STUDY ON SOME CHEMICAL TESTS AND ANTIMICROBIAL ACTIVITIES TESTS FROM LEAVES OF *ASYSTASIA GANGETICA* (L.) T. ANDERSON

Myat Myat Moe[1], Myat Kay Thwe Lwin [2]

Abstract

Asystasia gangetica (L.) T. Anderson is a medicinal plant which belongs to the family Acanthaceae. It is commonly known as the Creeping foxglove, Coromandel and Chinese violet, and Myanmar name Kyauk-kwè-pin (ေက်ာက္ကြဲပင္). In this study, *Asystasia gangetica* was collected from FAME Organic Pharming Project, Pyin-Oo-Lwin. Botanical studies were about morphological characters and sensory characters of powdered leaves. Some chemical studies such as preliminary examination of phytochemical tests, physico-chemical tests, nutritional values tests and safety tests from powdered leaves. The microbial studies, antimicrobial activities tests on seven tests from water and ethanol extracts of powdered leaves. They have been described for a role in alternative medicinal and culinary plant.

Key words: morphological characters, sensory characters, some chemical tests, antimicrobial activities tests

Introduction

Asystasia gangetica (L.) T. Anderson plants are grown in tropical and subtropical areas. It is native to India, Malay Peninsula and Africa (Weed Management Guide, 2003).

Genus *Asystasia* means inconsistency and relates to the fact that the corolla is more or less regular, which is unusual in the family Acanthaceae (Edwards & Getliffe Norris, 1993). The word *gangetica* is derived from the Ganges River in India where it is presumed the species occurs (Edwards & Getliffe Norris, 1993).

This plant, ground cover thrives in semi-shade and will also grow in sunny spots if it receives adequate moisture. It can be planted in any soil in the garden, but will do better if plenty of compost is added. Propagate from cuttings taken after flowering or by removing rooted runners (Edwards & Getliffe Norris, 1993).

Asystasia gangetica plant is an attractive, fast growing, spreading, perennial herbaceous groundcover that grows from 300 - 600 mm in height. It has green, oval-shaped leaves with rounded base occurring in opposite pairs. The flower is pale yellow and cream coloured with purplish spots or markings. The fruit is a club shaped capsule, splitting from tip to base (Adetula, 2004).

Asystasia gangetica leaves have been shown to contain large amounts of proteins; as well as amino acids, minerals, carbohydrate, lipids and fibres (Hamid & *et al*, 2011). The leaves are eaten as vegetable and used as herbal remedy in traditional African and Nigeria medicine for the management of asthma (Akah. & *et al*, 2003).

In Myanmar, alternative medicinal uses are for renal stones, indicating to the maintaining crystalloid-colloid balance, increasing urine output and eliminating the urinary sediments (FAME, 2011).

Therefore, this research aimed to find out the pure standardization in traditional value of *Asystasia gangetica* (L.) T. Anderson plant

[1] Dr, Professor and Head, Department of Botany, Dagon University
[2] Dr, Lecturer, Department of Botany, Pyay University

Materials and Methods

The specimens were collected from FAME Organic Pharming Project, Pyin-Oo-Lwin, during the flowering and fruiting period time, especially from August to February. After collection, family, genus and species of these plants were verified with the help of references and literatures such as Hooker (1885), Backer (1965), Dassanayake (1998), Hamid (2011) and Wikipedia (2014).

Preparation of fresh leaves was carried in air-dried method, at room temperature for four weeks. And then, dried leaves were powdered and were stored in air tight container or desiccator for prevention of moisture and contamination according to Trease and Evens (1978) and Venema (2013).

The qualitative chemical tests for various phytochemical constituents were carried out according to standard procedures used for the method by Central Council Research in Unani Medicine (1987) and Trease and Evans (1978).

The quantitative analysis for the determination of pH value, total ash, moisture contents, water soluble and ethanol soluble have been done according to British Pharmacopeia (1968) and W.H.O (1998).

