

## Study on Morphology, Histology and antimicrobial activities on the leaf of *Gynura procumbens* (Lour.) Merr.

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### Abstract

*Gynura procumbens* (Lour.) Merr. (Family Asteraceae) is a medicinal plant commonly found in wild and cultivated in Myanmar. Traditionally, the fresh leaves of *Gynura procumbens* were used as leafy vegetables for treated diabetes mellitus. In this paper, studies on the morphology, histology and antimicrobial activities of the leaf of *Gynura procumbens* (Lour.) Merr. (Pyar mee ywet), have been undertaken. The dried powdered leaves were pale brown, milky in odour and slightly sour in taste. The upper epidermal cells were slightly wavy, and the lower epidermal cells wavy with anomocytic type of stomata on both surfaces; trichome non-glandular, uniseriate; calcium oxalate crystals were present. Powdered forms of leaves were also studied which can be used to ascertain their identification and standardization for traditional medicine. The antimicrobial activities of extract of aqueous and organic solvents of this plant were tested in six different pathogenic microorganisms. The extracts of tested plant were found to be potent.

**Keywords:** *Gynura procumbens*, medicinal plant, histology, antimicrobial activities and diabetes mellitus

### INTRODUCTION

People have become increasingly aware of the overuse and misuse of antibiotic and are now very interested in alternative medicines produced by medicinal plants. Herbal medicine is a traditional medicine or folk medicine practice based on the use of plants and plant extracts. Human has been used herbal medicine for their health and used a variety of plants and plant products for treatment of various diseases. The therapeutic efficacies of many indigenous plants have been described by traditional herbal medicine practitioners. Traditional medicine (TM) is widely used and of rapidly growing health system and economic importance. In Asia and Latin America, population continues to use TM as a result of historical circumstances and cultural belief (WHO 2002).

Diabetes Mellitus is a major growing health problem in almost all countries of world-wide. The World Health Organization estimates that more than 180 million people have diabetes. Almost 80% of diabetes death occurs in undeveloped and developing countries (WHO 2008). Diabetes can be caused by the deficiency of insulin in the people or when the body cannot produce or use insulin correctly. Insulin is a hormone that changes the sugars from foods we eat into necessary energy for the body. This can affect the different parts of the body, including the heart, blood vessels, eyes, nerves and kidneys.

In Myanmar, a large number of herbal medicines are being used for the treatment of diabetes mellitus. Among them, *Gynura procumbens* were used by local people who believed that these plants have anti-diabetic activity. *Gynura* is a small genus of plants within the family Asteraceae (Kress *et al.* 2003). The plant *Gynura procumbens* is well known in Myanmar as Pyar mee ywet. This species was native to China and gradually distributed into Thailand, Malaysia, Indonesia and Myanmar. It is widely cultivated in Myanmar.

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*Gynura procumbens* is a decumbent perennial herbs; stem angular. Leaves simple, alternate, margin serrate and pubescent on both surfaces. Inflorescence terminal or corymbose; flowers orange yellow; achenes linear.

Histological studies of this plant showed numerous non glandular trichomes and anomocytic type of stomata on both surfaces of the leaves.

*Gynura procumbens* is valuable medicinal plant. This plant is able to decrease blood sugar level and also induce insulin secretion (Zang & Tan 2000). In Myanmar, the fresh leaves of this plant were used as food vegetable for treated diabetes.

Antibiotics represent one of the drugs that can be used in food animals both therapeutically to treat disease, as well as sub-therapeutically to improve the growth rate. Antimicrobial activities of plant extracts are now available from scientific information of traditional medicine.

These plants are also reputed for its effectiveness against pathogenic microorganisms (Fareed *et al.*, 2008, Mahady 2002 and Mar Mar Nyein *et al.* 1999). Therefore, antimicrobial study of these plants has been conducted in this part. Five different bacterial species and one species of yeast-like fungus were used to investigated the antimicrobial activities. *Bacillus subtilis.*, *B. pumalis*, *Staphylococcus aureus* are common Gram-positive bacteria and *Pseudomonas aeruginosa* and *Escherichia coli* are Gram negative bacteria and yeast-like fungus *Candida albican* were used in this antimicrobial testing because of their medical significance in causing common problems of microbial infections found in this community.

