Phytochemical Investigation of *Tecoma stans* (L.) H.B.K Leaves and its Brine Shrimp Lethality test

Aung Mya Thein[1] , Khin Cho Cho Oo[2]

Abstract

The medicinal plant, *Tecoma stans* (L.) H.B.K. belongs to the family Bignoniaceae and locally known as "Sein-ta-kyu". It is widely distributed as an ornamental plant and collected from the Yangon University Campus, Yangon Region, during 2015-2017. The leaves of *Tecoma stans* (L.) H.B.K have hypoglycemic action. Tecomine and tecostanine are hypoglycemic alkaloids. In this research, morphology, taxonomy of the vegetative and reproductive parts and histological characters of *Tecoma stans* (L.) H.B.K. was investigated with the help of available literature. The sensory characters of the powdered leaves were studied. Among them, the taste of leaves is bitter. Investigation on preliminary phytochemical tests of the powdered sample of leaves were examined for the presence or absence of primary and secondary metabolites. It was found that glycoside and tannin were especially abundant in the leaves of *Tecoma stans* (L.) H.B.K. The aqueous extract and 70 % ethanolic extracts of *Tecoma stans* (L.) H.B.K. was tested for in vivo Brine Shrimp Lethality Assay (BSLA). It was shown that the aqueous and 70 % ethanolic extracts were potent against the brine shrimp with LC_{50} values of 0.078 mg/mL and 1.318 mg/mL respectively.

Keywords : Morphological and histological characters, Phytochemical test, Brine shrimp lethality test

Introduction

Medicinal plants constitute an important natural wealth of a country. Bignoniaceae family is pantropical and subtropical with a few genera in the temperate parts of North America, Asia and the southern hemisphere (Heywood, 2007). Bignoniaceae family consists of 22 genera and 40 species. *Tecoma stans* (L.) H.B.K. belongs to the family Bignoniaceae and locally known as Sein-ta-kyu in Myanmar (Kress *et al*., 2003). Medicinal plants have a long history of use in most communities throughout the world. It has been confirmed by WHO that herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. The history of plants being used for medicinal purposes is probably as old as the history of mankind. Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Akereele, 1984). Phytochemical screenings of plants have revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins, etc. The study of bioactive compounds from plant sources and extracts in the chemical laboratory is often difficult due to the lack of suitable, simple and rapid screening procedure. One of the simplest biological responses to monitor is Lethality, since there is only one criterion, which is either dead or alive. A procedure for general toxicity screening that does not require too much specialization is therefore essential as a preliminary stage in the study of

[1] Dr., Lecturer, Department of Botany, Dagon University
[2] Dr., Associate Professor, Department of Botany, Dagon University

bioactive substances in plant extracts. A simple animal that has been used for this purpose is the brine shrimp, *Artemia salina* Leach. This method is a rapid, inexpensive, in-house bioassay for pharmacologically active plant extracts (Meyer *et al.,* 1982 and Sam, 1993). The present research aims to find the medicinal value of *Tecoma stans* (L.) H.B.K. and to promote the intensive application of Myanmar traditional medicine. The objectives of the present study are to verify the morphological and histological characters of various plant parts, to examine the phytochemical constituents and brine shrimp lethality tests from the leaves of *Tecoma stans* (L.) H.B.K.

Materials and Methods

Botanical studies

The specimens of *Tecoma stans* (L.) H.B.K. was collected from the Yangon University Campus, Yangon Region, during 2015-2017. These plants were classified and identified using Hooker (1885), Backer and (1965). In the histological study, *Tecoma stans* (L.) H.B.K. was prepared by freehand sectioning and examined according to the methods of Metcalfe and Chalk (1950), Esau (1965), Pandey (1988), Trease and Evans (2002) at the Department of Botany, University of Yangon.

The following reagents were used to examine the section cutting and powdered samples.

- Chloral hydrate solution B.P as clearing reagents.
- Solution of phloroglucinol B.P followed by concentrated hydrochloric acid for testing lignin.
- Acetic acid and concentrated sulphuric acid for testing calcium oxalate crystals.
- The oil globules were confirmed by using Sudan III and IV.

