbene *et.al*, 2016). This experiment was done at Department of Biotechnology, Mandalay Technological University.

1. Selection of the mice:

The strain of ICR albino mice was used in this study. The mice with 50-60 g of body weight and age of 10-12 weeks were selected to use and kept separately.

2. Induction of the diabetes to mice:

The selected mice were prepared to cause hyperglycemic effect by using adrenaline injection. 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 as shown in Figure 2(a). They were kept for 4 hours 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. After one week, the mice were used to test the hypoglycemic activity.

3. Determination of normal fasting blood glucose level:

In order to determine the normal blood glucose level, the mice were fasted overnight before the commencement of the experiment, to ensure stable blood sugar resulting blood drops were tested by Glucometer and test strips as shown in Figure 2(b) and (c).

4. Experimental design of groups of selected mice:

The selected mice were divided into 4 groups for the determination of hypoglycemic activity. They are tested plant sample, positive and negative control and normal group. Each group contained five mice and gave them markers by using sodium picrate solution.

5. Administration of the selected plant extracts, standard drug and water as treatment:

A total of 15 fasted mice were used to give orally for the determination of hypoglycemic activity. Group I mice were administered with the plant sample extracts of 1 g/kg of body weight. Group II mice were kept as a positive control, the standard drug; Glibenclamide was administered

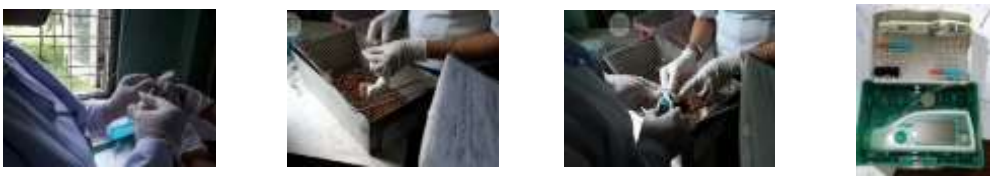
0.5 mg/kg of body weight. The remaining group was given orally with water 0.2 mL to each mouse. No fasting and no adrenaline injection group of mice were used as normal group.

6. Second induction of diabetes to mice:

After administration of the tested sample and the controls, all group mice were injected with adrenaline.

7. Screening of blood glucose level:

During the experimental procedure, three observations were performed at five times of 45 minutes interval after injection of the adrenaline by using Glucometer. The results were collected from each group for the data analysis.



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

Figure 2 Detection of hypoglycemic activity

(a) Induction of diabetes to mouse (b) Cutting the tail to collect blood

(c) Determination of blood glucose level and (d) Glucometer

E. Determination of Antioxidant Activity

Antioxidant activity of the ethanol extract from the stem of selected plant was determined by DPPH assay method by using APEL UV/Vis spectrophotometer. This determination was performed at Department of Chemistry, Panglong University.

1. Preparation of reagents:

In this experiment three solutions were prepared. They are DPPH solution, standard ascorbic acid solution and various concentrations of sample solution.

2. Preparation of 100 μM DPPH solution:

DPPH powder 0.004 g (4 mg) was weighed and it was thoroughly and gently dissolved in 100 mL of ethanol and stored in brown colored volumetric flask. It must be kept in the fridge for no longer than 24 hours before use.

3. Preparation of standard ascorbic acid solution:

Ascorbic acid (2mg) was dissolved in 20 mL of ethanol (Analar grade). This solution was thoroughly mixed at room temperature to obtain 100 μg/mL of standard solution. The various concentrations of standard solution (0. 25, 0. 5, 1, 2 and 4 μg/mL) were determined by using two-fold dilution methods. 1 mL of ascorbic acid and 3 mL of DPPH solutions were thoroughly mixed for about 15 min at room temperature. The absorbance of the mixture was measured at 517 nm.

4. Preparation of test sample solution:

Sample (0.01 g) was dissolved in 20 mL ethanol (Analar grade). This solution was thoroughly mixed at room temperature for 15 minutes to obtain 500 μg/mL of sample solution. The various concentrations of sample solution (0.625, 1.25, 2.5, 5 and 10 μg/mL) were prepared by using two-fold dilution method. 1 mL of sample solution and 3 mL of DPPH solution were

thoroughly mixed for about 15 minutes at room temperature. The absorbance of the mixture was measured at 517 nm.

