ACUTE ORAL TOXICITY AND ANTIOXIDANT ACTIVITY ON LEAVES OF *STROBILANTHES TONKINENSIS* LINDAU

(LAT-PHAT-HMWE)

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Abstract

Strobilanthes tonkinensis Lindau (lat-phat-hmwe) is a medicinal and aromatic plant native to Yunan Province, China. The specimen was collected from Sin Phyu Inn Village, Pyin Oo Lwin Township, Mandalay Region. In the present study, microscopical evaluation on the aqueous, 95% and 70% ethanolic extraction, acute oral toxicity test and antioxidant activity of leaves of *Strobilanthes tonkinensis* Lindau were investigated. In microscopical evaluation, the presence of glandular and non-glandular trichomes, cystoliths and diacytic stomata showed the distinct characters. The leaves were extracted with 70% and 95% ethanol and water using a reflux extraction method at Research Division, University of Traditional Medicine, Mandalay in 2023. In acute oral toxicity study, six female mice (25 ± 5g) were used for each extract by using main test of Organization for Economic Co-operation and Development (OECD) 425 guidelines (2008) at Department of Medical Research, Pyin Oo Lwin Branch. In this study, there are no toxic signs and mortality in mice at the various doses of extract (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD_{50} values of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg. Evaluation of antioxidant activity of *Strobilanthes tonkinensis* Lindau were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assays method at Department of Chemistry, University of Research Centre, Mandalay. In the antioxidant activity, the IC_{50} value of standard ascorbic acid is 4.271 µg/mL and the aqueous extracts, 70% and 95% ethanolic extracts are 63.618 µg/mL, 5.026 µg/mL and 2.925 µg/mL respectively. Thus, 95% ethanolic extract was the most effective antioxidant activity than the other extracts. Therefore, the leaves of *Strobilanthes tonkinensis* Lindau are non-toxic and a good source of phytoconstituents including with potential antioxidant and used safely in medicinal purposes.

Keywords*:* *Strobilanthes tonkinensis* Lindau, Microscopical characters, Extraction, Acute oral toxicity, Antioxidant activity

Introduction

The various types of bioactive compounds derived from medicinal plants serve as raw materials for new drug discovery. Therefore, plants are a source of medicinal compounds and play an important role in the maintenance of human health (WHO, 2013). *Strobilanthes tonkinensis* Lindau is a medicinal and aromatic plant belonging to the family Acanthaceae, native to Yunan Province, China and is now extensively distributed across Thailand and Vietnam including Myanmar. It has a unique aroma of glutinous rice when dried and is rich in biologically active compounds such as amino acids and flavonoids and aromatic components. According to the World Health Organization, plant extracts or their active constituents are used as folk medicine in traditional therapies by 80% of the world's population (WHO, 2011).

Extraction is a process whereby the constituents of a plant are removed using a solvent. Watery and ethanolic extracts have different properties. Ethanol dissolves alkaloids, flavonoids,

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glycosides, tannins and essential oils but does not dissolve proteins. Water dissolves flavonoids, glycosides, tannins, saponins, sugar, proteins and mineral salts but does not dissolve fats, resins and essential oils. Choice of solvent should be based on the polarity of the plant constituents of interest (Harborne, 1984).

The acute toxicity has been defined as the adverse effects occurring within a short time of administration of a single dose within 24 hours. The acute toxicity test was done to determine the symptoms consequent to administration of the test agent and the LD50 (medium lethal oral dose) of that agent. LD50 is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route.

In Myanmar, green tea leaves are mixed with the leaves of Strobilanthes tonkinensis Lindau also called the glutinous rice flavour leaves is now popular for the very noticeable and pleasant sticky rice aroma with green tea taste. Moreover, dried aromatic powders of *Strobilanthes tonkinensis* Lindau leaves is now used in the betel by mixing with other ingredients. It has been widely and commercially cultivated in Mohnyin Township, Kachin State neighboring with Chinese Province of Southern Yunnan, the place where the native and indigenous of this plant. It is also cultivated in Pyin Oo Lwin Township, Mandalay Region, Taunggyi Township and Nantsam Township, Shan State.