The nutritional values such as protein, fat, fiber and carbohydrates were also determined. The protein and fiber were also defined by acid alkali treatment. The fat content was determined by the soxhlet extraction method. The carbohydrate content was determined by the totally result of protein, fat, fiber, moisture and ash contents. The methods of Horwitz (2000), Kjeldahl (1883), Kirk & Sawyer (1991) were applied for investigation of nutritional value studies.

Safety tests of arsenic and aflatoxin for *A. gangetica* powdered leaves sample by using test kit and ELISA method (Leszczynska, *et al*., 1998).

Antimicrobial activities of water and ethanol leaves extracts were tested on seven pathogenic microorganisms, such as *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Agrobacterium tumefaciens* and *Candida albicans* by using agar-well diffusion method *in vitro* (Cruickshank, 1975).

*(These preparation and experiments was conducted at the Botany Department, Dagon University; FAME Pharmaceutical Laboratory; Small Scale Industries Department, Yangon and Nay-Pyi-Taw; Development Centre for Pharmaceutical Technology (DCPT).

Results

I. Botanical studies

1.1 Plant Systematics classification

Rank	Scientific Name
Kingdom	: Plantae
Division	: Angiosperms
Class	: Eudicots
Sub-class	: Asterids
Order	: Lamiales
Family	: Acanthaceae
Genus	: *Asystasia*
Species	: *gangetica*
*Binomial name	: *Asystasia gangetica* (L.) T.Anderson Pl. Zeyl. [Thwaites] 235. [Oct-Nov 1860] (According to International Plant Names Index, 015)

1.2 Morphological characters of *Asystasia gangetica* (L.) T. Anderson

This plant is a perennial evergreen herbaceous, ascending with creeping growth. It grows about less than 0.5 m tall to a height of about 1 m or more; quadrangular stems are lightly hairy (i.e. sparsely pubescent), branched, reddish-brown colour and develop roots (i.e. adventitious roots) where the joints (i.e. nodes) come into contact with moist soil. Simple leaves, opposite, bright green colour; lamina ovate to lanceolate, about 3 - 8 cm long and 1.5 - 4.5 cm width, base cuneate to cordate, apex shortly acuminate or acute, margin crenulate to entire, glabrous to sparsely pubescent with 4 - 6 lateral veins at each side of the midrib; petiolate present, slender, about 2 - 6 cm long. Inflorescence terminal racemes, lax or dense up to about 25 cm long, flowers arranged opposite or alternate with directed to one side. Flowers are bracteolate, at the base of the pedicel; bisexual, actinomorphic, complete, hypogynous, 5-merous; pedicelate up to about 3 mm long. Calyx (5) sepals, sub-synsepalous, basally connate, bright green colour, linear-lanceolate, about 5 - 9 mm long, thinly pubescent on the back. Corolla (5) petals, synpetalous, funnel-shaped, ventricose or narrow, about 3.5 cm long and about 2.5 cm width, 5 lobes, subequal, imbricate in bud, usually pale-yellow cream colour with purplish spots or markings inside lower lobe and tube. Androecium 4 / 2+2 stamens, didynamous, subequal, inserted and attached on the corolla tube; anthers oblong, about 2 mm long, parallel, dehiscent, pollen sac with pollen grains numerous, basifixed, introrse; filaments long, about 5 - 7 mm long; inferior. Gynaecium superior; ovary oblong, about 1mm long, densely pubescent; bicarpellary, syncarpous, bilocular, two ovules in each loculus; axile placentation; style filiform, about 2 cm long, glabrous except near the base; stigma bifid or two short lobes, about 1 mm long and 2 mm width. Fruits are club-shaped capsule, about 2 - 3 cm long, pubescent and glandular, bright green colour to reddish brown colour, splitting from tip to base, usually 4-seeded. Seeds ovoid, flattened, about 4 mm long, pale yellow to brown and black, crenate margins, tuberculate; supported by retinacula (persistent hook).