5. Two-fold serial dilutions:

A two-fold dilution reduces the concentration of a solution by a factor of two that is reduced the original concentration by one half. A series of two-fold dilutions is described as two-fold serial dilutions.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function (Lee *et.al*, 2004; Vertuani, 2004).

Results and Discussion

A. Preliminary Phytochemical Test of the Stem of *Dracaena angustifolia* (Medik.) Roxb.

Preliminary phytochemical tests were carried out by standard qualitative methods. These results are shown in Table 1.

Table 1 Preliminary Phytochemical Test of the Stem of *Dracaena angustifolia* (Medik.) Roxb.

No.	Test	Reagent used	Observation	Results
1.	Alkaloid	Dragendorff's reagent	Orange ppt	+
2.	Flavonoid	EtOH, Conc: HCl, Mg ribbon	Pink color solution	+
3.	Glycoside	10 % Lead acetate	White ppt	+
4.	Phenolic	10 % $FeCl_3$	Green color solution	+
5.	Polyphenol	1 % $K_3[Fe(CN)_6]$, 1 % $FeCl_3$	Greenish blue color solution	+
6.	Lipophilic	0.5 N KOH solution	Deep blue color solution	+
7.	Saponin	Shaked with H_2O	Frothing	+
8.	Sugar	Benedict's solution	Brick red ppt	+
9.	Steroid	$(CH_3CO)_2O$, Conc.H_2SO_4	Blue color solution	+
10.	Terpene	$(CH_3CO)_2O$, $CHCl_3$, Conc.H_2SO_4	Reddish brown color solution	+
11.	Tannin	1% $FeCl_3$	Yellowish brown ppt	+

(+) present of constituents, (–) absence of constituents, (ppt) precipitate

B. Antimicrobial Activity of Crude Extracts of the Stem of *Dracaena angustifolia* (Medik.) Roxb.

Antimicrobial activity of various solvent extracts of the stem of *Dracaena angustifolia* Medik.) Roxb. was determined by applying agar well diffusion method and tested on six selected microorganisms. These results are tabulated in Table (2) and Figure (3). As the results of antimicrobial activities, ethyl acetate, ethanol and acetone extracts responded high activity on all tested organisms. Methanol extract showed high activity on *Bacillus subtilis, Candida albicans, Escherichia coli* and medium activity on *Pseudomonas aeruginosa, Bacillus pumius*. *n*-Hexane extract revealed no activity on all tested organisms. Consequently, resulting data can be shown that the stem of *Dracaena angustifolia* Medik. possess the potency for the treatment of diseases related to microorganisms tested.

Table 2 Antimicrobial Activity of the Stem Bark of *Dracaena angustifolia* (Medik.) Roxb. (Nant thar ku)

Solvent extracted	Inhibition zones (diameter, mm)					
	B. subtilis	***S. aureus***	***B. pumilus***	***P. aeruginosa***	***C. albicans***	***E. coli***
MeOH	20 (+++)	18 (++)	17 (++)	17 (++)	20 (+++)	20 (+++)
EtOH	25 (+++)	25 (+++)	21 (+++)	25 (+++)	25 (+++)	25 (+++)
Acetone	24 (+++)	24 (+++)	21 (+++)	21 (+++)	25 (+++)	25 (+++)
EtOAc	35 (+++)	40 (+++)	38 (+++)	40 (+++)	40 (+++)	42 (+++)
n-hexane	-	-	-	-	-	-

10 mm ~ 14 mm (+) - low activity, 15 mm ~ 19 mm (++) - medium activity, 20 mm above (+++) - high activity

