Antioxidant activity and hypoglycemic effect of *Stephania venosa* (Bl.) Spreng. tubers

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Abstract

Stephania venosa (Bl.) Spreng., a kind of Myanmar traditional medicinal plant locally known as “Taung-kyar-kyat-thway”, was collected from Saymon Taung of Popa reserved forest. The morphological characters of this plant were identified and the phytochemical and physiochemical investigations on *S. venosa* (Bl.) Spreng. tubers were tested. The extract of *S. venosa* (Bl.) Spreng. was experimented and found that there was no acute toxicity. It was also detected that it had antioxidant activity. When it was experimented on adrenaline induced hyperglycemic rats, hypoglycemic effect was observed.

Introduction

- Medicinal plants constitute an important natural wealth of a country.
- The Menispermaceae is a family predominantly of lianas of tropical lowlands and most species grow in tropical rain forest, but there are some subtropical and warm-temperate species (Heywood, 2007).
- This family has (18) genera and (43) species (Kress *et al*., 2003). *Stephania venosa* (Bl.) Spreng. is locally known as “Taung-kyar-kyat-thway”.
- This plant is widely distributed in Myanmar and is collected from Saymon Taung, Popa reserved forest.
- The plants are identified using Hooker (1875), Backer (1963), Dassanayake (1995) and HU QI-ming *et al*., (2007).

- Tubers of this plant have been utilized as one of medicinal ingredients in Thailand, Malaysia, Japan, Australia and France.
- Its tubers have been used as a traditional medicine by local people of Myanmar for antipyretic, hypertension, and nerve tonic, diuretic and sedative.
- Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals.
- The investigations on phytochemical and physicochemical properties tubers of *S. venosa* (Bl.) Spreng. were carried out.
- Today, lethality studies are , as yet, conducted as more of toxicology investigations but often only as a first step towards providing some insight into the relative potency of new chemicals (Patrick, 1988) .

- The acute toxicity of 70 % ethanolic extract from tubers of S. *venosa* (Bl.) Spreng. is tested on albino mice.
- Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk (Miller *et al.*, 2000).
- The free radical scavenging activity in tubers of *Stephania venosa* (Bl.) Spreng. has been substantially investigated.
- The hypoglycemic effect of 70% ethanolic extracts of *S. venosa* (Bl.) Spreng. tubers is investigated on the adrenaline – induced hyperglycemic rats.

Aim and Objectives

- To find out the medicinal properties of *Stephania venosa* (Bl.) Spreng. [Taung-kyar-kyat-thway].
- To perform the preliminary phytochemical tests, the physicochemical investigation
- To ascertain the antioxidants activity of 70% ethanolic extract of tubers of *S. venosa* (Bl.) Spreng.
- To verify of the hypoglycemic effect of *S. venosa* (Bl.) Spreng.

Materials and Methods

- The specimens of *Stephania venosa* (Bl.) Spreng. were collected from Saymon Taung, Popa reserved forest.