The collected plants were thoroughly washed with water to remove impurities and cut into small pieces. Then, the samples were on air-dried in shade for two weeks. When constant weight was obtained the samples were pulverized by a grinding machine. This powder was stored in the airtight container for further study.

Chemical studies

Preliminary phytochemical investigation on leaves of *Tecoma stans* (L.) H.B.K. was carried out to examine the plant constituents.

According to the methods of Marini Bettolo, *et al*., (1981), Central Council for Research in Unani Medicine (1989) and Trease and Evans (2002) were applied for the investigation of phytochemical studies. The results were as shown in Figure (1) and Table (1).

Fig. 1 Phytochemical Test

Acute toxicity test of aqueous and 70 % ethanolic extracts of *Tecoma stans* (L.) H.B.K. leaves on brine shrimp lethality test

Brine shrimp lethality test was employed to determine the toxicity of aqueous extract and 70 % ethanolic extract of *Tecoma stans* (L.) H.B.K. This test was conducted at the Department of Botany, University of Yangon.

Hatching of brine shrimp larvae

Dried brine shrimp cysts (1 g) were incubated in a hatching container with the aeration artificial seawater (42 g/l) at 28 - 30 °C under the light source. An aquarium stone aerator provides suitable oxygenation.

Fig. 2 Hatching of brine shrimp larvae

Non-hatched eggs lay at the bottom and empty capsules float on the water surface. After 48 hrs of incubation at room temperature, the active brine shrimp nauplii were collected by using Pasteur pipette and ready to transfer to the test solution. Artificial seawater was prepared into 1 litre of distilled water, NaCl, KBr, KCl, Na_2SO_4 and $MgCl_2$.

Preparation of stock solution

Artificial seawater 10 ml was placed in a beaker and aqueous extract of leaves 60 mg was added to get stock solution.

Procedure for Brine Shrimp toxicity test

Firstly, 5 beakers are filled with 1 ml of artificial seawater and 1 ml of stock solution was added to the first beaker and mix thoroughly. Afterward, according to the serial dilution method, 1 ml of mixed solution (i.e, extract and artificial seawater) from the first beaker was added to the second beaker and simultaneously continue 1 ml each from second to third, third to fourth and fourth to the fifth beaker. The sixth beaker was kept as a control. Then, the previously prepared brine shrimp nauplii 10 numbers each was collected by using Pasteur pipette and transferred to the 5 beakers containing tested sample solution and incubated for 24 hrs. The beakers were examined at 6 hrs interval and the number of death nauplii in each beaker was counted and recorded. The procedure of these tests was repeated three times.

The mortality percentage was calculated using the following formula.

$$\% \text{ of mortality} = \frac{\text{number of mortality}}{\text{number of test shrimps}} \times 100$$

The same procedure was also repeated three times for 70% ethanolic extract of leaves.

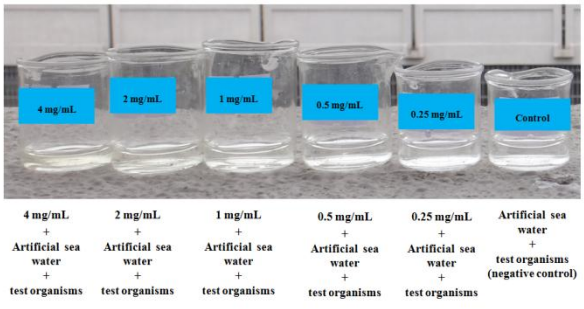

Fig.3 Brine shrimp toxicity test of aqueous extract of *Tecoma stans* (L.) H.B.K.

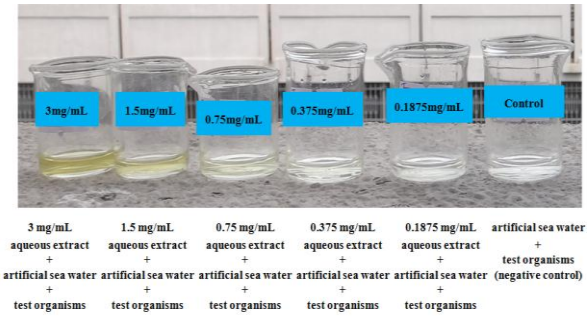

Fig.4 Brine shrimp toxicity test of 70 % ethanolic extract of *Tecoma stans* (L.) H.B.K.

