

## Morphological Characters of *Atalantia monophylla* Correa. (Taw-shauk) and Antispasmodic Activity of its Root

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### Abstract

*Atalantia monophylla* Correa. (Taw-shauk), belonging to the family Rutaceae is used as lubricant in Myanmar traditional medicine. They are large aromatic shrubs, sharp spines at the nodes. Most of the Rutaceae family are wing petiole but this plant is not wing petiole, the tip emarginate and gland dotted. *Atalantia monophylla* Correa. is grown in hilly regions of the Monywa District in Sagaing Region. The leaves, fruits and roots are used as traditional medicine for the treatment of antispasmodic activity, snake-bite, emollient and paralysis. Morphological characters and pharmacological activities were investigated in this work. Acute toxicity of aqueous and 50% ethanolic extracts of Taw-shauk root were tested on Albino mice model. Both extracts were free from acute toxic or harmful effects up to maximal permissible dose of 12 g/kg. The antispasmodic activity of 95% ethanolic extract of *A. monophylla* Correa. root was investigated on acetylcholine induced contraction of isolated rat intestine. The 95% ethanolic extracts (6 mg) of Taw-shauk roots could completely block the contraction caused by Ach (0.1 mg) transiently, where as the action of Ach (0.1 mg) was completely blocked by atropine (5 mg). These results provided a scientific proof to use *A. monophylla* Correa. root as an antispasmodic agent in Myanmar traditional medicine.

### Introduction

*Atalantia monophylla* Correa. (Taw-shauk in Myanmar) belongs to the family Rutaceae consists of about 150 genera and 900 species (Trease and Evans, 2002). This plant used in this research was wildy grown on the slopes of Mae Sam Mhe at Monywa District in Sagaing Region.

The root is used as antispasmodic agent. It can also be applicable in snake bite, emollient, chronic rheumatism, paralysis, bruises, sprains and swellings. The leaf juice is an ingredient in a compound liniment used in hemiplegia. Decoction of the leaves is applied for itchness and other skin complaints. Berries yield warm oil which is a valuable application in chronic rheumatism and paralytic limbs (Nadkarni, 1999). *A. monophylla* Correa and *A. roxburghiara* have muscle relaxant effects on intestinal smooth muscle. The aim of present study was to investigate the antispasmodic activity of Taw-shauk root via its muscle relaxant action on acetylcholine induced isolated rat intestine.

### Materials and Methods

#### Sampling

Roots of *Atalantia monophylla* Correa. used in this research were collected from widely grown in hilly regions of Monywa District in Sagaing Region, during the flowering and fruiting periods extending from October to January. The collected roots were washed with water to remove impurities. After washing and cleaning, the sample was air dried and ground to get powder and stored in air-tight container.

#### Acute Toxicity Test

#### Preparation of Aqueous and 50% Ethanolic Extracts of *A. monophylla* Correa. Root

Aqueous extract was prepared by heating 100 g of root powder in a conical flask with 1

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L of distilled water for 6 h in a water bath at 60°C. The mixture was filtered using cheese cloth and the filtrate was evaporated to dryness on a boiling water bath. The residue obtained was then kept in desiccators.

50 % EtOH extract was prepared by macerating 100 g of root powder in a stoppered conical flask with 1 L of 50% ethanol for one week. The mixture was filtered by using filter paper and the filtrate was evaporated to dryness on a boiling water bath. The residue obtained was kept in desiccators.