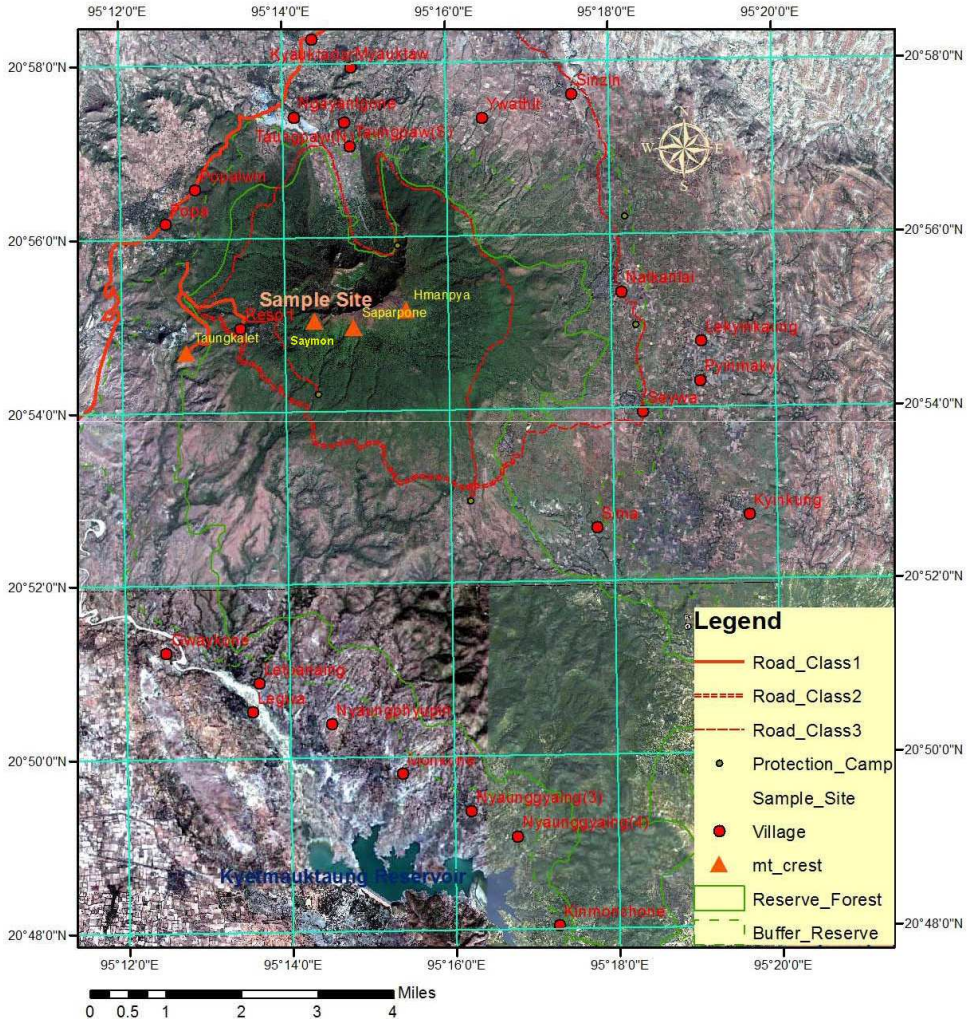


Figure 1. Sample site of Saymon Taung, Popa reserve forest area

Preparation of crude extract

- The fresh tubers were washed and chopped into small pieces and dried under shady place with good ventilation.
- (100)g of powdered tuber of *Stephania venosa* (Bl.) Spreng. was introduced into a 1000 ml conical flask and extracted with 700 ml of 70% ethanol by decoction for 6 hours.
- The solution was filtered and the solvent were evaporated to dryness using water bath at 100 °C so as to obtain a paste.
- The extract was stored in a desiccator at 4 °C until use.

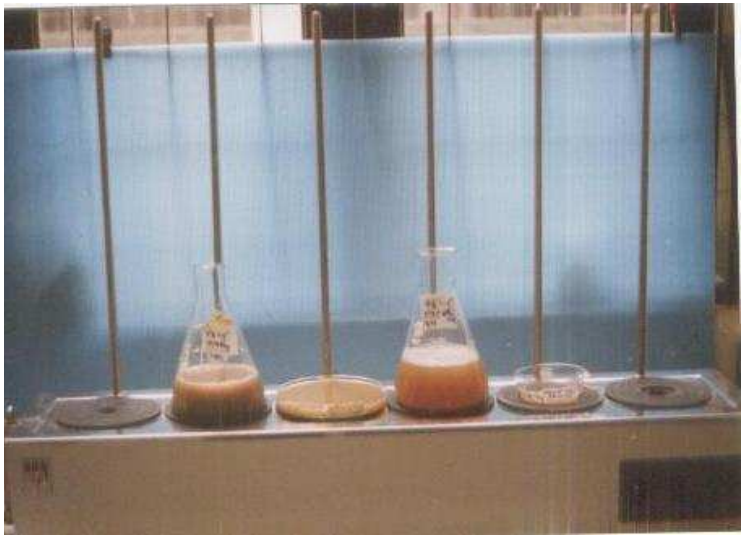


Figure 2. 70% ethanol extraction

Phytochemical and Physicochemical investigation of tubers of *Stephania venosa* (Bl.) Spreng.

- Phytochemical and physiochemical investigation on tubers of *Stephania venosa* (Bl.) Spreng. were undertaken according to the method of British Pharmopoeia (1973), Central Council for Research in Unani Medicine (1989) and Quality Control Methods for medicinal plant materials (1998).

Acute toxicity assay

- Albino mice (body weight 25-35 g) were used in the present study. The acute toxicity test was done according to the method described by Litchfield and Wilcoxon (1949).

Antioxidant activity assay

- Antioxidant activity was evaluated by the 1, 1-Di-phenyl-2-Picrylhydrazyl (DPPH) assay in accordance with the method of Tomoko Yamaguchi (1998).