Results

Morphological characters of *Tecoma stans* (L.) H.B.K.

Scientific Name - *Tecoma stans* (L.) H.B.K.
Myanmar Name - Sein-ta-kyu
English Name - Yellow Bell (or) Yellow Trumpet
Family - Bignoniaceae

Perennial small tree, 1.5 - 2.0 m high, stem terete with warty. **Leaves** unipinnately compound, imparipinnate, leaflets 5 - 7 pairs, opposite, lamina lanceolate, 2.7 - 10.8 cm long and 0.8-3.2 cm wide, the tips acuminate, the margins serrate, the bases cuneate, both surfaces glabrous, petiolules 0.2 - 1.4 cm long, glabrous, petioles 2.5 - 6.3 cm long, glabrous. **Inflorescence** terminal or axillary, racemose, the peduncles cylindrical, 0.6 - 1.0 cm long and 1.5 mm wide, pedicel 0.8 - 1.5 cm long and 1.7 mm wide, glabrous, bracteates, bracteolate. **Flowers** bright yellow, 3.5 - 5.6 cm long and 0.8 - 1.6 cm in diameter, complete, bisexual, zygomorphic, pentamerous, hypogynous. **Sepals** (5), synsepalous, tubular, 5 - dentate, glabrous. **Petals** (5), synpetalous, campanulate-funnel shaped, the tube widened above the base, segments of limb 5 and subequal, the tubes 1.0 - 1.2 cm long and 3.0 - 4.5 mm wide, the limbs 3.3 - 3.6 cm long and 1.2 - 1.5 cm wide. **Stamens** 2 + 2 + 1st, didynamous, epipetalous, stamens, inserted at the top of the narrow part of the tube, posterior staminode small, hairy at the insertion of the stamens. the filaments 1.3 - 1.7 cm long, inserted, the anthers dithecous, divergent, dorsifixed, introrse, longitudinal dehiscence. **Ovary** superior, oblongoid, 0.5 mm long and 1.0 mm in diameter, glabrous, disc present, bicarpellary, syncarpous, bilocular, the placentation axile, the style long and slender, about 2.2 cm long, the stigma 2 lipped. **Fruits** capsule, long-linear, coriaceous, 2.5 - 17.6 cm long and 4.0 - 9.0 mm wide, compressed contrary to the septum, bivalve. **Seeds** thinly discoid, wing hyaline, 2.0 - 6.0 mm long and 2.0 - 5.1 mm wide. Flowering and fruiting time is from February to October.

Morphological characters of *Tecoma stans* (L.) H.B.K.