The present study focuses on the investigation of morphology, histology and antimicrobial activities of *Gynura procumbens*, which has not been scientifically investigated. The aim of this research is extensive application of plants promoting the Myanmar traditional medicine scientifically. To achieve this aim to verify both vegetative and reproductive parts, to become the histological characters of leaves and powder and to evaluate the antimicrobial activity of different crude extracts by using agar well diffusion method.

## MATERIALS AND METHODS

The specimens of *Gynura procumbens* were collected from the Chanmyatharsi and Amarapura Township, Mandalay Division, during the flowering period of years 2006 – 2008. The collected fresh specimens of both vegetative and reproductive parts of the plant were identified by using Hooker (1876), Kartikar and Basu (1935), Backer (1963), Kurz (1877) and Dassanayake (2000). Taxonomic descriptions were accompanied with the photograph of natural habitats and parts of the plant with measurement. Herbarium specimens were also prepared and kept in the herbarium of the Botany Department of Mandalay University.

The collection specimens were washed and cut into small pieces then air dried in room temperature about 14-20 days. After that, these were homogenized by blender to get powder and stored in air tight containers for histological and antimicrobial studies.

### Histological studies of plant

The histological characters of fresh specimens leaves and powders were prepared by the literature of Metcalfe and Chalk (1950), Esau (1953) and Evans (2002). The histological characters of fresh specimens were examined by preparing free hand sections with the help of the following reagents.

1. Chloral hydrate solution as clearing reagent.
2. Concentrated sulphuric acid B.P for testing calcium oxalate crystals.

Antimicrobial activities of crude extracts from the leaves of *Gynura procumbens* have been investigated. Antimicrobial activity of the leaves of *Gynura procumbens* were conducted by using 95% ethanol, distilled water, ethyl acetate and petroleum-ether (60 – 80°C) extracts. In this study, agar well diffusion method was used to screen the antimicrobial activity. These experiments were conducted in the laboratory of Development Center for Pharmaceutical Technology (D.C.P.T).

#### **Extraction**

The powders of leaves were extracted by using 95% ethanol, distilled water, ethyl acetate and petroleum ether (60 – 80°C) for about 2 weeks and then filtered. The solvents were then evaporated by using water bath to obtain a paste.

#### **Test organisms**

The different solvents extracts were tested against six pathogenic microorganisms by using agar well diffusion method. The strains of six pathogenic microorganisms were *Bacillus subtilis* (JAP-0221215), *Bacillus pumalis* (IFO-12102), *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa* (IFO-3080), *Candida albican* (IFO-1060), and *Escherichia coli* (ATCC-25922).

#### **Experimental procedure**

Nutrient agar medium was prepared according to method described by Cruickshank (1975). Nutrient agar was prepared and 20 – 25 ml of the medium was poured into test tube and plugged with cotton wool and sterilized at 121°C for 15 minutes in an autoclave. Then the test tubes were cool down to 30 – 35°C and poured into sterilized petridish and 0.1 – 0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2 – 3 hours. After this, 10 mm plate agar well made with the help of sterilized agar well cutter. After that about 0.2 ml of extracts were introduced into the agar well and incubated at 37°C for 24 – 48 hours. The inhibition zone appeared around the agar well, indicating that the sample extracts inhibit the pathogenic microorganisms.

## **RESULT**

### **1. Morphological Character**

#### ***Gynura procumbens* (Lour.) Merr., Enum, Philipp. Fl. Pl. 3.618.1923**

*G. sarmentosa* DC.