Hypoglycemic activity assay

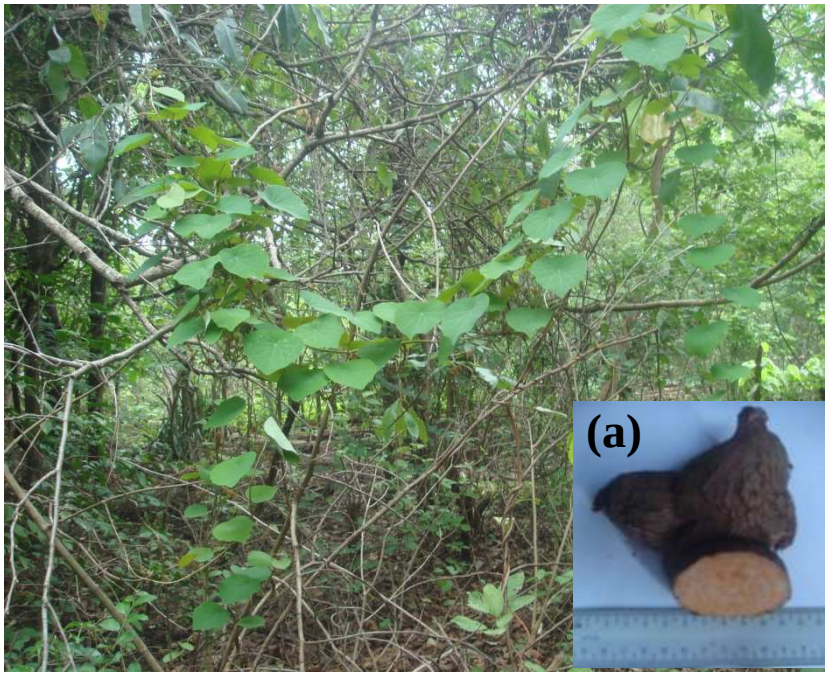
- The hypoglycemic activity of 70% ethanolic extracts are experimented on adrenaline induced hyperglycemic rats, according to the method of Gupta *et al*., (1967).

Findings and Discussions

Scientific name - *Stephania venosa* (Bl.) Spreng.
Myanmar name - Taung-kyar-kyat-thway
English name - not found
Family - Menispermaceae
Distribution - Widely distributed in Myanmar

Outstanding characters

Habit - Perennial dioecious plant, twinner with large underground tubers.



Habit **(a) tuber**

Leaves

- peltate, petiole

  reddish-green in colour.

leaves

Inflorescences

- compound umbellate

  cymose, many

  flowered;

- bracts lanceolate.

male inflorescences

female inflorescences

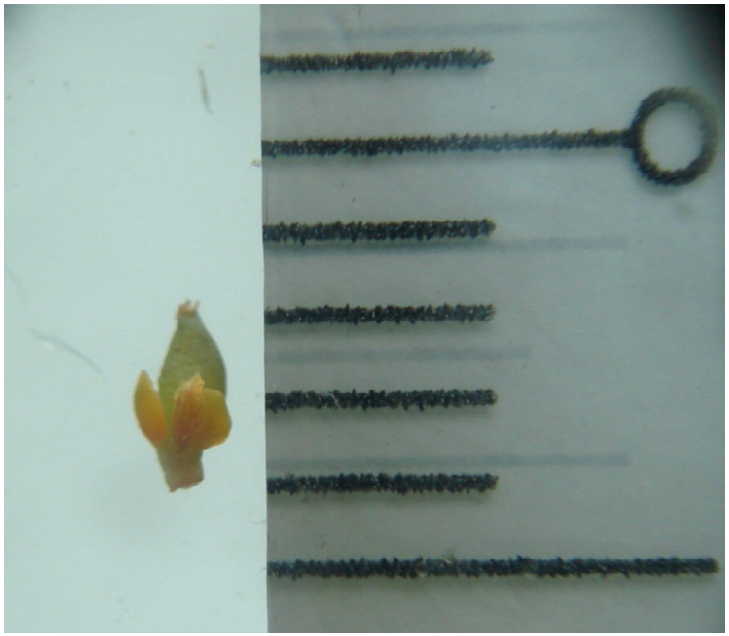
Staminate flowers

- actinomorphic;
- sepals 6 in 2 whorls, free, yellowish orange;
- petals 3, free, yellowish orange, lateral margins often inflexed;
- stamens connate in a peltate synandrium, basifixed, dehiscing transversely.

male flower

Pistillate flowers,

- zygomorphic, hypogynous;
- sepals rarely reduced to 1;
- petals reduced to 2, free, smaller than the male flowers, yellowish orange;
- staminodes absent;
- ovary oblongoid, often swollen on one side, basal placentation, the stigma laciniate.



female flower

Fruit

- Drupe with style remnant near base.

Seed

- horseshoe-shaped.

  Flowering and fruiting from August to December

Fruits

Phytochemical investigation

Table 1. Qualitative analysis of *Stephania venosa* (Bl.) Spreng. tubers

No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloid	1% HCl	Dragendroff's Mayer's Wagner's	White Reddish brown	+ +
2	Terpene	Pet-ether	$CHCL_3$, Acetic Anhydride, H_2SO_4 con.	pink colour	–
3	Carbohydrate	H_2O	10% α-napthol, H_2SO_4 con.	red ring	+
4	α-amino acid	H_2O	Ninhydrin	violet	+
5	Reducing sugar	H_2O	Fehling's Soln:	Red ppt	+
6	Glycoside	95%EtOH, 10% lead acetate	10% lead acetate	white	+
7	Flavonoid	EtOH	HCl/Mg	Pink	+
8	Steroid	$CHCL_3$	Acetic anhydride, H_2SO_4 con.	greenish blue	+
9	Saponin	H_2O	Distilled water	Frothing	+
10	Tannin	H_2O	1% ferric chloride, H_2SO_4 dil.	Yellowish	+
11	Phenolic compound	EtOH	1% ferric chloride	Deep blue	+
12	Starch	H_2O	Iodine	Blue black	+