Fig.5 Plants in normal habit


Fig.6 Compound leaves


Fig.7 Inflorescence


Fig.8 L.S of flower


Fig.9 Calyx

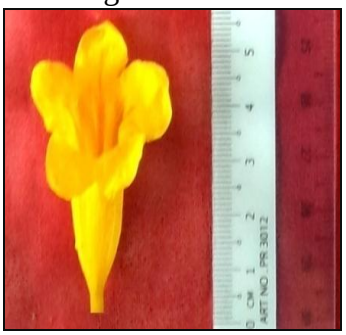
Fig.10 Corolla


Fig.11 Stamens

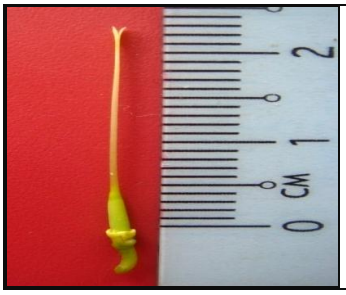

Fig.12 Pistil

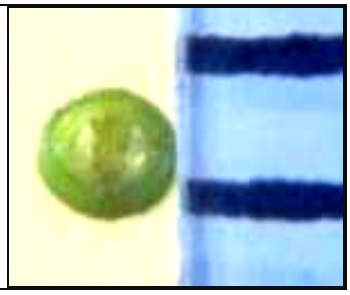
Fig.13 T.S of ovary

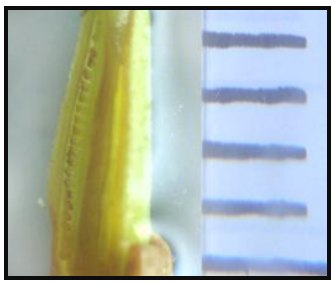
Fig.14 L.S of ovary


Fig.15 Fruit

Fig.16 Seeds

Histological characters of leaves of *Tecoma stans* (L.) H.B.K. Lamina

In the surface view of the lamina, the cuticle is striated and thin walled parenchymatous cells. Trichomes are present on both surfaces. Anomocytic type of stomata is present on the lower surface only. In the transverse section, the upper epidermis of cuticle layer is thicker than the lower surface. The upper and lower epidermal cells are barrel-shaped. Peltate trichomes are present on both surfaces. Calcium oxalate crystals and oil globules abundantly occurr in lamina.Vascular bundles are closed collateral types. Xylem always presents towards the upper epidermis. It consists of vessels, tracheids, fibres and xylem parenchyma. Phloem lies towards the lower epidermis.

Midrib

In surface view of midrib, upper epidermal cells are thick walled parenchymatous cell and rectangular in shape with oil globules. In transverse section, epidermal cells are rounded to oval shaped parenchymatous cells. Peltate trichomes and pigments colour are present on both surfaces. Vascular bundles are heart shaped and opened collateral type.

Petiole

In the surface view of petiole, epidermal cells are thin-walled, polygonal in shape and elongated along the axis of parenchymatous cells. In transverse section, petioles are oval-shaped in outline and winged petioles are present. Vascular bundles are heart-shaped, opened collateral type. Each bundle lies in the middle region and more fibrous and pigments colour on the lower side. The second layer is larger and small bundles alternating with each other as in the midrib. Xylem lies on the upper epidermis and consists of vessels, fibres and xylem parenchyma.

Petiolule

In the surface view of petiolule, both epidermal cells are irregular and rectangular in shape, thick walled parenchymatous cell. In tansverse section, petiolule is more or less rounded in shape. Vascular bundles are heart shaped and opened collateral type.

Rachis

In the surface view of rachis, the upper epidermal cells are rectangular in shape and thin-walled collenchymatous cells. In transverse section of rachis, oval-shaped in outline and occurred as winged rachis. Vascular bundles are heart shaped and opened collateral type. Xylem lies towards the upper epidermis and consists of fibres, tracheids and xylem parenchyma. Peltate trichomes and calcium oxalate crystals are present in parenchymatous cells.

Histological characters of leaves of *Tecoma stans* (L.) H.B.K.

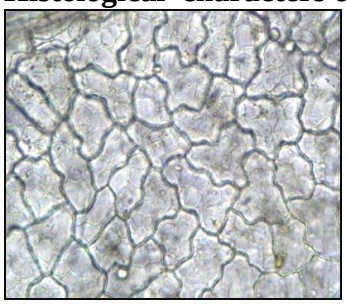

Fig.17 upper epidermis of lamina with calcium oxalate crystals (X100)

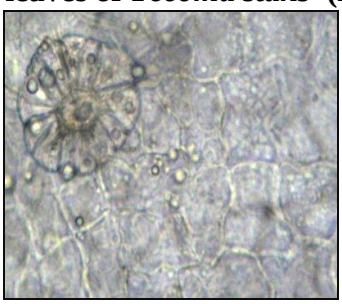

Fig.18 upper epidermis of lamina with oil globules and peltate trichome (X400)

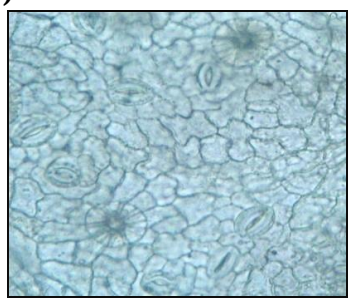

Fig.19 lower epidermis with peltate trichome and stomata (X100)

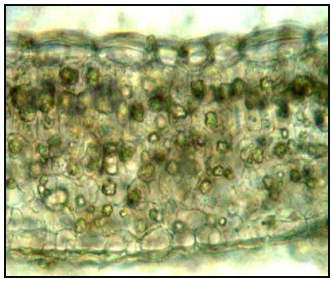

Fig.20 T.S of lamina showing spongy mesophyll cells and oil globules (X100)