Strobilanthes tonkinensis Lindau is one of the edible Myanmar indigenous plants and popularly marketable for its characteristic scent. It is a natural source of locally available useful medicinal plants. However, there were only a few researches for this plant. Therefore, this present research focused on the evaluation of pharmacological activities of these medicinal and edible leaves. For safety use of this plant, the present study aimed to examine the acute oral toxicity of the leaves of this plant. Moreover, this investigation also intended to evaluate the antioxidant activity of the leaves of this plant.

Materials and Methods

Plant samples collection and preparation

The plant samples were collected from Sin Phyu Inn Village, Pyin Oo Lwin Township, Mandalay Region. The collected leaves samples were cleaned thoroughly to remove impurities. Then, the leaves were air-dried, grinded and kept in the air-tight containers for the study of diagnostic characteristics.

Extraction from leaves of *Strobilanthes tonkinensis* Lindau

In this study, the aqueous extract, 95% and 70% ethanolic extract were extracted by reflux extraction method at Research Division, University of Traditional Medicine, Mandalay in 2023.

Aqueous extraction

The aqueous extract will be done by 100 g of leaves of *Strobilanthes tonkinensis* Lindau with 500 ml of distilled water by using reflux apparatus for 3 hours at 60°C for 3 times. The extract will be filtered by using filter paper and filtration will be concentrated by using a vacuum rotary evaporator at 60°C. The concentrated extract will be made powder by freeze dryer to get dry powder extract.

95% and 70 % ethanolic extraction

The powder of leaves of *Strobilanthes tonkinensis* Lindau was successively extracted with 95% and 70 % ethanol and water by using Soxhlet apparatus 6 hrs. The ethanol-soluble fraction was dried by rotary evaporator under reduced pressure and concentrated on water bath at 60ºCat Research Division, University of Traditional Medicine. (Alekhya *et al*., 2013; Tailor & Goyal, 2014).

Figure 1 Extraction of leaves by Soxhlet

Figure 2 Removal of solvent by Rotary Evaporator

Figure 3 Drying of water extract by Freeze Dryer

Acute oral study from leaves of *Strobilanthes tonkinensis* Lindau

Acute oral toxicity study from aqueous and 70% ethanolic extract from leaves of *Strobilanthes tonkinensis* Lindau was performed at Department of Medical Research, Pyin Oo Lwin Branch. (OECD) 425 guidelines (2008).

Acute oral toxicity study from aqueous extract and 70% ethanolic extract from leaves of *Strobilanthes tonkinensis* Lindau

The two extracts of aqueous and 70 %ethanolic extract were tested on adult healthy 12 female albino mice of 25 ± 5g for acute oral toxicity. It was carried out according to Organization for Economic Co-operation and Development (OECD) guideline 425.The main test was performed because there is no previous report about the toxicity and lethality of the leaves of *Strobilanthes tonkinensis* Lindau.

The mice were fasted food but not water for 4 hours prior to dosing. The fasted body weight of the first mice was determined and initiated at 175 mg/kg. This mouse was observed carefully each interval for 30 minutes, 1 hr, 4 hours and then 24 hrs. After 48 hours observation, the next mice were selected by using a factor of 3.2 times the original dose if the first mice survived. Therefore, the similar dose progression was done. The second mice were given 550 mg/kg, the third mice were 1750 mg/kg. the fourth mice were 5000 mg/kg and then the fifth and sixth mice were 5000 mg/kg. Body weight for each was determined for 7th day, 14th day.

Preparation of animals

For acute toxicity test, only female mice (body weight 25 ± 5g) were used. This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in those case where differences are observed, females are

generally slightly more sensitive. The animals were provided by Laboratory Animal Service Division, Department of Medical Research, POL Branch. The animals were kept in the cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals maintained in laboratory with unlimited supply of food and water (OECD, 2008).