+ = Present, - = Absent

Physicochemical properties

Table 2. Quantitative analysis of *Stephania venosa* (BL.) Spreng. tubers

Physicochemical characters	**Quantity determined(%)**
Moisture content	1.05
Total Ash	3.49
Acid insoluble ash	31.21
Water soluble ash	2.34
Ethanol soluble matter	19.53
Water soluble matter	2.44
Chloroform soluble matter	1.21
Petroleum-ether soluble matter	0.14

Acute toxicity of 70% ethanolic extract of *Stephania venosa* (Bl.) Spreng. Tuber

- The 70% ethanolic extract showed that minimum dose is 3g/kg and maximum dose is 12g /kg within 14 days.
- Therefore, it was observed that 70% ethanolic extracts were free from acute toxic or harmful effect below 6g /kg dose.
- According to method for calculation of LD_{50}, LD_{50} of 70% ethanolic extract of tuber of *Stephania venosa* (Bl.) Spreng**.** was found to be 10.7g/kg and its confidence limits was between 7.13g/kg-16.05g/kg.

Antioxidant activity of 70% ethanolic extract of tubers of *Stephania venosa* (Bl.) Spreng. by DPPH Assay Method

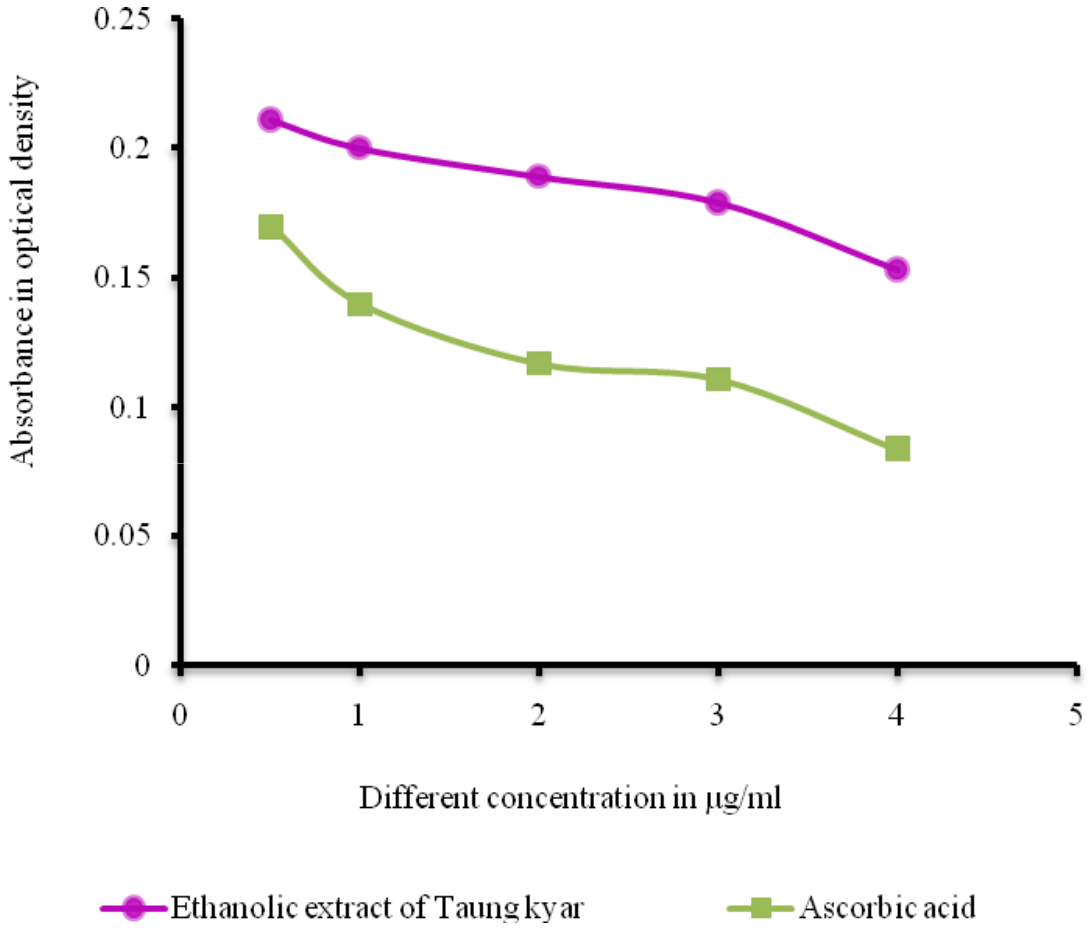


Figure 3. Absorbance effects of different concentration of 70% ethanolic extract from *Stephania venosa* (Bl.) Spreng. and the standard ascorbic acid and on *in vitro* DPPH assay