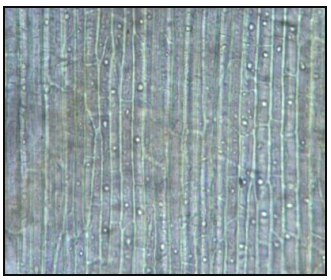

Fig.21 Surface view of midrib showing epidermal cell (X100)

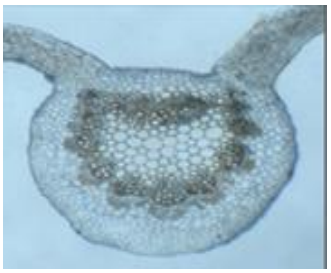

Fig.22 T.S of midrib showing vascular bundles (X400)

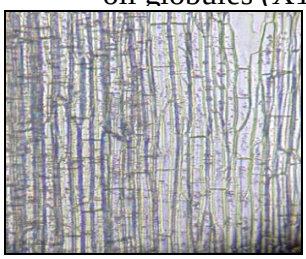

Fig.23 Surface view of petiole showing epidermal cell (X100)

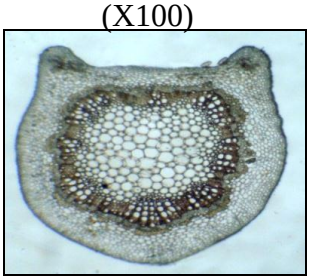

Fig.24 T.S of petiole showing vascular bundles (X400)

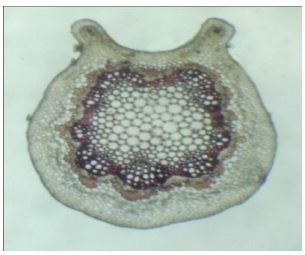

Fig.25 T.S of petiolule showing close-up view of vascular bundles (X400)

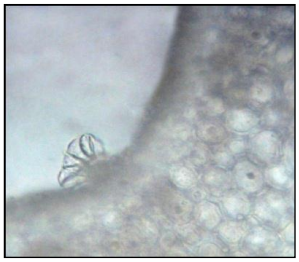

Fig.26 T.S of rachis showing peltate trichome (X400)

Diagnostic characters of powdered leaves

In the powdered sample of *Tecoma stans* (L.) H.B.K., fibre, oil globule, pitted vessel, scalariform vessel, spiral vessel, calcium oxalate crystal, epidermal cell with stomata and peltate trichome were observed.

Sensory characters of the leaves

Sample / Sensory Characters	Leaves
Color	Darkgreen
Odour	Strongly pungent
Taste	Bitter
Texture	Granular

Diagnostic characters of powdered leaves of *Tecoma stans* (L.) H.B.K.

Fig.27 Powdered leaves

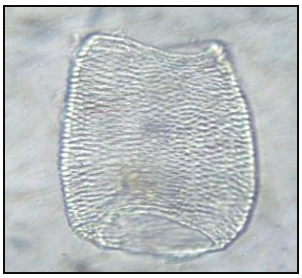

Fig.28 Pitted vessel (X400)

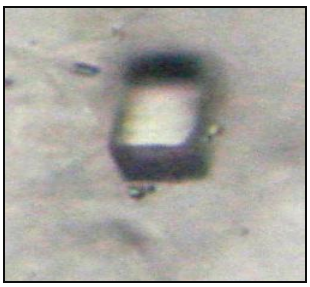

Fig 29 Calcium oxalate crystal (X400)

Fig.30 Oil globule (X400)

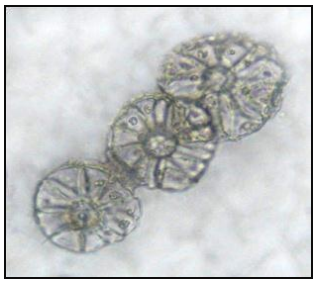

Fig.31 Peltate trichomes (X400)