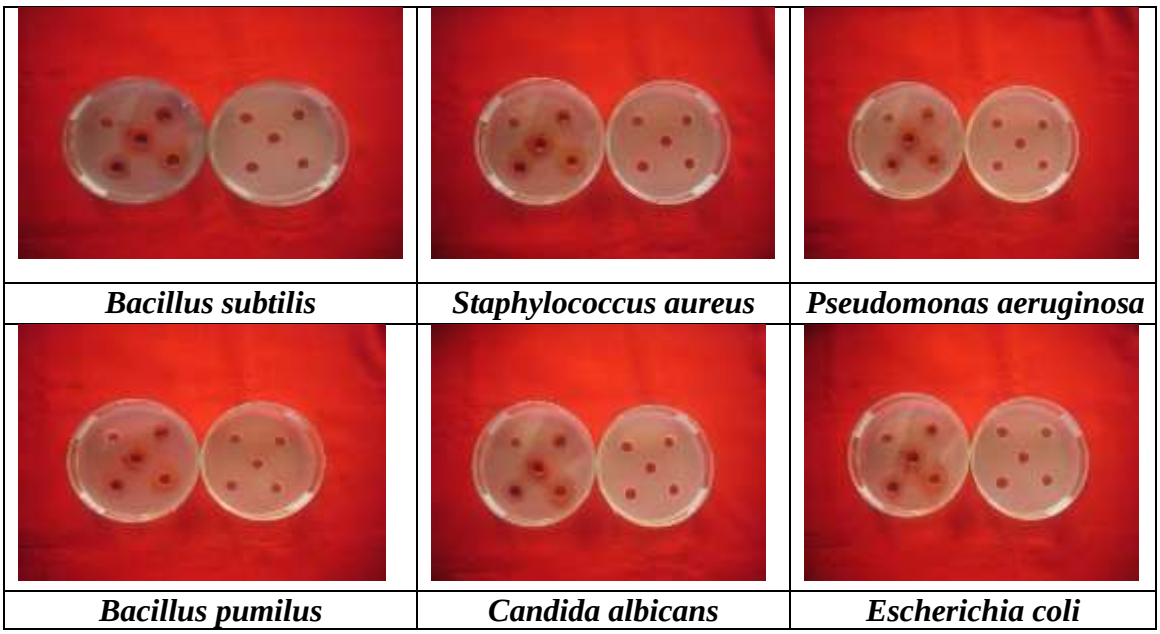


Figure 3 Antimicrobial activities (inhibition zones) of *Dracaena angustifolia* (Medik.) Roxb.

C. Determination of the Hypoglycemic Activity of Crude Extract on Adrenaline Induced Mice

The blood glucose levels of fasted animals at five times of 45 minutes intervals after adrenaline injection measured. From the measured glucose levels, the mean blood glucose values were calculated as shown in Table 3 which was also graphically presented in Figure 4. The significant hypoglycemic activity was observed in *Dracaena angustifolia* (Medik.) Roxb. and comparable to diabetic control. According to the results of the experimental carried out on diabetic mice, it had been shown that the ethanol plant extract has highly significant activity than the negative control (water) and consistent hypoglycemic effect. Positive control (Glibenclamides) was also highly significant than the water. The ethanol plant extract has highly significant activity than the negative control (water) and also effective as positive control (Glibenclamide). Therefore, ethanol plant extract has potential to use as routine drug like the positive control.

Table 3 Hypoglycemic Activity of Plant Extract, Positive Control (Glibenclamide) and Negative Control (Water)

Groups	Dose	Blood Glucose Level (mg/dL)					
		0 min	45 min	90 min	135 min	180 min	225 min
Sample extract (Nant thar ku)							
1	1 g/kg	130	153	130	104	84	70
2	1 g/kg	128	150	116	101	86	72
3	1 g/kg	124	146	122	103	82	68
4	1 g/kg	132	152	128	98	81	69
5	1 g/kg	125	148	132	95	79	67
Mean		128	150	126	100	82	69
Positive control (Glibenclamide)							
1	0.5 mg/kg	122	144	98	75	62	50
2	0.5 mg/kg	114	147	102	80	63	52
3	0.5 mg/kg	120	143	103	81	64	56
4	0.5 mg/kg	117	140	102	82	65	55
5	0.5 mg/kg	118	148	108	83	60	48
Mean		118	144	102	80	63	52
Negative control (Water)							
1	0.2 mL/kg	131	156	136	122	114	103
2	0.2 mL/kg	115	152	128	112	102	94
3	0.2 mL/kg	121	158	132	118	105	96
4	0.2 mL/kg	118	155	127	115	101	93
5	0.2 mL/kg	114	149	130	116	99	91
Mean		120	154	131	116	104	95

Table 4 Hypoglycemic Activity of Ethanol Extract of Selected Plant, Positive Control (Glibenclamide) and Negative Control (Water)

Groups	Dose	Blood Glucose Level Mean ± SD (mg/dL)					
		0 hr	45 min	90 min	135 min	180 min	225 min
Ethanol plant extract (Nant thar ku)	1 g/kg	128 ±3.35	150 ±2.87	126 ±6.56	100 ±3.71	82 ±2.71	69 ±1.94
Glibenclamide (Standard drug)	0.5 mg/kg	118 ±3.04	144 ±5.96	102 ±3.64	80 ±3.12	63 ±1.94	52 ±3.5
Diabetic negative control (water only)	0.2 mL/kg	120 ±6.80	154 ±3.53	131 ±3.60	116 ±3.39	104 ±5.89	95 ±4.64