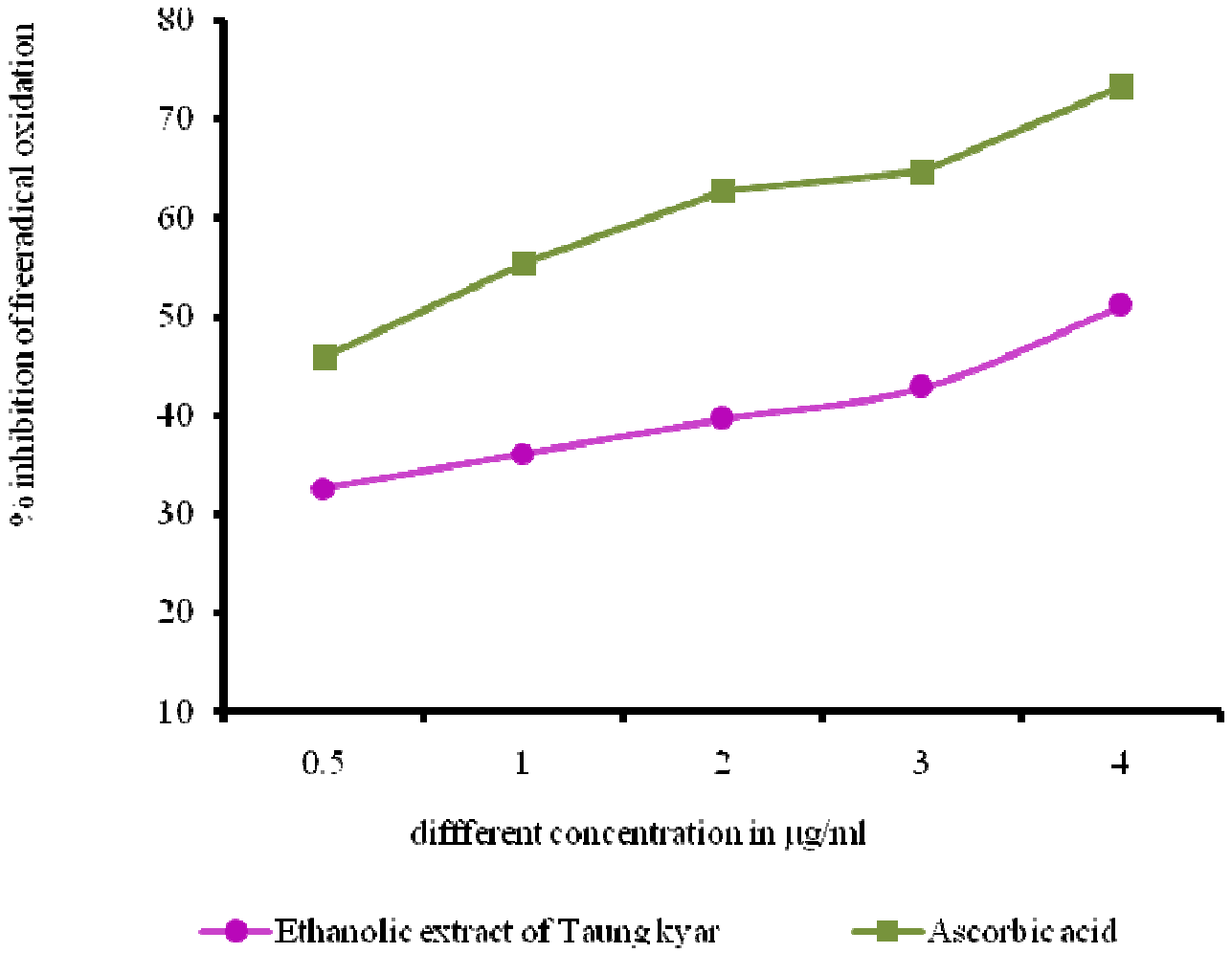


Figure 4. Percent inhibition of free radical oxidation of the ascorbic acid and different concentration of 70% ethanolic extract from *Stephania venosa* (Bl.) Spreng. *in vitro* DPPH assay

