# Investigation of Antimicrobial activity and acute toxicity test of *Tecoma stans* (L.) H.B.K. Leaves

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

## Abstract

Pharmacognosy is the study of medicinal drugs derived from plants or other natural sources. The medicinal plants of *Tecoma stans* (L.) H.B.K. locally known as sein-ta-kyu in Myanmar belongs to the family Bignoniaceae had been studied. It was collected from Kamayut Township, Yangon Region during 2015-2017. In this research, Antimicrobial activities of *Tecoma stans* (L.) H.B.K. were also investigated at the Pharmaceutical and Food Research Department (PFRD) by using Agar-well diffusion method with nine pathogenic microorganisms. Methanolic extract showed the most significant activity on *Candida albicans* while pet-ether and chloroform extracts did not show any activity on *Vibrio Cholerae, Klebsiella Pneumoniae* and *Proteus mirabilis.* The acute toxicity test was carried out with aqueous extract and 70% ethanolic extract from leaves of *Tecoma stans* (L.) H.B.K. on albino mice. It was observed that the minimum dose of 4g/kg and the maximum dose of 16 g/kg of aqueous extract and 70% ethanolic extracts were free acute toxic or harmful effect.

***Keywords***: Antimicrobial activity and Acute toxicity test.

## Introduction

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 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 purpose is probably as old as the history of mankind. (Akereele, 1984).

Herbal plants are widely distributed in Myanmar. Among them, *Tecoma stans* (L.) H.B.K. belongs to the family Bignoniaceae and locally known as "Sein –ta- kyu" in Myanmar, Yellow Elder (or) Yellow Bells in English has been included. (Kress *et al*., 2003)

Bignoniaceae family is pantropical and subtropical with a few genera in temperate part of North America, Asia and the southern hemisphere. This family is primarily tropical family of about 110 genera and about 750 species (Lawrence, 1964)

Teoma is not a toxic because this plant is used in Latin America as a remedy for diabetes and also for feeding cattle and goats in Mexico. In south East Asia, *Tecoma Stans* (L.) H.B.K. is only planted as ornamental one and it is also credited with antidiabetic properties. (lemmens and Burnyapraphatsara, 2003)

Microoganisms live almost everywhere on earth where there is liquid water, including hot spring, on the ocean there is liquid water, including the spring, on the ocean floor and deep inside with the earth's crust. Microorganisms are critical to nutrient recycling in ecosystems as they act as decomposers. They are very diverse and include bacteria, fungi, cyanobacteria, unicellular (single-celled) algae. Bacteria

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

are pathogenic microorganisms with a relatively simple and primitive form of cellular organization. These cells are smaller than those of protozoa and fungi (Cruickshank *et al.,* 1975).

Antimicrobial activities of different solvents extracts of *Tecoma stans* (L.) H.B.K. were tested with *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae* and *Proteus mirabilis*.

Chemicals can have a wide range of effects on our health. Depending on how the chemical will be used, many kinds of toxicity tests may be required. One way is to carry out lethality testing (the $LD_{50}$ tests) by measuring how much of a chemical is required to cause death. The most common $LD_{50}$ test is the acute toxicity test, in which animals are given a single dose of chemical and the $LD_{50}$ is determined over a 24 hours time period. (Canadian Centre for Occupational Health and Safety, 2013).

The aims of the present research are 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 investigate the antimicrobial activity of differen solvent extracts and to determine the acute toxicity from the leaves of *Tecoma stans* (L.) H. B. K.

## Materials and Methods

### Antimicrobial activities of various solvent extracts of *Tecomastans* (L.) H.B.K. leaves

### Apparatus used

Autoclave, clean bench, conical flask, hot air sterilizer, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipettes, water bath and loops.

### Microorganisms

The various solvent extracts were tested against nine pathogenic microorganisms by using agar-well diffusion method. The extent of antimicrobial activity was measured at the diameter zone of inhibition. The test organisms include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae and Proteus mirabilis*. The test was conducted at the Pharmaceutical and Food Research Department (PFRD).