SD = Standard Deviation

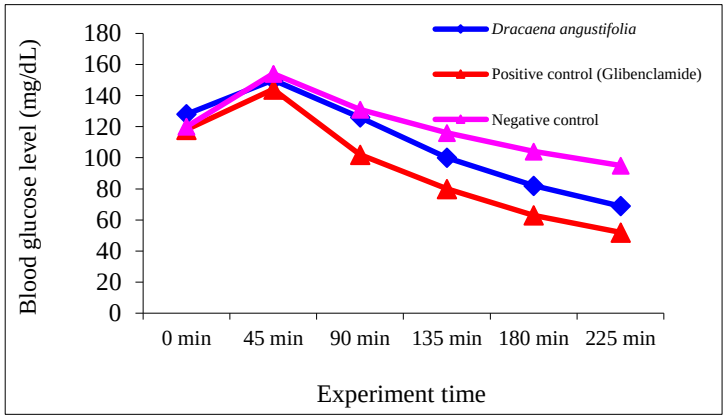


Figure 4 Level of fasting blood sugar during hypoglycemic activity test with *Dracaena angustifolia*, positive control and negative control

D. Determination of Antioxidant Activity of Stem of *Dracaena angustifolia* (Medik.) Roxb

Antioxidant activity of ethanol extract of stem of *Dracaena angustifolia* (Medik.) Roxb. was expressed as percentage of DPPH radical inhibition and IC_{50} values (μg/mL). Free radical scavenging activities values of ascorbic acid and sample extract in percentage range from 47.39% to 57.86 % and 31.25 % to 76.25 % respectively. The results of antioxidant activity using DPPH assay method in sample extract and ascorbic acid used as a positive control are shown in Figures 5 and 6 and Tables 5 and 6.

Table 5 Absorbance Values and % Inhibition of Standard Ascorbic Acid

Standard	Concentration (μg/mL)	% Inhibition	IC $_{50}$ value(μg/mL)
Ascorbic acid	0. 25	47.39	0.90
	0.50	49.58	
	1.00	51.49	
	2.00	51.67	
	4.00	57.86	

Table 6 Absorbance Values and % Inhibition of Sample Extract

Sample	Concentration (μg/mL)	% Inhibition	IC $_{50}$ value(μg/mL)
Dracaena angustifolia	0.625	31.25	3.17
	1.250	44.42	
	2.500	49.69	
	5.000	63.58	
	10.000	76.25	