The flowering and fruiting period is from November to April. The morphological characters were shown in Figures (1 to 10).

Fig. (1) Habit

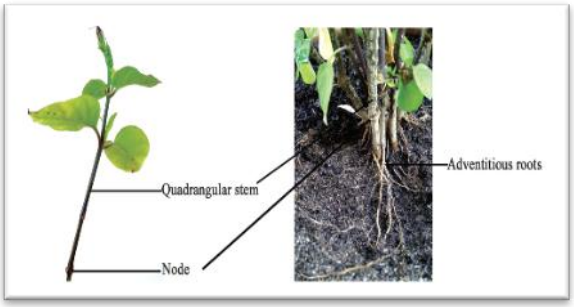


Fig. (2) Stem and root

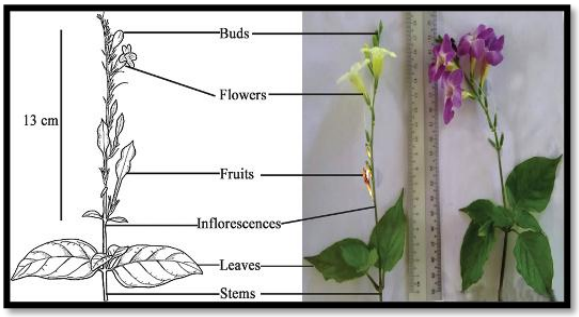


Fig. (3) Stems, Leaves and inflorescences

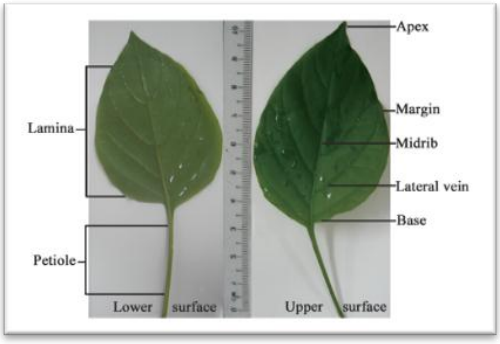


Fig. (4) Members of leaf

Fig. (5) Inflorescences

Fig. (6) Flowers

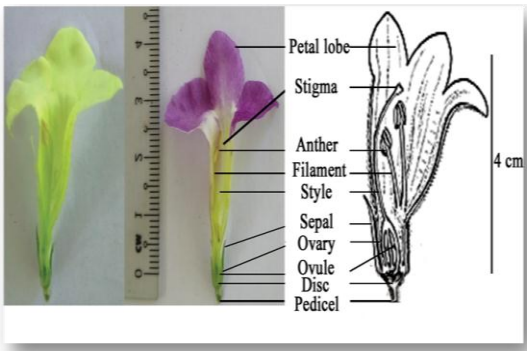


Fig. (7) L.S of Flower

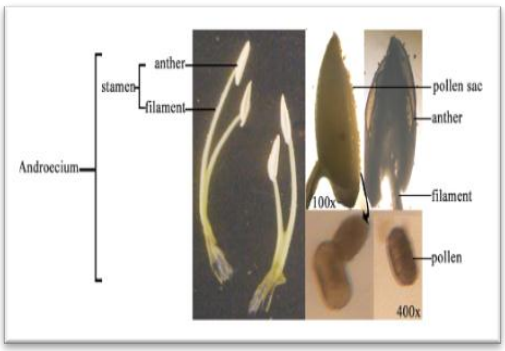


Fig. (8) Androecium

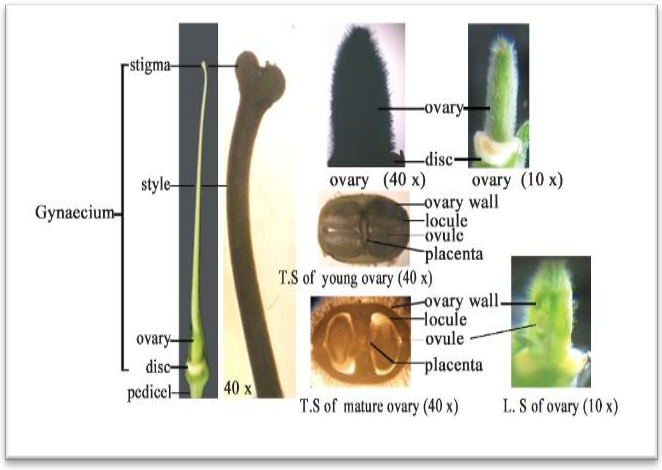


Fig. (9) Gynaecium

Fig. (10) Fruits and seeds

1.3 Sensory characters of *Asystasia gangetica* powdered leaves

The sensory characters of *A. gangetica* powdered leaves were dark green in colour. The odour pleasant smell and taste were pleasant (characteristics). The texture was fibrous and fine. These are shown in the following Figures 11 to 13.

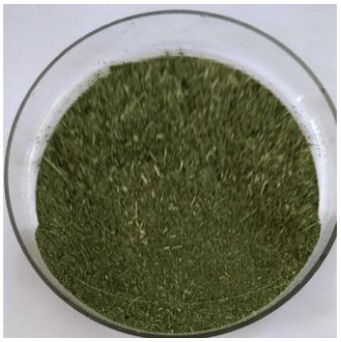


Fig. (11) Fibrous leaves powder

Fig. (12) Fine leaves powder

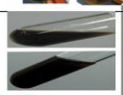
Fig. (13) Fine leaves powder stored in the sterile desiccator for prevention of moisture and contamination

II. Some chemical studies

2.1 Qualitative investigation (Phytochemical Test) on powdered leaves extracts of *Asystasia gangetica*

The qualitative investigation was carried out on the powdered leaves of *A. gangetica*. The preliminary phytochemical test was done to find out the types of phytoorganic constituents such as alkaloids, glycosides, reducing sugars, α-amino acid, saponins, flavonoids, phenolic compound, carbohydrates, starches and tannins. According to the procedures mentioned from the results are summarized in Table (1).