Decumbent or procumbent perennial succulent herbs, up to 25 cm high, stem angular, striate, much branched. Leaves simple, alternate, blades ovate-oblong or spatulate, 3.2 – 9.5 cm long, 1.5 – 3.5 cm wide, apex acute or obtuse, base attenuate, slightly serrate along the margin, pubescent on both surfaces; petiole 1.0 – 2.3 cm long, pale green, pubescent. Inflorescence axillary or terminal corymbose, head consists of more than 60 flowers; capitulum orange-yellow, about 10 mm in diameter, homogenous involucre bracts cylindrical, phyllaries 9 – 12, arranged in 2 series narrow, equal, about 7 mm long 1 mm wide, receptacle flat; flowers disciform, bisexual, regular, epigynous, pentamerous, sessile; pappus hairs. Corolla tubular, orange-yellow; tube slender, about 8 mm long; 1.4 mm wide; lobe 5, linear, minute. Stamens 5, epipetalous, inserted; filament short; anther dithecous. Ovary unilocular, single ovule in each cell; stylar arms slender. Achenes linear, fusiform, 3.0 – 4.0 mm long with numerous pappus hairs copious, slender, edible.

Flowering periods – September to November

English Name – Unknown

Myanmar Name – Pyar mee ywet

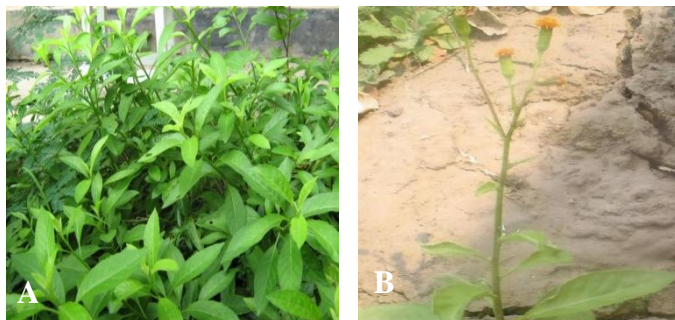


Figure 1 A. Habit & B. Inflorescence of *Gynura procumbens* (Lour.) Merr

## 2. Histological characters

### Sensory characters of powdered leaves of *Gynura procumbens* (Lour.) Merr.

The dried powdered leaves were pale brown, milky in odour and taste is slightly sour.

### Macroscopical characters of the dried leaves

*Gynura procumbens* leaves was about 3.2 – 9.5 cm long, 1.1 – 3.8 cm broad. The upper and lower surfaces were pale green in colour. The leaf pinnately compound, the midrib prominent at the lower surface, the lateral veins was reticulate, margin serrate and the tip acuminate, the base attenuate. The surfaces were pubescent and the texture thick and smooth. The petiole was about 1.1 – 2.3 cm long, tomentose, crescent shape.

### Microscopical characters of powdered leaves

The upper epidermal cells were slightly wavy, and the lower epidermal cells wavy with anomocytic type stomata on both surfaces, trichomes non-glandular, uniseriate, and tapering at the apex. Palisade parenchyma 1- to 2-layered below the upper epidermis, spongy parenchyma 5 – 8 layers of cells, calcium oxalate crystals (prism shape), vessels, tracheids and fibers were present in the powdered leaves.