Preparation and administration of doses

The 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau was dissolved in distilled water for required concentration to be administered. Animals were fasted prior to dosing. Food but not water was withheld for 3-4 hours in mice. The fasted body weight of each animal was determined. The dose was calculated according to fasted body weight. By using cannula, doses of the test substances administered. Then, food was withheld for 1-2 hours (OECD, 2008). Body weights of mice were measured and recorded once a week. At the end of the test period, the mice were weighed again.

Figure 4 Preparation and administration of doses

Clinical observations

The mice were observed continuously during the first 30 minutes after dosing and observed periodically for the next 24 hours to monitor the systemic toxic effects and then daily thereafter, for 14 days. All observations were systematically recorded with individual worksheet being maintained for each animal. The observations of parameters were recorded skin and fur changes, eyes, mucous membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. Salivation, convulsion, cyanosis, tremors, and diarrhoea did not occur in all animals for 14 days (OECD 2008).

Screening of Antioxidant Activity on leaves of *Strobilanthes tonkinensis* Lindau by DPPH free Radical Scavenging Assay Method

The antioxidant activity of aqueous extract and 70% and 95% ethanolic extract were measured by using DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging assay method at Department of Chemistry, University of Research Centre, Mandalay in 2023 (Neha & Mishra, 2011).

Preparation of Test Sample Solution for Crude Extract

About 0.01g of leaf extract was dissolved in 10 ml of 95 % ethanol under vigorous shaking. This solution was used as a stock solution (1 mg/ mL). This stock solution was diluted with ethanol to obtain desired concentrations of 25, 50, 100, 200, 400 μg/mL.

Preparation of 60 μM DPPH Solution

DPPH powder (0.0024 g) was thoroughly and gently dissolved in ethanol (100 mL). This solution was freshly prepared and stored in brown coloured reagent bottle. It was kept in the refrigerator for no longer than 24 hours.

Preparation of Standard Ascorbic Acid Solution

Ascorbic acid (0.01g) was dissolved in 100 mL of 95 % ethanol. It was diluted with ethanol to obtain the standard solution with the concentrations of 1, 2, 4, 8 and 16 μg/mL).

Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

The control solution was prepared by mixing 2 mL of 60 μM DPPH solution and 2.0 mL of ethanol. Then, the test sample solutions were also prepared by mixing the test solution (2.0 mL) and 60 μM DPPH solution (2.0 mL) with various concentrations. The blank solution was also prepared by mixing the test sample solution (2.0 mL) and 95 % ethanol (2.0 mL). Furthermore, these solutions were allowed to stand at room temperature for 30 minutes. Moreover, the absorbance of each solution was measured at 519 nm by using UV-1800 spectrophotometer.

% inhibition value was calculated by using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}} \times 100$$

$\text{Abs}_{\text{control}}$ = absorbance of control solution

$\text{Abs}_{\text{sample}}$ = absorbance of test sample solution

$\text{Abs}_{\text{blank}}$ = absorbance of blank solution

Results

Morphological and Microscopical characters of *Strobilanthes tonkinensis* Lindau

Aromatic herb; stem and branches quadrangular; inflorescence terminal and axillary spike, leaves simple, opposite; flowers white, bisexual, zygomorphic, hypogynous, bracteate, bracteoate, glandular trihomes present; calyx (5), lobes linear; corolla (5), tubular; stamens 4, didynamous, carpels (2), glandular stipitate in upper portion of ovary, yellow disc at the base of ovary. In microscopical evaluation the presence of glandular trichomes, cystoliths and diacytic stomata showed the distinct characters. SEM images also revealed the presence of cystoliths and glandular trichomes on the surfaces of leaves. The results were shown in Figure:5–18.