Table. 1 Phytochemical test for water and ethanol powdered leaves extracts

No.	Constituents	Extracts	Test Reagent	Observation	Results
1	Alkaloid	H_2O EtOH	1.Mayer's reagent 2.Dragendroff's reagent	1. Not change white ppt 2. Not change orange ppt	- -
2	Glycoside	H_2O EtOH	10 % lead acetate solution	White ppt 	+ +
3	Reducing sugar	H_2O EtOH	Benedict's solution	Brick red ppt 	+ +
4	α-amino acid	H_2O EtOH	Ninhydrin Reagent	Deep pink & violet 	+ +
5	Saponin	H_2O EtOH	Distilled water	Foaming 	+ +
6	Flavonoid	H_2O EtOH	dilHCL & small piece of zinc/ magnesium	Reddish brown colour 	+ +
7	Phenolic compound	H_2O EtOH	3% Ferric chloride solution	Brown colour 	+ +
8	Carbohydrate	H_2O EtOH	10% α-napthol +Conc: H_2SO_4	Red ring 	+ +
9	Starch	H_2O EtOH	Iodine solution	Bluish black colour 	+ +
10	Tannin	H_2O EtOH	1% Ferric chloride solution	Yellowish brown/ Bluish black 	+ +

(+) = presence, (-) = absence

2.2 Test for quantitative properties (Physico-chemical Test) of *Asystasia gangetica* powdered leaves

To identify the structures of isolated compounds, they were firstly characterized by determination of their physical properties such as pH value, total ash, moisture contents, water soluble and ethanol soluble matters. From the results of physico-chemical properties, the moisture content was usually determined by drying to constant weight and taking the loss in weight as moisture. Total ash content was also examined and recorded. The solubility of powdered leaves was carried out to find the number of total solids soluble in solvent. All these values were useful for the quality control system regarding the physico-chemical ash and impurities whenever it was used for medicinal purposes. The different soluble matter contents were shown in Table (2).