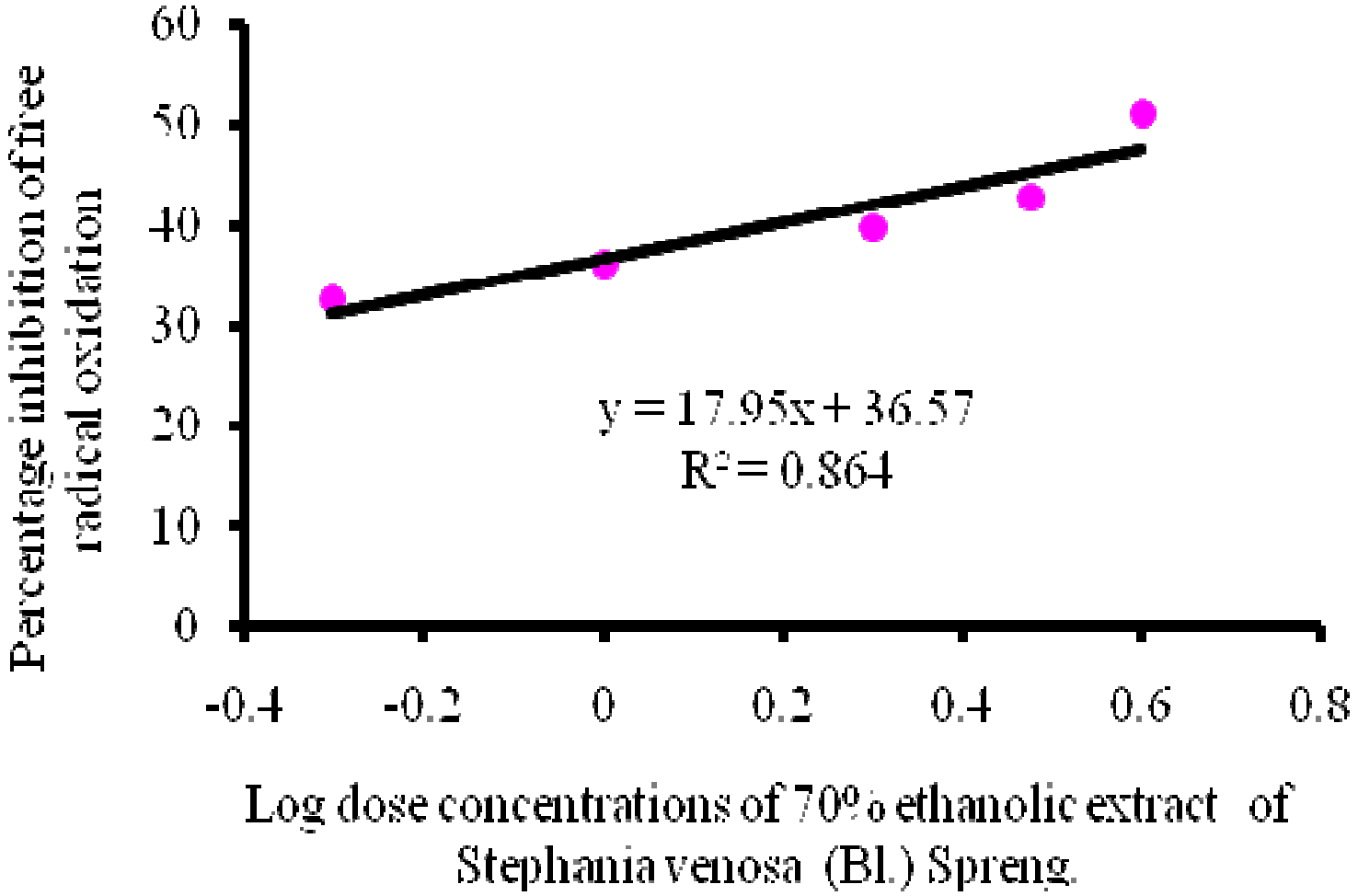


Figure 5. Relationship between mean percent inhibition of free radical oxidation and different log concentration of 70% ethanolic extract from *Stephania venosa* (Bl.) Spreng.

The points represent the mean values of observation in each group. R^2 is 0.864. The coefficient, R is 0 .929 ($P<0.05$) IC_{50} = 5.601 µg/ml