Figure 5 Habit

Figure 6 Inflorescence

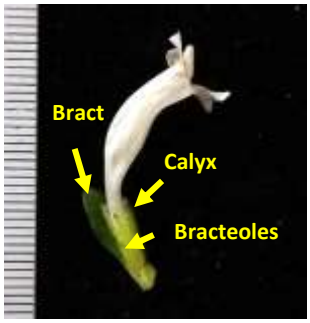

Figure 7 Flower

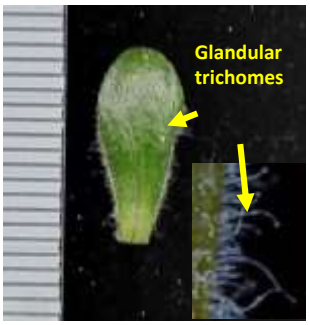

Figure 8 Bract

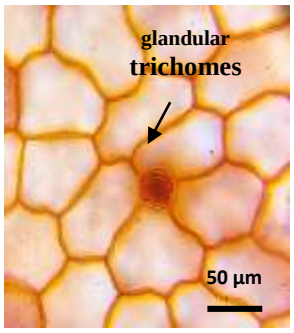

Figure 9 Adaxial surface view of lamina

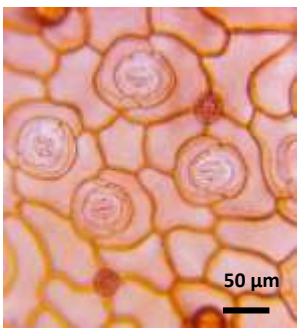

Figure 10 Abaxial surface view of lamina

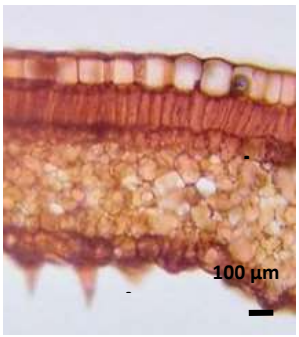

Figure 11 T.S of lamina

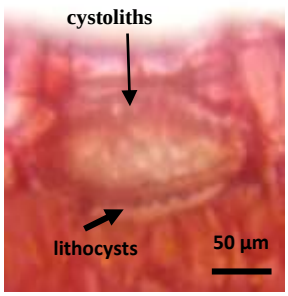

Figure 12 Cystolith within lithocyst in the epidermal layer of T.S of lamina

Figure 13 Cystolith

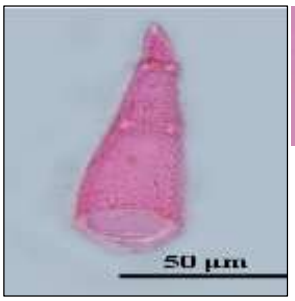

Figure 14 Non-glandular trichome

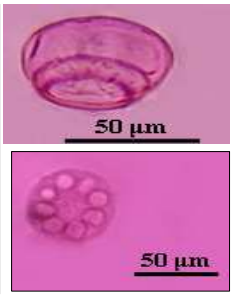

Figure 15 Glandular trichome

Figure 16 Spiral vessels

Figure17 Aseptate fiber

Scanning electron microscopy (SEM)

Figure 18 SEM images of leaves of *Strobilanthes tonkinensis* Lindau

Acute Toxicity Study from Aqueous Extract and 70% Ethanolic Extract of *Strobilanthes tonkinensis* Lindau

Acute toxicity test was done by using main test of OEDC 425 guideline (2008). In the acute toxicity study, there were no toxic signs and mortality in mice at the various doses of 70 % ethanolic extract of *Strobilanthes tonkinensis* Lindau (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period.

Observations signs of toxicity and mortality of the mice were recorded. There were no toxic signs and mortality during the observation period of 14 days. Skin and fur changes, eyes, mucous membranes, motor activity and behavioral patterns were found to be normal. Salivation, convulsion, paralysis of limbs, tremors and diarrhea were not found in all mice and was shown in table (5). Therefore, the LD50 value of 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg (raw powder 35.7 g/kg) body weight.