Table (2) Physico-chemical tests on powdered leaves

No.		Composition	Contents (%) of powdered leaves
1		pH value	6.95 %
2		Total ash	8.69 %
3		Moisture content	4.59 %
4		Water soluble matter	27.76 %
5		Ethanol soluble matter	7.17 %

2.3 Nutritional value investigation on powdered leaves of *Asystasia gangetica* plant

For quality control assessment of the medicinal plant materials, nutritional values such as protein, fat, fiber and carbohydrate of *A. gangetica* powdered leaves were also determined. The results for these contents are summarized in Table (3) and test report (1).

Table (3) Nutritional values on powdered leaves

No.	Composition	Contents (%) of powdered leaves
1	Protein	19.17 %
2	Fat	18.06 %
3	Fiber	30.66 %
4	Carbohydrate	18.83 %

2.4 Safety tests on powdered leaves of *Asystasia gangetica*

Some safety tests of arsenic and aflatoxin for powdered leaves were firstly determination by quality control assessment of the medicinal and culinary plant. These test results were shown in Table (4) and test report (2).

Table. (4) Safety tests on powdered leaves

No.	Composition	Result of powdered leaves	Standard
1	Arsenic	Not detected	Not more than 5 ppm
2	Aflatoxin	8.459 ppb	< 20 ppb

Tests Report – 1 Tests Report – 1

REPUBLIC OF THE UNION OF MYANMAR
Ministry of Agriculture, Livestock and Irrigation
Small Scale Industries Department
Thudama Main Road, North Okkalapa 137 (A), Yangon, Myanmar
Phone:662956

LABORATORY REPORT

1. Reference Letter of Dr. Myat Kay Thwe Lwin , Dated 8 March 2018
2. Sample Asystasia gangetica leaves powder
3. Sender Dr. Myat Kay Thwe Lwin ,"ဟသ်တ တက္ကသိလ်"
4. Objective To analyse the quality of sample by Chemical Test.
5. Date of received 8.3.2018

CHEMICAL RESULTS

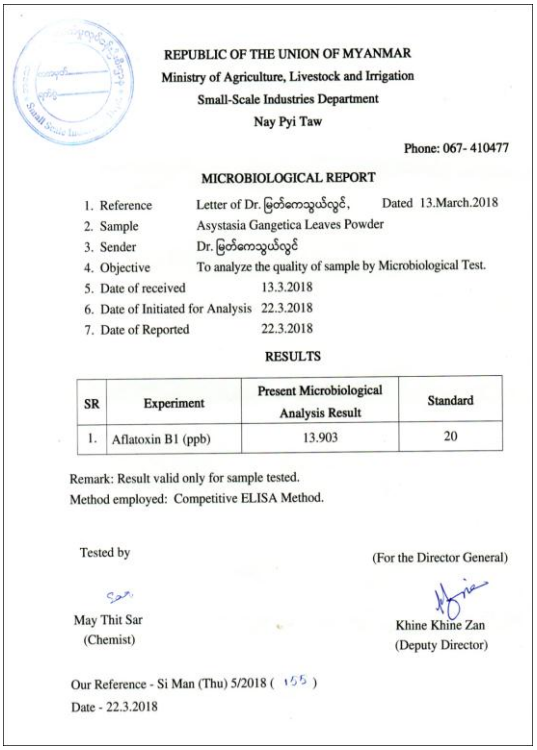
No	Experiment	Present Chemical Analysis Results
1	Protein (%)	19.17
2	Fat (%)	18.06
3	Fiber (%)	30.66
4	Arsenic	Not Detected

Remark : Results valid only for sample tested.
Method employed : Chemical Analysis of Food by David Pearson, Kjeldahl Method and Arsenic Test Kit.