### Procedure for antimicrobial activity

The study of antimicrobial activities was performed by agar-well diffusion method. Nutrient agar was prepared according to the method of Cruickshank (1975). Nutrient agar was boiled and 25 ml of the medium was poured into each test tube and plugged with cotton wool and sterilized at 121 °C for 15 minutes in an autoclave. Then, the tubes were cooled down to 30-35°C and the content was poured into sterilized petridishes and 0.1-0.2 ml of test organism was also added into the dishes. The agar was allowed to set for 2-3 hours. Then, 10 mm plate agar-well was made with the help of sterilized agar-well borer. After that, about 0.2 ml of sample was

introduced into the agar-well and incubated at 37 °C for 24 hours. The inhibition zone appeared around the agar-well, indicating the presence of antimicrobial activity. The extent of antimicrobial activity was measured with the help of transparent ruler at the diameter zone of inhibition including the agar-well.

**Table 1 Types of microorganisms and their diseases**

| No. | Types of microorganisms | Diseases |
|---|---|---|
| 1. | *Bacillus subtilis* | Ropiness and spoilage of food |
| 2. | *Staphylococcus aureus* | Skin infections and food poisoning |
| 3. | *Pseudomonas aeruginosa* | Pneumonia, urinary tract infection, septic shock, gastrointestinal infection, skin and soft tissue infections |
| 4. | *Bacillus pumalis* | Eye infection, soft tissue and cutaneous infections |
| 5. | *Candida albicans* | Oral and vaginal infection, skin and cardiac infections |
| 6. | *Escherichia coli* | Urinary tract infections, neonatal meningitis, septicemia, diarrhoea and dysentery |
| 7. | *Vibrio cholerae* | Diarrhoea, vomiting and abdominal cramps |
| 8. | *Klebsiella pneumoniae* | Pneumonia, urinary tract infections, lower biliary tract surgical wound site infection |
| 9. | *Proteus mirabilis* | Wound infections, septicemia, urinary tract infections and pneumonias |

(Cruickshank, 1975)

**Acute toxicity test of aqueous extract and 70 % ethanolic extractfrom*Tecoma stans* (L.) H.B.K. leaves by using albino mice**

**Materials**

Animal used - 70 albino mice weighing 25-35g
Drug used - Different doses of aqueous extract and 70% ethanolic extract of leaves
Apparatus - Mice cages, animal balance, 18 gauge dosing needle, disposable syringes 1ml to 2ml, rubber glove and mask
Dose schedule - 4 g/kg body weight of mice
- 8 g/kg body weight of mice
- 16 g/kg body weight of mice
Period of observation - 14 days

**Methods**

Acute toxicity of aqueous extract and 70% ethanolic extract of *Tecoma stans* (L.) H.B.K was evaluated by the method of Litchfield and Wilcoxon (1949). Seventy albino mice, weighing (25-35)g were used in this study. Mice were separated into 7 groups and each group contained 10 mice.

Each group was placed separately in the seven mouse cages. Food was held for the period of 18 hours before administration of drugs.At first, the mice were individually marked with picric acid staining on the parts of the body and weighed. Required doses were calculated based on the body weight of the mice.

Group I serve as control group and was administered 10 ml/kg of distilled water. Group II, III and IV were treated orally with 4 g/kg, 8 g/kg and 16g/kg body

weight of aqueous extracts while Group V, VI and VII were treated orally with 4 g/kg, 8 g/kg and 16 g/kg body weight of 70% ethanolic extract respectively.After given the extracts orally, each group of mice was kept in seven cages with free access to water and food. They were observed carefully for 14 days.