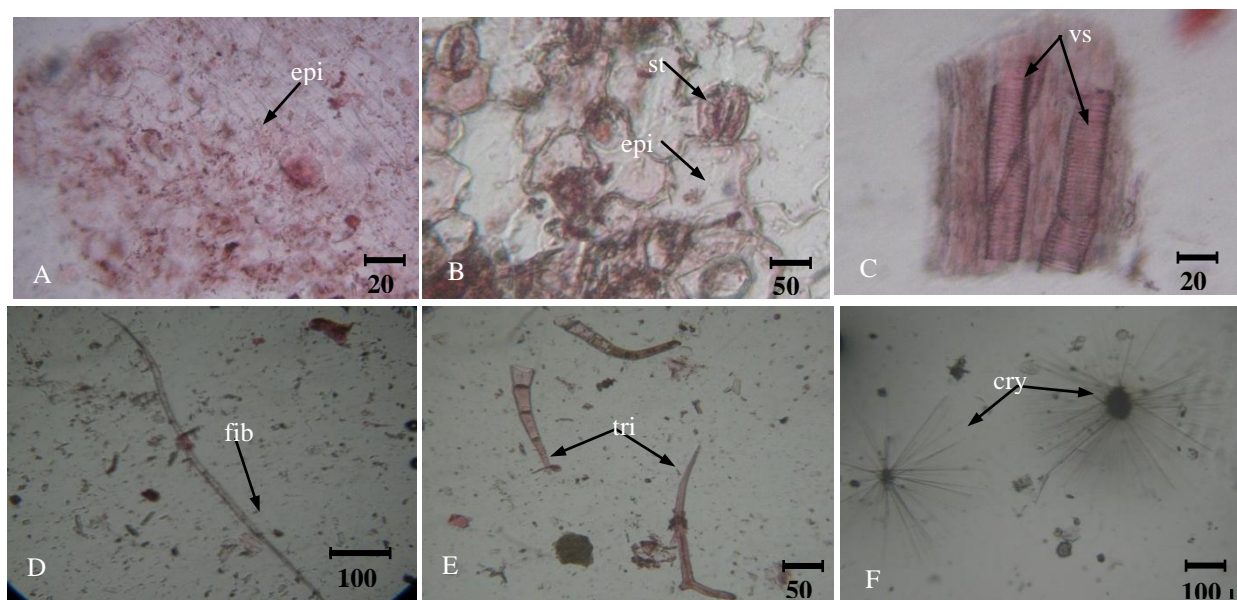


Figure 2 Powdered leaf of *Gynura procumbens* (Lour.) Merr.  
 (A) Adaxial surface of lamina, (B) Abaxial surface of lamina, (C) Vessel element, (D) Fiber element, (E) Fragment of trichome, (F) Needle shape crystal

st = stomata

vs = vessel

epi = epidermal cell

fib = fiber

cry = crystal

tri = trichome

### 3. Antimicrobial activities

The antimicrobial activities of *Gynura procumbens* were investigated. The results were shown in Table (3) and Figure (3.1 – 3.2). In the present study, the extracts of all these plants displayed different activity on the tested microorganisms. The aqueous and petroleum ether extracts of used plants appeared to have no effect on all tested microorganisms.

Table (3) and Figure (3.1, 3.2) showed that ethyl acetate extracts of *Gynura procumbens* was highly effective extract against all tested microorganisms causing maximum inhibition zone 20 – 21 mm. Ethanol extract showed the medium activities 15 mm zone against *Bacillus subtilis* and *B. pumalis*, minimum inhibitory zone range from 12 to 14 mm against *Candida albican*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**Table 3 Antimicrobial activity of various solvent extracts of the leaves of *Gynura procumbens* (Lour.) Merr.**

Solvents	Organisms					
	<i>Bacillus subtilis</i>	<i>Bacillus pumalis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>E.coli</i>	<i>Candida albican</i>
Pet-ether	–	–	–	–	–	–
EtOAc	20 mm (+++)	21 mm (+++)	20 mm (+++)	20 mm (+++)	20 mm (+++)	20 mm (+++)
EtOH	15 mm (++)	15 mm (++)	14 mm (+)	13 mm (+)	14 mm (+)	12 mm (+)
H <sub>2</sub> O	–	–	–	–	–	–

Agar well = 10 mm

10 mm – 14 mm (+)

15 mm – 19 mm (++)

20 mm – above (+++)