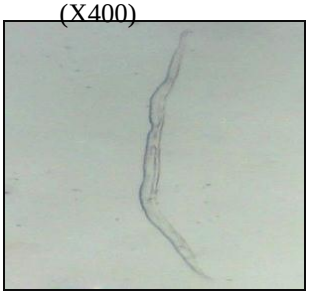

Fig.32 Fibre (X100)

Fig.33 Spiral vessels (X400)

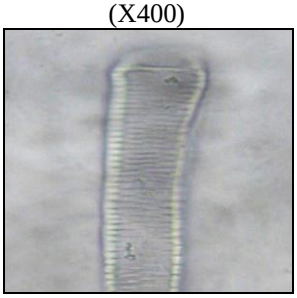

Fig.34 Scalariform vessel (X400)

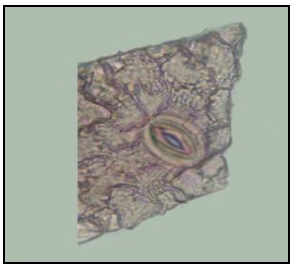

Fig.35 Fragment of epidermal cell with stomata (X400)

Phytochemical test of *Tecoma stans* (L.) H.B.K. leaves

In qualitative analysis, the phytochemical tests were carried out in order to know the presence or absence of alkaloid, α-amino acid, carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid in the leaves but starch and cyanogenic glycoside was absent. The results are shown in Table (1).

Table.1 Table showing the phytochemical test of *Tecoma stans* (L.) H.B.K. leaves

No.	Test	Extract	Test Reagents	Observation	Result
1.	Alkaloid	1% HCl	(1) Mayer's Reagent (2) Wagner's Reagent (3) Dragendroff's reagent	White ppt Brown ppt Orange ppt	+ + +
2.	α-amino acid	H_2O	Ninhydrin solution	Purple spot	+
3.	Carbohydrate	H_2O	10% α -naphthol + conc-H_2SO_4	Red ring	+
4.	Starch	H_2O	I_2KI solution	No change in color	-
5.	Reducing sugar	H_2O	Benedict's solution	Brick red ppts	+
6.	Cyanogenic glycoside	H_2O	(1) conc-H_2SO_4 acid (2) Sodium picrate paper	No change in color	-
7.	Glycoside	H_2O	10% lead acetate solution	White ppts	+
8.	Phenolic compound	H_2O	Ferric chloride	Deep blue color	+
9.	Saponin	H_2O	Distilled water	Frothing	+
10.	Tannin	H_2O	Ferric chloride	Deep blue color	+
11.	Flavonoid	EtOH	(1) Mg turning (2) Conc HCl acid	Pink color	+
12.	Steroid	P.E	Acetic anhydride + conc-H_2SO_4	Blue green color	+
13.	Terpenoid	P.E	Acetic anhydride + conc-H_2SO_4	Deep pink color	+

(+) present (-) absent (ppt) precipitate

Acute toxicity test of aqueous and 70% ethanolic extracts of *Tecoma stans* (L.) H.B.K. leaves on brine shrimp lethality test

The number of dead, alive brine shrimp and the mean percentage mortality were plotted against the logarithm of concentrations. The value of lethal concentration for plant extracts showed two graphic procedures.

Table.2 Table showing the number of alive and dead larvae from aqueous extract of *Tecoma stans* (L.) H.B.K. leaves

No.	Concentration (mg/mL)	Alive	Dead
1	3	1	9
2	1.5	3	7
3	0.75	6	4
4	0.375	7	3
5	0.1875	8	2

Table.3 Table showing accumulated dead, alive and mortality percentage from aqueous extract of *Tecoma stans* (L.) H.B.K. leaves

No.	Concentration (mg/mL)	Log concentration	Dead	Alive	Accumulated Dead	Accumulated Alive	Ratio Dead: Total	Mortality %
1	3	0.5	9	1	25	1	25 / 26	96
2	1.5	0.2	7	3	16	4	16 / 20	80
3	0.75	-0.1	4	6	9	10	9 / 19	47
4	0.375	-0.4	3	7	5	17	5 / 22	23
5	0.1875	-0.7	2	8	2	25	2 / 27	7