**Acute toxicity of the aqueous extracts and 70% ethanolic extracts from the leaves of *Tecoma stans* (L.) H.B.K.**

**Fig. 1 Digital balance**

**Fig. 2Weighing albino mouse in balance**

**Fig. 3 Mice cages contain 10 mice each**

**Fig. 4 Groups of four mice cages**

**Fig. 5 Administration of extract suspension to mice**

## Results

**Antimicrobial activities of various solvent extracts of *Tecoma stans* (L.) H.B.K. leaves**

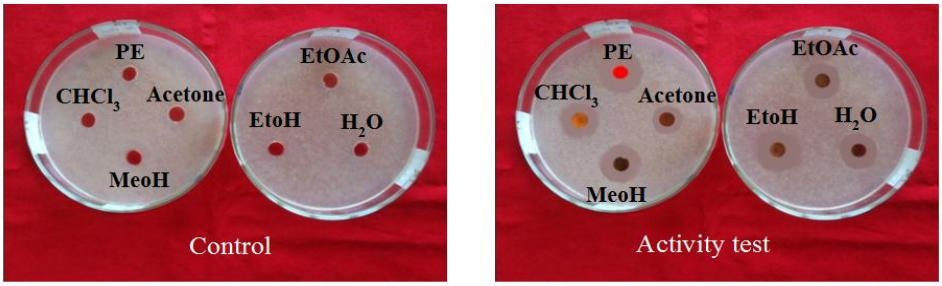
**Screening for antimicrobial activity**

Antimicrobial activity was studied with 70% pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol and watery extracts. Agar-well diffusion method was used to determine the zone of inhibition of microbial growth at particular concentration of various extracts which are as shown in Figures.

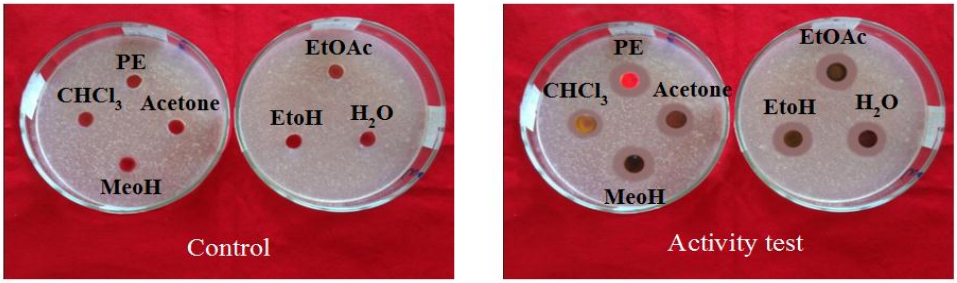
**Table 2 Table showing antimicrobial activity against nine test organisms by using different solvent extracts of *Tecoma stans* (L.) H.B.K. leaves**

| Extracts | Organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***Bacillus subtilis*** | ***Staphylococcus aureus*** | ***Pseudomonas aeruginosa*** | ***Bacillus pumalis*** | ***Candida albicans*** | ***Escherichia coli*** | ***Vibrio cholerae*** | ***Klebsiella pneumoniae*** | ***Proteus mirabilis*** |
| Pet-ether | 20 mm | 20 mm | 20 mm | - | - | - | - | - | - |
| $CHCl_3$ | 15 mm | 15 mm | 13 mm | 21 mm | 16 mm | 12 mm | - | - | - |
| MeOH | 17 mm | 20 mm | 20 mm | 21 mm | **22 mm** | 20 mm | 20 mm | 20 mm | 20 mm |
| Acetone | 13 mm | 18 mm | 17 mm | 16 mm | 16 mm | - | 13 mm | 13 mm | 13 mm |
| EtOAc | 17 mm | 18 mm | 16 mm | 13 mm | 15 mm | 12 mm | 13 mm | - | 16 mm |
| EtOH | 19 mm | 17 mm | 16 mm | 13 mm | - | 13 mm | 12 mm | - | 13 mm |
| D/W | 17 mm | 17 mm | 16 mm | 14 mm | - | - | 12 mm | - | 13 mm |