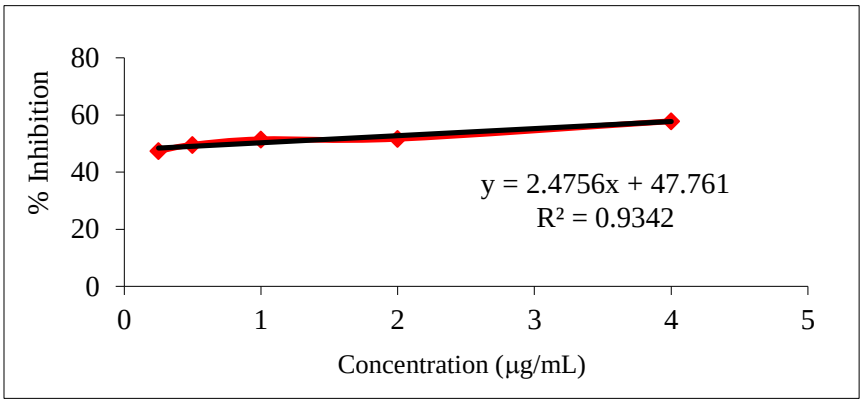


Figure 5 Plot of % inhibition vs concentration of standard ascorbic acid

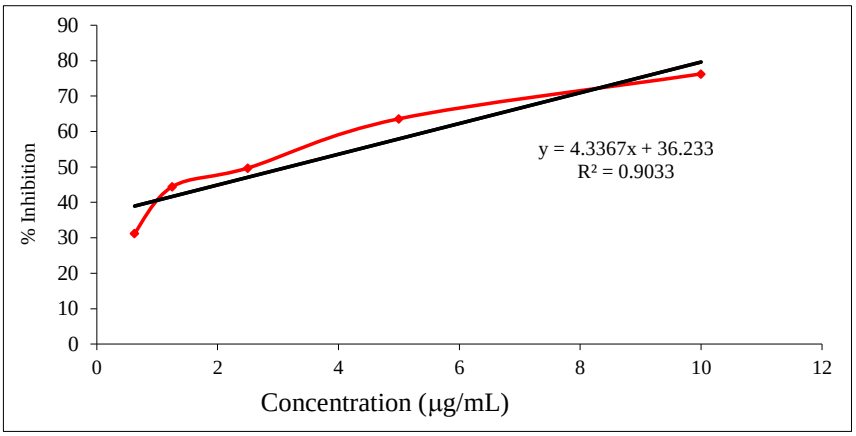


Figure 6 Plot of % inhibition vs concentration of ethanol extract of *Dracaena angustifolia* (Medik.) Roxb.

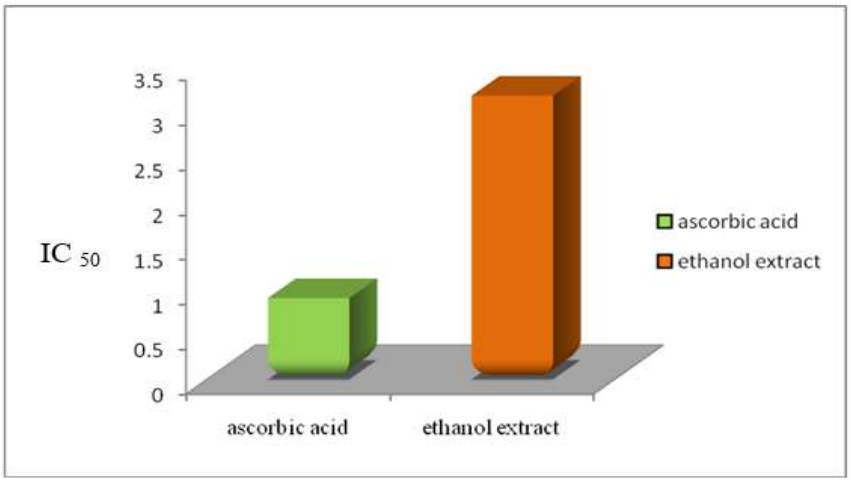


Figure 7 Comparison of IC_{50} values of standard ascorbic acid and ethanol extract of *Dracaena angustifolia* (Medik.) Roxb.

The IC_{50} value is a parameter used to measure antioxidant activity and it is defined as the sample extract concentration required for 50 % scavenging of DPPH radicals under experimental condition employed. The smaller IC_{50} value corresponds to a higher antioxidant activity. According to above IC_{50} values, the ethanol extract of the stem of *Dracaena angustifolia* (Medik.) Roxb. was found to exhibit significant antioxidant property which is comparable to standard ascorbic acid. Moreover, in accordance with Figure 5 and 6, increase in concentration implies

increase in % inhibition of oxidation. From these results, it is also observed that increase in concentration shows to increase in % inhibition, it means that increase the free radical scavenging activity.

Conclusion

In this research work one of Myanmar traditional medicinal plants, *Dracaena angustifolia* (Medik.) Roxb. was chosen for chemical and pharmaceutical investigation. Preliminary phytochemical screening showed that the selected plant has valuable phytochemical constituents. In accordance with antimicrobial activity determination, the selected plant has effective antimicrobial activity. So, various solvent extracts of the selected sample should be used for treatment of microorganism infections. The significant findings were that the test plant extract possessed high remarkable hypoglycemic effects like the standard drug, glibenclamide. It was found to possess effective hypoglycemic activity, which supports the traditional application of this plant in treatment of diabetic. IC_{50} value of the ethanol plant extract has greater than standard ascorbic acid. But the free radical scavenging activity of plant extract has effective activity like that of standard ascorbic acid. Therefore, the ethanol extract of selected plant should be used as antioxidant for maintaining of human health, protection of cancer, improve blood circulation and regulate blood pressure. From the results of experimental data, the stem of *Dracaena angustifolia* (Medik.) Roxb. has effective bioactivities. So, with the good quality control of the pharmaceutical preparation from the stem of *Dracaena angustifolia* (Medik.) Roxb., it may be used for a variety of medicinal purposes.

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