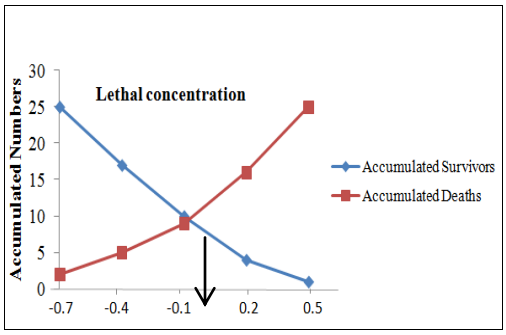

Fig.36 Accumulated Numbers Vs Log concentration of aqueous extract of *Tecoma stans* (L.) H.B.K. leaves

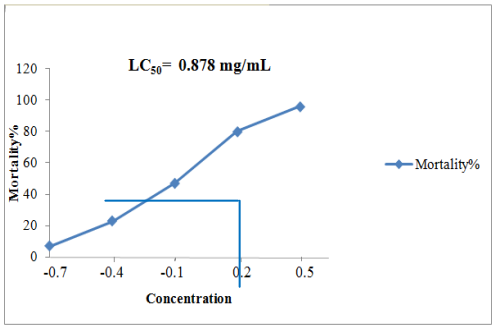

Fig.37 Mortality % Vs Log concentration of aqueous extract of *Tecoma stans* (L.) H.B.K. leaves

Table.4 Table showing the number of alive and dead larvae from 70 % ethanolic extracts of *Tecoma stans* (L.) H.B.K. leaves

No.	Concentration (mg/mL)	Alive	Dead
1	4	2	8
2	2	4	6
3	1	6	4
4	0.5	8	2
5	0.25	9	1

Table.5 Table showing accumulated dead, alive and mortality percentage from 70% ethanolic extract of *Tecoma stans* (L.) H.B.K. leaves

No	Concentration (mg/mL)	Log concentration	Dead	Alive	Accumulated Dead	Accumulated Alive	Ratio Dead:Total	Mortality %
1	4	0.6	8	2	21	2	21 / 23	91
2	2	0.3	6	4	13	6	13 / 19	68
3	1	0	4	6	7	12	7 / 19	37
4	0.5	-0.3	2	8	3	20	3 / 23	13
5	0.25	-0.6	1	9	1	29	1 / 30	3

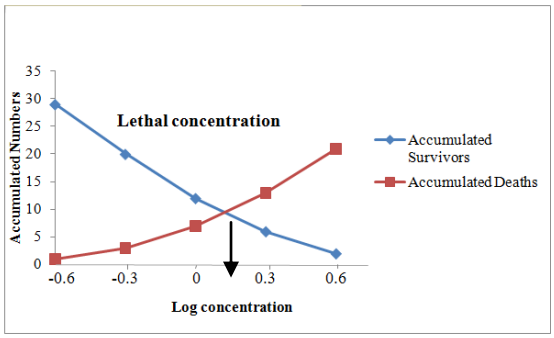

Fig.38 Accumulated numbers Vs Log concentration of 70 % ethanolic extracts of *Tecoma stans* (L.) H.B.K. leaves

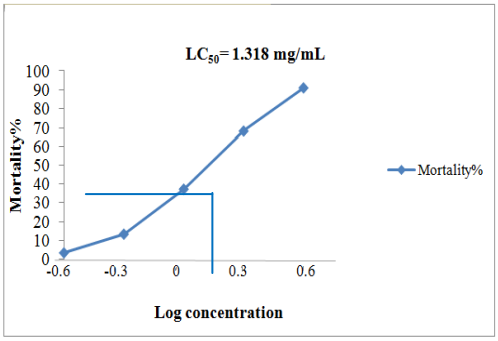

Fig.39 Mortality % Vs Log concentration of 70 % ethanolic extracts of *Tecoma stans* (L.) H.B.K. leaves