Tested by
(Chemist)
Kyi Pyar Khaing
B.Sc Chemistry

Our Reference – Si Mam (Tha) 5/2018 (1095)
Dated- 3·4·2018

Checked by
(For the Director General)
Dr. Kyu Kyu Hlaing
Assistant Director
B.Sc(Hons),M.Sc,
M.Res,Ph.D.(Chemistry)



REPUBLIC OF THE UNION OF MYANMAR
Ministry of Agriculture, Livestock and Irrigation
Small-Scale Industries Department
Nay Pyi Taw
Phone: 067- 410477

MICROBIOLOGICAL REPORT

1. Reference Letter of Dr. မြတ်ကေသွယ်လွင်, Dated 13.March.2018
2. Sample Asystasia Gangetica Leaves Powder
3. Sender Dr. မြတ်ကေသွယ်လွင်
4. Objective To analyze the quality of sample by Microbiological Test.
5. Date of received 13.3.2018
6. Date of Initiated for Analysis 22.3.2018
7. Date of Reported 22.3.2018

RESULTS

SR	Experiment	Present Microbiological Analysis Result	Standard
1.	Aflatoxin B1 (ppb)	13.903	20

Remark: Result valid only for sample tested.
Method employed: Competitive ELISA Method.

Tested by
May Thit Sar
(Chemist)

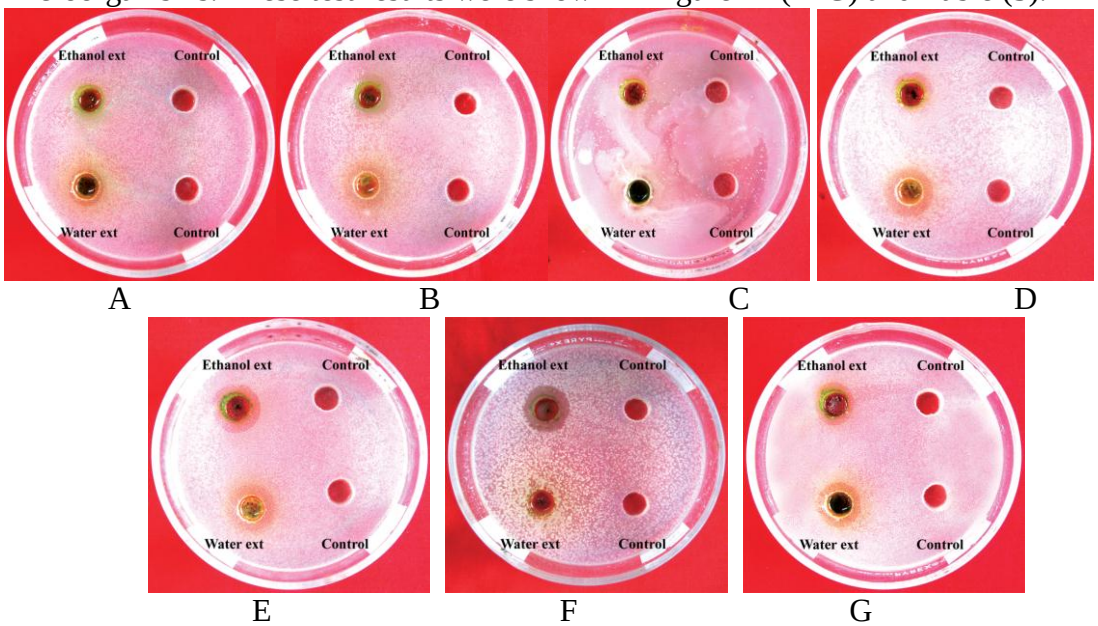
(For the Director General)
Khine Khine Zan
(Deputy Director)

Our Reference - Si Man (Thu) 5/2018 (155)
Date - 22.3.2018

III. Microbial studies

3.1 Antimicrobial activities of water and ethanol powdered leaves extracts from *Asystasia gangetica*

In these experiment, water and ethanol powdered leaves extracts on *A. gangetica*. These results were shown on ethanol and water extracts effective on seven microorganisms. These test results were shown in Figure 14 (A-G) and Table (5).