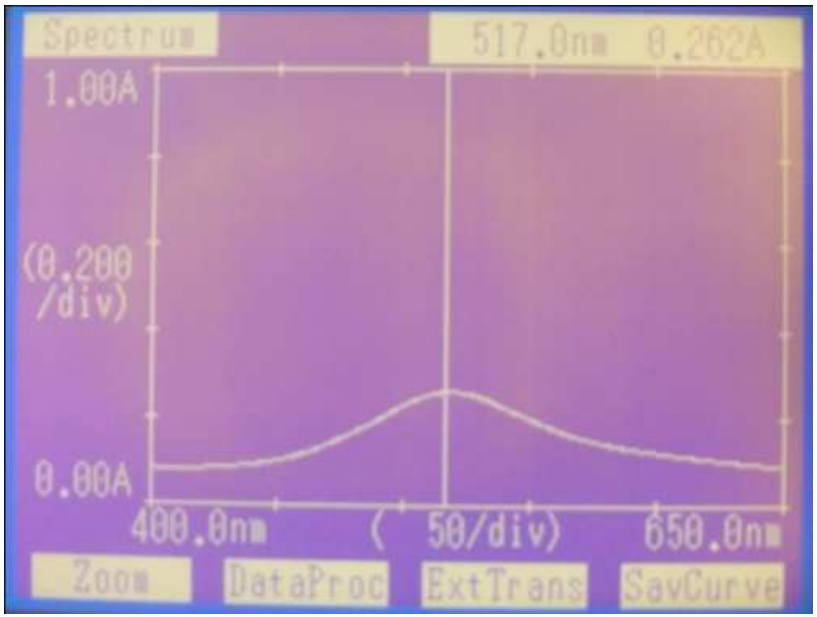


Figure 6. Peak and wavelength of using DPPH in ethanol

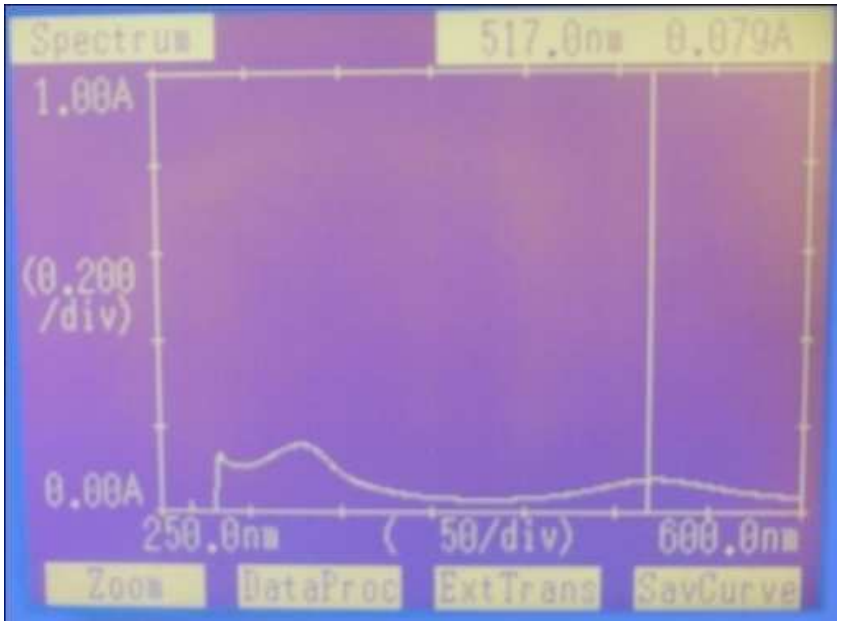


Figure 7. Peak and wavelength of using crude extract solution + 60 µM DPPH solution

Hypoglycemic activity of 70% ethanolic extract from tuber of *Stephania venosa* (Bl.) Spreng.

Table 3. Percent inhibition effect (Mean ± SEM) of 70 % ethanolic extract of *Stephania venosa* (Bl.) Spreng. 3 g/kg, 4 g/kg and Glibenclamide 4mg/kg on adrenaline- induced hyperglycemic rat model

group of rats	Percent inhibition of hyperglycemia			
	1 hr	2 hr	3 hr	4 hr
Ethanolic extract 3g/kg (n=6)	-1.46 ± 7.51	14.83±18.19	9.59±12.07	25.16±14.44
Ethanolic extract 4g/kg (n=6)	9.12 ± 7.49	23.91±17.98	42.45±10.51	47.21±14.39
Glibenclamide 4mg/kg (n=6)	39.82±7.37	45.78±13.68	58.02±10.59	91.52±11.53