Acute toxicity test was done for aqueous extract by using main test according to OECD 425guideline (2008). It showed that at the dose of 5000mg/kg of watery extract of *Strobilanthes tonkinensis* Lindau there was no lethality of the mice up to 14 days observation period. Therefore, median lethal dose (LD_{50}) of extract was supposed to be greater than 5000 mg/kg.

Clinical observations

For evaluation of toxicity, no significant changes were observed in toxic parameters. Skin and fur changes, eyes, mucous membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. Salivation, convulsion, cyanosis, tremors, and diarrhoea did not occur in all animals. There were no abnormalities detected.

Body weight

Single oral doses of 175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg of *Strobilanthes tonkinensis* Lindau were administered to the mice. The animals were observed for 14 days. There were no significance changes in body weight of the mice before and after administration of the tested drug.

Table 1 Result of acute toxicity of aqueous extract of *Strobilanthes tonkinensis* Lindau on mice after single oral administration of the test drug according to main test in OECD 425 guideline

Different doses of test drug (mg/kg)	Number of tested mice	Observed period (Day)	Body weight (g)			Observed death
			0 week	1st week	2nd week	
175	1	14	24g	24g	24g	0
550	1	14	22g	22g	23g	0
1750	1	14	23g	22g	22g	0
5000	1	14	22g	21g	20g	0
5000	1	14	29g	25g	26g	0
5000	1	14	23g	22g	22g	0

Table 2 Result of acute toxicity of 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau on mice after single oral administration of the test drug according to main test in OECD 425 guideline

Different doses of test drug (mg/kg)	Number of tested mice	Observed period (Day)	Body weight (g)			Observed death
			0 week	1st week	2nd week	
175	1	14	21g	21g	20g	0
550	1	14	22g	22g	21g	0
1750	1	14	23g	21g	21g	0
5000	1	`14	24g	23g	23g	0
5000	1	14	26g	24g	25g	0
5000	1	14	26g	24g	24g	0

Antioxidant Activities of leaves of *Strobilanthes tonkinensis* Lindau

Antioxidant activities of aqueous extract and 70% ethanolic extract were determined by DPPH radical scavenging method and compared with standard ascorbic acid. DPPH scavenging activity of standard and sample solutions showed by reducing the purple coloured radical DPPH to the yellow coloured non-radical form DPPH.DPPH is a stable organic, free radical with an absorption band at 517 nm. The absorbance and % inhibition values at various concentrations of standard ascorbic acid and solution of two different extracts.

Antioxidant activities of standard and test sample solutions were expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/mL). The IC_{50} value of standard ascorbic acid is 4.271 µg/mL and the aqueous extracts, 70% and 95% ethanolic extracts are 63.618 µg/mL, 5.026 µg/mL and 2.925 µg/mL respectively.

Table 3 Scavenging activity of standard Ascorbic acid at different concentrations

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ascorbic acid	1	0.228	32.34	4.271
	2	0.166	43.91	
	4	0.129	50.74	
	8	0.109	67.65	
	16	0.086	90.91	

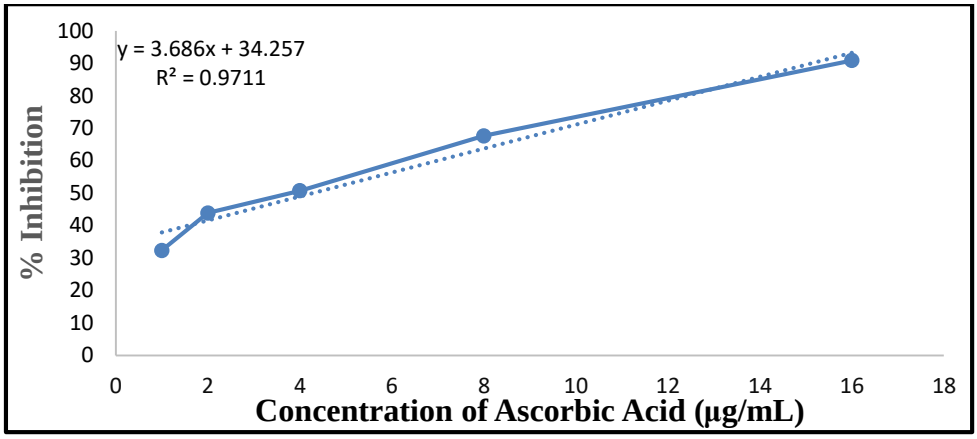

Figure: 19 Percent Inhibition at Different Concentration of Standard Ascorbic Acid