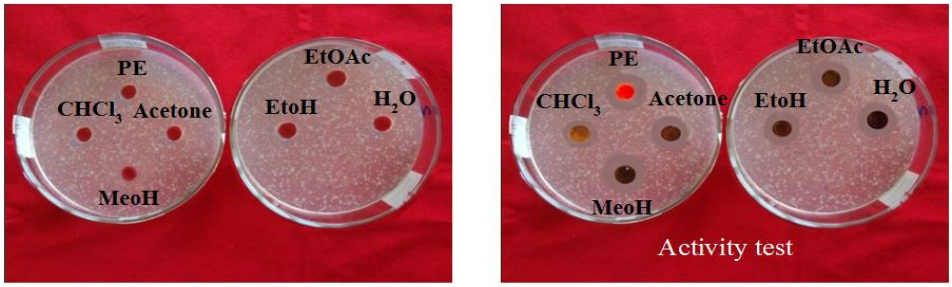
Agar well - 10 mm, 10 mm ~ 14 mm, 15 mm ~ 19 mm, 20 mm above



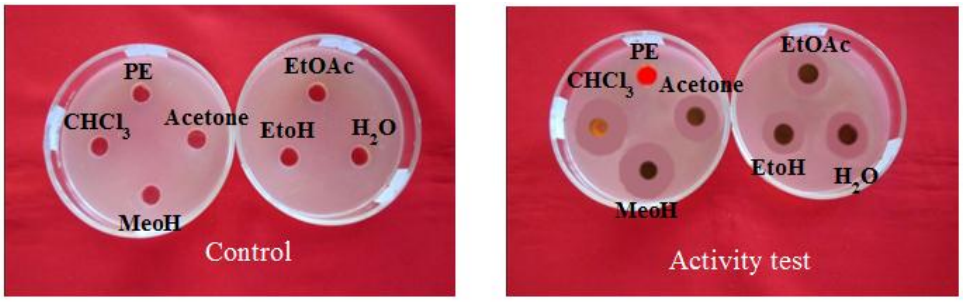

*Bacillus subtilis*



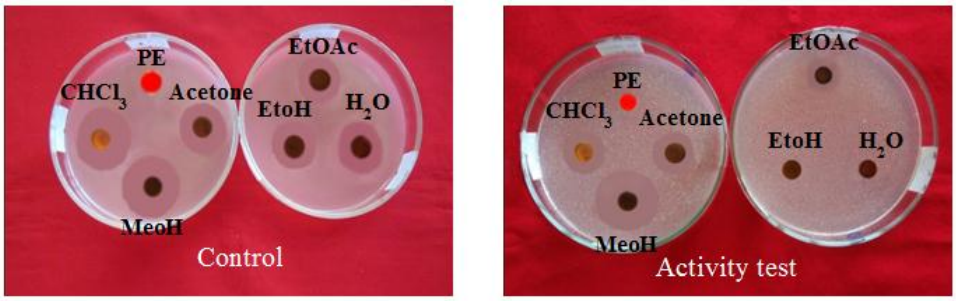

*Staphylococcus aureus*



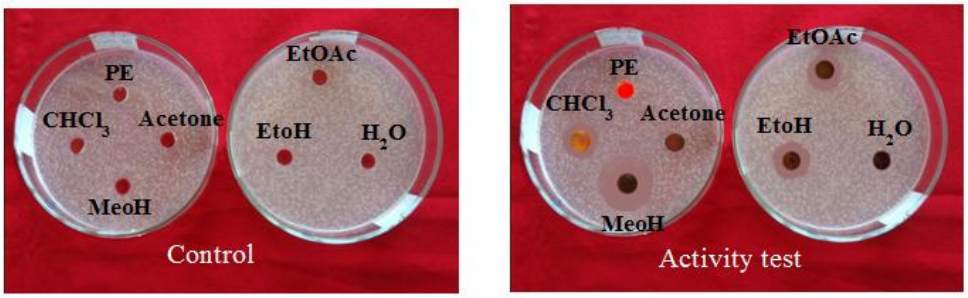

*Pseudomonas aeruginosa*



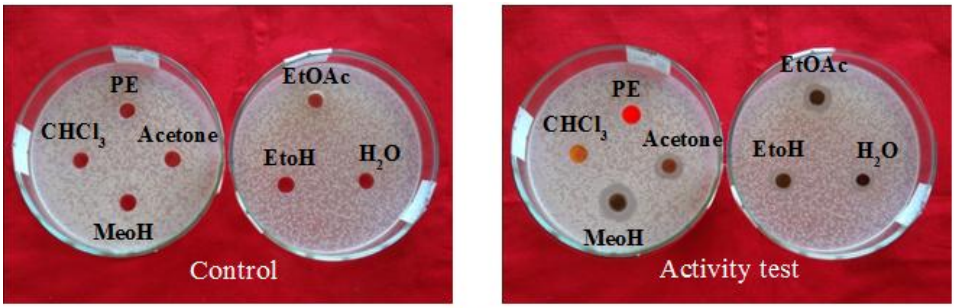

***Bacillus pumalis***




***Candida albicans***




***Escherichia coli***




***Vibrio cholerae***

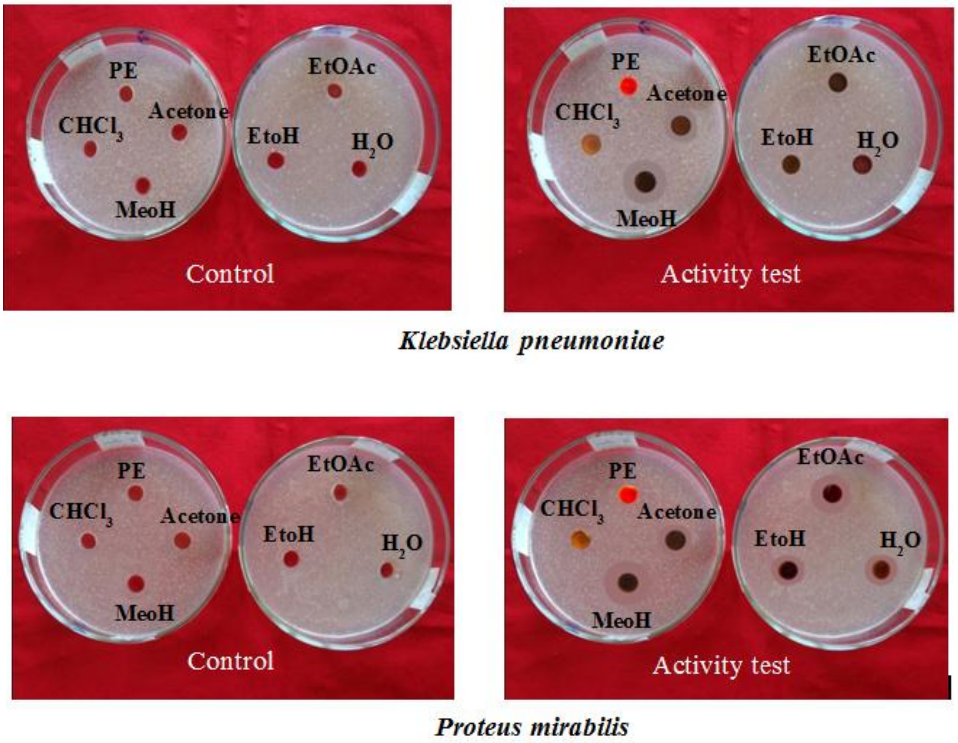



**Fig 6. Antimicrobial activity of different solvent extracts of *Tecoma stans* (L.) H.B.K. leaves**

**Acute toxicity test of aqueous extract and 70% ethanolic extract from*Tecoma stans* (L.) H.B.K. leaves by using albino mice**

The aqueous extract and 70% ethanolic extract of leaves from *Tecoma stans* (L.) H.B.K. showed no lethal effects.Therefore, it was observed that aqueous extract and 70% ethanolic extract were free from acute toxic or harmful effect as shown in Table.