A-*Bacillus pumilus*, B-*Bacillus subtilis*, C-*Escherichia coli*, D-*Pseudomonas aeruginosa*,E-*Staphylococcus* aureus, F-*Agrobacterium* tumefaciens, G-*Candida albicans*

Fig. (14) Antimicrobial activities test

Table (5) Antimicrobial activities screening on the powdered leaves extracts

Sample	Solvents	Test microorganisms						
		Bacillus pumilus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Agrobacterium tumefaciens*	*Candida albicans*
Asystasia gangetica leaves extracts	Ethanol	14mm	12mm	12mm	13mm	15mm	15mm	14mm
	Water	14mm	12mm	11mm	14mm	15mm	11mm	14mm

Agar well size - 10 mm

Discussion and Conclusion

In this research, the whole plant of *A. gangetica* is studied. It is perennial herbaceous with usually ascending, branched, quadrangular stems with rooting at the lower nodes. Bright green colour oval-shaped leaves with terminal racemes inflorescences were lax or dense flowers arranged opposite or alternate with directed to one side. Five merous bisexual flowers are hypogynous. Bracteolate is at the base of the pedicelate. Sub-synsepalous calyx are basally connate with linear-lanceolate. Syn-petalous of corolla are funnel-shaped, subequal, imbricate in bud, usually pale yellow and cream colour with purplish spots or markings inside lower lobe and tube. The fruit is a club shaped capsule, splitting from tip to base usually 4-ovoid seeds present, supported by retinacula (persistent hook). These characters were in general agreement with those mentioned by Hooker (1885), Dassanayake (1998), Backer (1965) & Edwards (1993).

The sensory characters of powder leaves were found to be dark green colour, pleasant smell odour, pleasant (characteristics) taste and fibrous/fine texture. Those could be used for the identification and standardization in traditional values of drug. All characters were mentioned by Trease and Evens (1978).

Physico-chemical properties of powder leaves were showed in pH value (6.95), total ash content (8.69%), moisture content (4.59%), water soluble matter (27.76%) and ethanol soluble matter (7.17%). Those could be used for the organic matter determined. These statements were in general agreement with those mentioned by British Pharmacopeia (1968) and W.H.O (1998).

Phytochemical screening of water and ethanol extracts leaves powdered were showed the presence of glycoside, reducing sugar, a-amino acid, saponin, flavonoids, phenolic compound, carbohydrate, starch and tannin whereas alkaloid were not detected in both extracts. All characters were mentioned by Central Council Research in Unani Medicine (1987), Trease and Evans (1978) and Hamid & *et al* (2011).

Some nutritional values tests showed in protein content (19.17%), fat content (18.06%), fiber content (30.66%) and carbohydrates content (18.83%) respectively. And then, safety tests of powdered leaves were showed in not detected on arsenic and aflatoxin. These all statements were in general agreement with those mentioned by Horwitz (2000), Kjeldahl (1883), Kirk & Sawyer (1991), Leszczynska, *et al*. (1998) and Hamid, *et al*. (2011).

Microbial aspects for antimicrobial activities tests of water and ethanol leaves extracts were showed in the efficiency on seven strains of microorganisms. But, aqueous extract show of *Escherichia coli* and *Agrobacterium tumefaciens* antimicrobial activities are little against.

All characters mentioned above can be used for identification and standardization in beneficial effect of alternative medicinal and culinary plant. Therefore, the present research deals with traditional medicine were given knowledge for local people and useful for the investigation of effective pharmacological research in the coming future.

Acknowledgements

Firstly, I would like to say thanks to Prof. Bang and his project team members for their good care and for their full support. And then, my deepest appreciation goes to Dr. Khin Maung Lwin (FAME), and all staff from FAME Pharmaceuticals, Yangon and FAME Organic Pharming Project, Pyin-Oo-Lwin, for their encouragement and help throughout this work..

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