Table 4 Scavenging activity of aqueous extract on leaves of *Strobilanthes tonkinensis* Lindau at different concentrations

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Watery extract	25	0.801	41.125	63.618
	50	0.543	49.559	
	100	0.422	58.941	
	200	0.256	64.400	
	400	0.089	86.205	

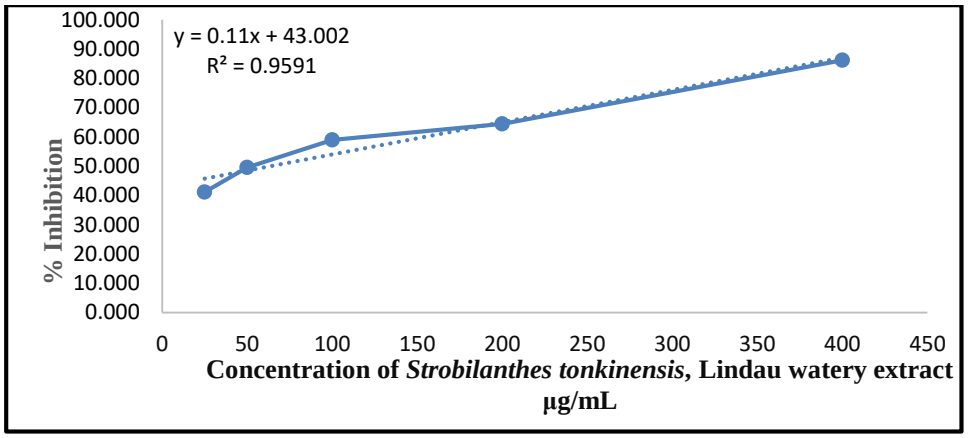

Figure 20 Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau aqueous extract µg/mL

Table 5. Scavenging activity of 70% ethanolic extract on leaves of *Strobilanthes tonkinensis* Lindau at different concentrations

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
70% Ethanol	2.5	0.192	42.169	5.026
	5	0.153	53.916	
	10	0.141	57.530	
	20	0.102	69.277	
	40	0.018	94.578	

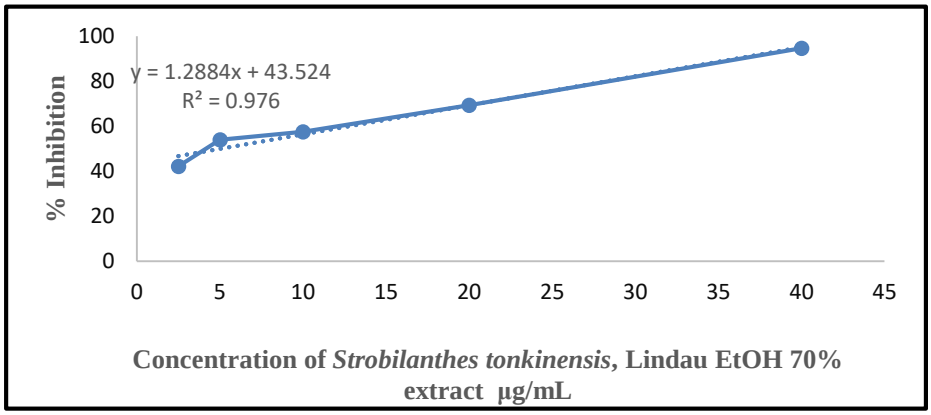

Figure 21. Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau EtOH 70% extract µg/mL

Table 6 Scavenging activity of 95% ethanolic leaves extract of *S. tonkinensis* Lindau at different concentrations