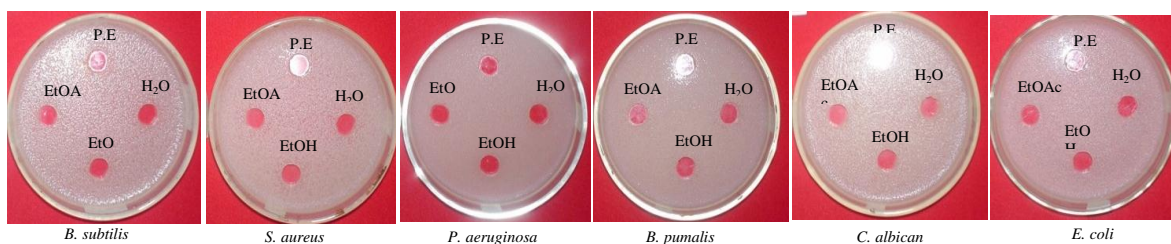


Figure 3.1 Control system of different solvents on test organisms

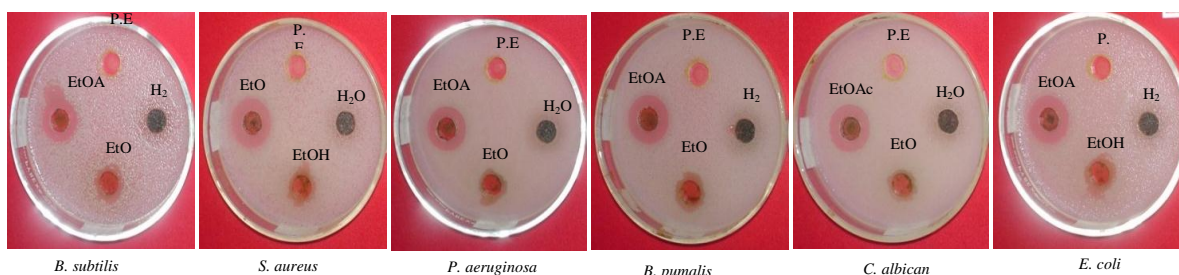


Figure 3.2 Treatment of different solvent extracts from the leaves of *Gynura procumbens* (Lour.) Merr. on test organisms

## DISCUSSION AND CONCLUSION

Morphological characters of *Gynura procumbens* is a decumbent perennial succulent herb; stem angular; leaves simple, alternate, ovate-oblong or spatulate, apex acute or obtuse, base attenuate, margin serrate, pubescent on both surfaces; inflorescence head, axillary or terminal corymbs; head consists of more than 60 flowers; corolla tubular, orange yellow; achenes linear with numerous pappus hairs. These characters are agreed with in those mentioned by Saengsai (2003).

Microscopical characters of *Gynura procumbens* showed the lacunar type of collenchyma. This finding agreed with Fahn (1974). He reported that the Lacunar type collenchyma was found in the petiole of family Asteraceae. Esau (1953) stated that the development of the collenchymatous thickening on the walls facing the intercellular spaces in Asteraceae. Anomocytic type stomata and vessel with pitted thickening were found. This finding agreed with Metcalfe & Chalk (1989). They reported that the members of family Asteraceae consisted of ranunculaceous or anomocytic type of stomata and vessels with helical and scalariform thickening.

Multicellular uniseriate non-glandular trichomes and numerous calcium oxalate crystals are present in the leaves of *Gynura procumbens*. Evans (2002) reported that calcium oxalate crystals assisted in the identification of drugs.

Antimicrobial activities of aqueous and organic solvent were tested in against six different microorganisms including Gram-positive bacteria: *Bacillus subtilis*, *B. pumalis* and *Staphylococcus aureus*; Gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*; yeast-like fungus *Candida albican*. These microorganisms significance in causing common problems of bacterial and fungal infection found in the community. Screening of the leaves extracts by agar-well technique in the study gave the zone diameter of range from 12 mm to 27 mm, which indicated substances possessing antimicrobial activity are present in it. The efficiency of the solvent used in the extraction varied with the plant material. Ethyl acetate extracts from the leaves of *Gynura procumbens* were observed more effective than ethanol extracts.

In present study, crude extract of *Gynura procumbens* had highly actively to fight against six tested microorganisms. This finding agrees with Mahady (2002). It was reported that the *Gynura procumbens* has antimicrobial activities and tremendous potential for plant antimicrobial agents to substitutes for conventional antibiotics. According to results, the extracts of these plants may be the therapeutic agents for treating infectious diseases caused by six tested microorganisms. In microbial assay, the leaves ethyl acetate extract of *Gynura procumbens* more antimicrobial activate than the other solvents extract. However, polar solvent like aqueous extract did not exhibit the best zone.

At present, the Government of Myanmar also encourage for development of scientific research on herbal and traditional medicine. Safe and effective traditional medicine have been identified and sought for the treatment of diseases. There was so many information on the diabetes activity regarding as wider application in traditional medicines in *Gynura procumbens*. Because of its country wide distribution and cheap availability of these traditional medicinal plants have been found to be an attractive potential substitute to more expensive western medicine. Effective solvent extracts from these plants will be tested and confirmed for its antidiabetic activity in the near future.



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