**Table 3. Acute toxicity test of aqueous extract and 70% ethanolic extract of *Tecoma stans* (L.) H.B.K. leaves**

| Group No | Number of mice | Type of Drugs | Dosage g/kg | Observed period | Dead tested | Observed of % death |
|---|---|---|---|---|---|---|
| I | 10 | Distilled water | 10 ml/kg | 14 days | 0/10 | 0% |
| II | 10 | Aqueous extract | 4 g/kg | 14 days | 0/10 | 0% |
| III | 10 | | 8 g/kg | 14 days | 0/10 | 0% |
| IV | 10 | | 16 g/kg | 14 days | 0/10 | 0% |
| V | 10 | 70% ethanolic extract | 4 g/kg | 14 days | 0/10 | 0% |
| VI | 10 | | 8 g/kg | 14 days | 0/10 | 0% |
| VII | 10 | | 16 g/kg | 14 days | 0/10 | 0% |

## Discussion and Conclusions

In the present research, antimicrobial activities of different solvents extracts with nine pathogenic microorganisms were tested. The results showed that the methanolic extract was more effective than other solvents extracts.

Pranay Dogra (2009) reported that the antimicrobial activity of petroleum, chloroform and methanolic extracts showed inhibitory effects for *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa.*

According to Rajendran (2011), ethyl acetate extracts were tested for antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans.*

The results of the present study with methanolic extract showed that the most significant antimicrobial activity against *Candida albicans,* which causes oral and vaginalinfection, skin and cardiac infections.

The result of acute toxicity study revealed that the aqueous extract and 70% ethanolic extract neither have lethality nor toxic reactions up to the maximum permissible dose until the end of the observation period. So, the minimum dose of 4g/kg and the maximum dose of 16g/kg of aqueous extract and 70% ethanolic extracts were free from acute toxic or harmful effect. All the animals were alive, healthy and active during this period. (http://www. Acute toxicity).

Several members of Bignoniaceae family, including *Tecoma, Podranea, Pandorea, Bignonia* and *Mansoa* are frequently grownas ornamentals, at least in certain areas of the tropics. A great many species are known in cultivation, if only rarely. Several of the rare species of Bignoniaceae produce excellent wood but are often not recognized by lumberjacks(http://www.download/Bignoniaceae.htm).

## Acknowledgements

I would like to express my sincere gratitude to Dr. Myat Myat Moe, Professor and Head, Department of Botany, Dagon University, for the permission to use various department facilities during the study period.

I also acknowledge to Dr. Sandar Hlaing, Professor, Department of Botany, Dagon University, for her interesting advices and suggestions on this study.

## References

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

Cruickshank, S. 1975. **Handbook of Bacteriology**. 10th Ed., E. & S. Churchill Livingstone Ltd., Edinburgh, 121-125.

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.

Lawrence, G.H.M. 1964. **Taxonomy of vascular Plants**. 10th ed. The macmillian, company, New York. London.

Lemmens R. H. M. J and N. Burnyapraphatsara. 2003. **Plant Resources of South East-Asia**. No.12 (3). **Medicinal and Poisonus plants**. Prosea foundation, Bagan 16122, Indonesia.

Litchfield, J. T and F. A. 1949. **A Simplified Method of Evaluating Dose Effect Experiments Journal of Pharmacology and Experiments.** *Journal of Pharmacology and Experimental Therapeutic.* **96**, 99-113. Standard Research Laboratories. Stanford: American Cyanamid Company.

Pranay Dogra. 2009. **Study of Antibacterial and Anticancer Activity of Selected Trifoliate Plants**. Pg. 4-8, Issue 2, Volume 1, Biofrontier, India.

Rajendran, A. 2011. **Isolation, Characterization, Pharmacological and Corrosion inhibition studies of Flavonoids obtained from Nerium oleander and *Tecoma stans* (L.) H.B.K**. No. 2, Pg. 1005-1013, Vol III. International Journal of Pharm Teach Research, India.

**Canadian Centre for occupational Health and Safety**. 2013. Canada.

**Website**

http://en Wikipedia, Pharamacognosy

http://www.Acute toxicity

http://www.download/Bignoniaceae.htm