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
95% Ethanol	2.5	0.132	48.031	2.925
	5	0.117	53.937	
	10	0.109	57.087	
	20	0.083	67.323	
	40	0.031	87.795	

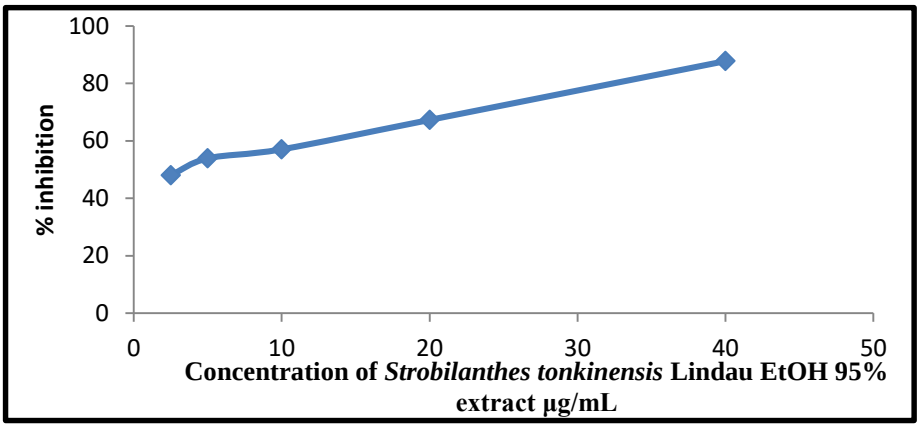

Figure 22. Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau EtOH 95% extract µg/mL

Discussion

In this study, the extraction with aqueous and 70 % ethanol from leaves of *Strobilanthes tonkinensis* Lindau and their acute oral toxicity test and antioxidant activity were carried out. The extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time and composition of the sample. Under the same extraction, temperature, solvent and composition of sample are the most important parameters. In this study, extracts of *Strobilanthes tonkinensis* Lindau were obtained by using water and 70% ethanolic extract. So, for extraction study, Reflux method was used.

The study on acute oral toxicity was performed in 6 numbers of female mice (25 ± 5g) for each extract and by using main test of Organization for Economic Co-operation and Development (OECD) 425 guidelines (2008) because there is no previous report in the international including Myanmar about the toxicity and lethality of the leaves of *Strobilanthes tonkinensis* Lindau. So, the main test was investigated. In this toxicity study, there are no toxic signs and mortality in mice at the various doses of extract (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD_{50} values of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg.

In the antioxidant activity of aqueous extract and 70% ethanolic extract was determined using DPPH solution. The antioxidant activity is expressed in terms of IC_{50} (µg/mL) values. The IC_{50} of a compound is inversely related to its antioxidant capacity. A lower IC_{50} value indicates higher antioxidant activity. The reference standard used was ascorbic acid. The obtained values for antioxidant activity of aqueous extract and 70% ethanolic extract by DPPH methods were ranged from 63.618 to 5.026 µg/mL. The highest activity was found in ethanolic extract and the others showed lower capacity to inhibit DPPH radicals. In comparison, ethanolic extract showed higher activity than the aqueous leaves extract of *Strobilanthes tonkinensis* Lindau.

Conclusion

Strobilanthes tonkinensis Lindau is a medicinal and aromatic plant. In Myanmar, green tea leaves are mixed with the leaves of *Strobilanthes tonkinensis* Lindau because of its strong aroma of glutinous rice. There was no information about the toxicity of *Strobilanthes tonkinensis* Lindau. According to the result, this aromatic odour may be due to the release of essential oils from the glandular trichomes present on the leaf surfaces. The information such as the presence of trichomes and crystal characters can help in identification and to detect the adulteration of other drugs in this medicinal plant. The medium lethal dose, LD_{50} was more than 5000mg/kg body weight. Therefore, the leaves of *Strobilanthes tonkinensis* Lindau were practically non-toxic and may be relatively harmless. These leaves may be used safely in medicinal purposes.

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