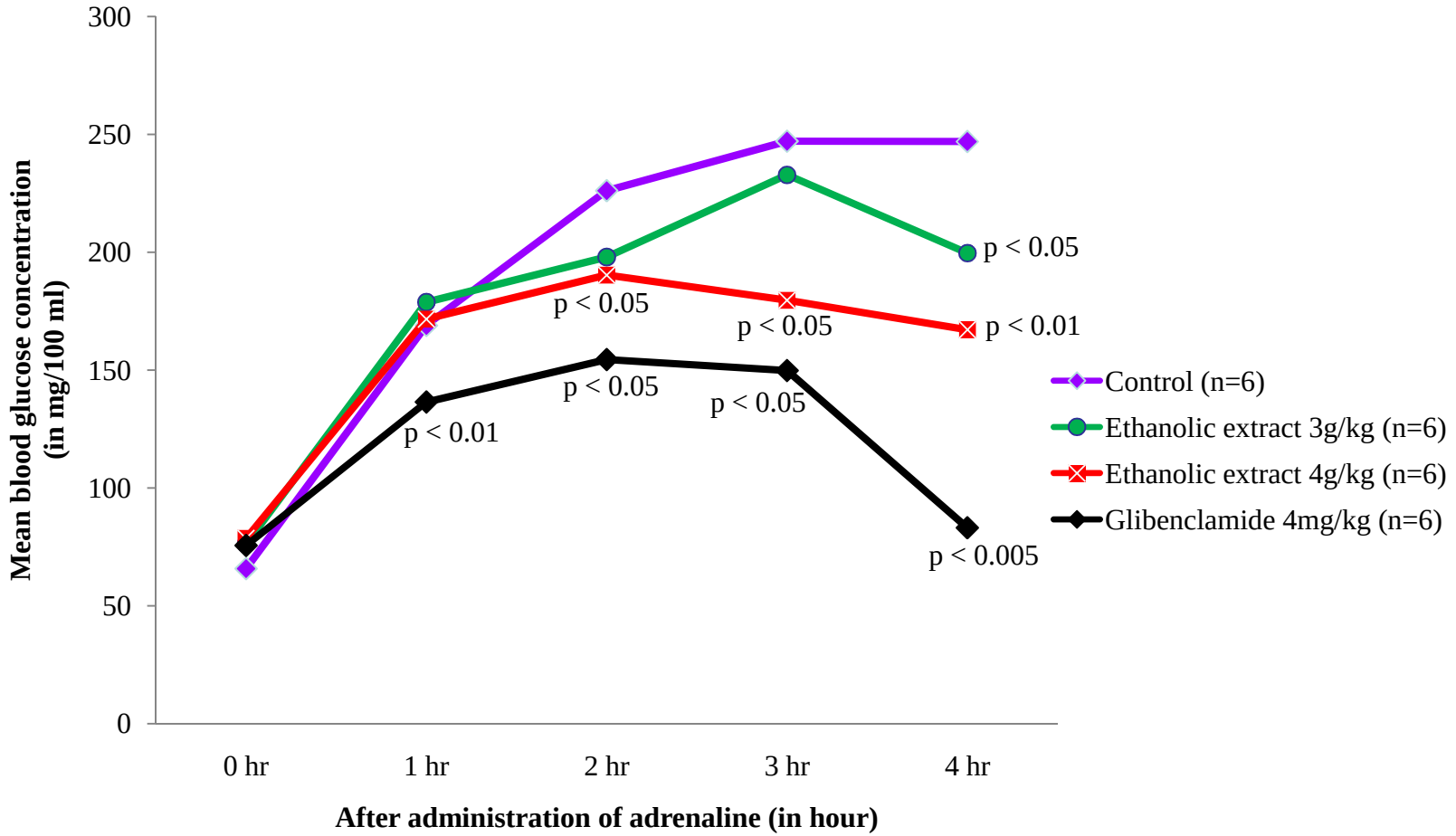


Figure 8. Time course effect of 70% ethanolic extract of *Stephania venosa* (Bl.) Spreng. 3 g/kg, 4 g/kg and standard Glibenclamide 4 mg/kg on adrenaline induced hyperglycemic rat model.

Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

Conclusion and Suggestion

- Collection of this plant was made especially from Saymon Taung, Popa reserved forest and morphological details of this plant were studied for identification. *Stephania venosa* (Bl.) Spreng. was chosen as focus of the present research because it is known to be widely used in Myanmar as folklore medicines and at the same time there is abundant literature on it (Fitoterapia, 2004 and Thai Pharmaceutical and Health Science Journal, 2007).

- Phytochemical investigation was carried out on the powder of tuber of *S. venosa* (Bl.) Spreng.. Alkaloid is especially abundant in this tuber powder. In quantitative analysis, the solubility of the tuber powder in ethanol was found to be high. Therefore the tuber powder exhibited higher solubility in polar solvents. These values are usually used for preparation of crude plant drugs in all the pharmacopeia.

- In acute toxicity study, LD_{50} of 70% ethanolic extract of tubers of *S. venosa* (Bl.) Spreng. was 10.7g/kg and its confidence limit was between 7.13 g/kg-16.05g/kg. The oral route of administration was chosen because it is the intended route for use in human. The 70% ethanolic extract was free from toxicity or harmful effect below 6g/kg dose.

- In the antioxidant activity, the radial scavenging activity of 70% ethanolic extract was found probably due to presence of relatively more polar constituents in the extract. *S. venosa* (Bl.) Spreng. "Taung-kyar-kyat-thway" can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug.

- In hypoglycemic activity, 4 g/kg of 70% ethanolic extract of *S.venosa* (Bl.) Spreng. tuber was able to reduce glucose level in blood in adrenaline-induced hyperglycemic rat model.The present study concludes that the plant might be useful as an alternative medicine for the treatment of diabetes mellitus with standard drug therapy. It should further be studied clinically on human being as hypoglycemic agent in the form of traditional medicine to be safety and extensively used by public.

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Thank You