Discussion and Conclusion

In this study, the morphological characters of *Tecoma stans* (L.) H.B.K. agrees with those described by Hooker (1885) and Backer (1965). In this research, the plants are perennial small tree, unipinnately imparipinnate compound leaves, terminal or axillary raceme, flowers are bright yellow, bisporangiate and zygomorphic. Stamens are 4 + 1st, didynamous, epipetalous, anther dithecous and stigma is 2-lipped. In microscopical study, the anticlinal wall of the lower epidermis are more wavy than the upper epidermis. Peltate trichome, oil globules and calcium oxalate crystals are present. Stomata types are anomocytic on the lower surface of lamina only. Heart shaped vascular bundle, collateral and opened type are found in midrib, rachis and petiole. Prism shaped calcium oxalate crystals are also found in lamina, midrib, rachis and petiole. These characters are in agreement with Esau (1953), Metcalfe and Chalk (1950) and Pandey (1988). In this study, investigation of preliminary phytochemical test on the leaves of *Tecoma stans* (L.) H.B.K showed that alkaloids, α-amino acid, carbohydrate, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid, reducing sugar and terpenoid were present but starch and cyanogenic glycoside were not observed. Glycoside and tannin were especially abundant in the leaves of *Tecoma stans* (L.) H.B.K. Glycosides play many important roles in living organisms and numerous plant-produced glycosides. These characters are in agreement with Marini Bettolo, et al., (1981) and Central Council for Research in Unani Medicine (1987). The analysis revealed that the lethality concentration (LC_{50}) of aqueous extract, 70% ethanolic extracts were 0.878 mg/ml and 1.318 mg/ml respectively. The degree of lethality was directly proportional to the concentration of the extract. The brine shrimp lethality of these plant extracts was found to be concentration-dependent. The LC_{50} of 70% ethanolic extracts indicates that it has a wide margin of safety. According to Meyer *et al.,* the crude plant extract is non-toxic (inactive) if it is greater than 1000 μg/ml (1mg/ml). So, the LC_{50} of 70% ethanolic extracts was considered to be non toxic. Brine shrimp lethality test is very useful because it provides a preliminary screen that can be supported by a more specific bioassay. These characters are an agreement with describing by Sam T. W., 1993. Thus, some useful drugs of therapeutic importance may develop out of the research specific works.

Acknowledgements

We would like to express our deepest thanks to Professor Dr. Myat Myat Moe, Head of Botany Department, Dagon University, for her permission and support in the department and her kind understanding throughout this research paper.

We want to express our gratitude to Professor Dr. Khin Lat Lat Mon, Department of Botany, Dagon University, for her advice and encouragement.

References

Akereele O. 1984. **WHO's traditional medicine program**. Progress and perspective. WHO Chron, Vol. 38, pg. 76-81, Geneva.

Backer, C. A. 1965. **Flora of Java**. Vol. II, Wolters Neordhoff N. V. Groningen, The Netherlands

Esau, K. 1953. **Plant Anatomy**. John Wiley and Sons, Inc. New York, London.

Heywood, V.H., D.M. Mode, I.B.K. Richardson and W.T. Stearn, (Eds.), 2007. **Flowering Plants of the World**. London: Oxford University Press.

Hooker, J.D. 1885. **The Flora of British India**. Asclepiadeae to Amarantaceae, Vol. IV, Reeve Co., Ltd., The Oast House, Brock, NR. Ashford, Kent. England.

Kress, J.W., A.D. Robert, E. Farr, and Yin Yin Kyi. 2003. **A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar**. Department of systematic Biology – Botany, National Museum of Natural History, Washington DC, USA.

Marini-Bettolo, G.B. 1981. Plant Screening by chemical and chromatographic procedure under field condition. *J. Chromato*. 213. 113. 127.

Metcalfe, C. R., and L. Chalk, 1950. **Anatomy of the Dicotyledons**. The Clarendon Press.

Meyer, B. N. 1982. **Brine Shrimp: A convenient general bioassay for active plant constituents.** Plant Med. No. 45, Pg 31-34. Philippines.

Pandey, S.N and A. Chadha. 1988. **Plant Anatomy and Embryology**. Vikas Publishing House Pvt. Ltd., New Delhi.

Sam, T. W. 1993. **Bioactive Natural Products**. Toxicity testing using the brine shrimp: Artemia salina. CRC Press, New York.

Trease, G.E. and W.C. Evans., 2002. **A Text Book of Pharmacognosy**, 15th Edition. Edinburgh, London, New York.

Central Council for Research in Unani Medicine. 1987. Phytochemical Standards of Unani Formulations, New Delhi.