### **Theory**

The purpose for conducting toxicity tests on animals is to evaluate the nature and the degree of harmful effects or deaths. Acute toxicity test is a single test to determine the symptoms consequence to administration of the test agents and to determine the LD<sub>50</sub> of those agents. The route of administration selected on animal would be intended route for its administration to human. Drug is given to an animal on one or at most two occasions. Usually the acute lethality of a compound is determined on the basis of deaths occurring in 24 hours but the survivors should be observed for at least two weeks in order to detect delayed effects. (Loomis, 1968)

### **Method**

The lethal activity and LD<sub>50</sub> of the samples were investigated according to the Litchfield and Wilcoxon method (1949). Samples were administered via oral route. Seventy albino mice of both sexes, weighing (25-35 g) were used in this study. Mice were fasted overnight but water was allowed and alibitum. They were randomly selected, weighed and separated into 7 groups and each group contains 10 mice (Figure 1). Group 1 served as control group and was administered distilled water only at a dose of 0.1 mL/10 g. Group 2, 3 and 4 were respectively administered with various doses of aqueous extracts as 3 g/kg, 6 g/kg and 12 g/kg body weight. Similarly, Group 5, 6 and 7 were administered with 50% ethanol extract at doses of 3 g/kg, 6 g/kg and 12 g/kg respectively. After given the extracts orally, food and water were supplied. Mice were observed carefully for their mortality and behaviour responses for 24 hours, there after once daily for 14 days. The mortality within the groups was recorded and LD<sub>50</sub> was calculated.

### **Determination of Antispasmodic Activity**

#### **Extraction and Preparation of Test Samples**

100 g of the root powder was immersed under 1 L of 95 % EtOH at room temperature for ten days with occasionally shaking. The mixture was then filtered through a filter paper. The filtrate thus obtained was heated over boiling water bath to remove the solvent. 95% EtOH extract was prepared according to this procedure.

Predetermined amount of ethanol extracts were then dissolved in distilled water (10 mL) to get the desired concentrations, immediately prior to use.

#### **Animals**

Albino rats of both sexes weighing 180-250 g were used in this study. The animals were caged in wire-mesh cages. Rats were deprived to food 24 hours prior to the experiment.

### **Theory**

Isolated rat intestine has no regular pendular movements (i.e. continuous contraction and relaxation). The effect of a drug on the intestine may be either stimulatory (contraction) or

inhibitory (relaxation). Thus, using specific antagonists, possible mechanism for action of 95% EtOH extract of Taw-shauk root can be studied.

### Method

Antispasmodic effect of 95% ethanolic extract of Taw-shauk root was conducted *in vitro* using isolated rat intestine. The smooth muscle preparation was done according to method described by Magnus (1904).

Animal was scarified; using chloroform and gastro intestinal content were then discarded from the animal. After discarding, 10 cm nearest to the gastrointestinal junction, the intestine muscle strips (15 to 20 mm) was cut and transferred to a dish containing tyrode solution which was gassed with O<sub>2</sub>. Cotton threads were fastened to both ends of the intestine muscle strip which was vertically mounted in a 50 mL organ bath filled with tyrode solution. The solution in organ-bath was gassed with oxygen and kept at 37 °C by a large heated water jacket. The contraction and relaxation measurements of the intestine muscle were recorded isometrically as 1 g tension via a force displacement isometric transducer (Ugo Basili), connected to 2 channel recorder (Ugo Basili). A 30-45 minutes period for stabilization was followed by a 10 minutes period during which basal activity was recorded.

The relaxation effect of 95% ethanolic extract of *A. monophylla* Correa. (2 - 6 mg/mL bc) were studied on acetylcholine (0.1 mL) induced contraction on isolated rat intestine. Ach (0.1 ml/bc alone) was administered into the bath and recording was done. Once the plateau of contraction elicited by stimulant was achieved, the extract was added to the organ bath non-cumulatively. After recording the spasmolytic effect for each experiment, the tissue preparation was washed three times with Tyrode solution followed by at least 30 sec for rest. The same protocol was carried out in presence of other samples.

Each experiment was performed on 7 segments of ileum. Segments without signs of spontaneous activity were discarded. The photograph of isolated rat intestine was shown in Figure 3. The apparatus set-up to measure the contraction and relaxation of ileum smooth muscle is shown in Figure 4.

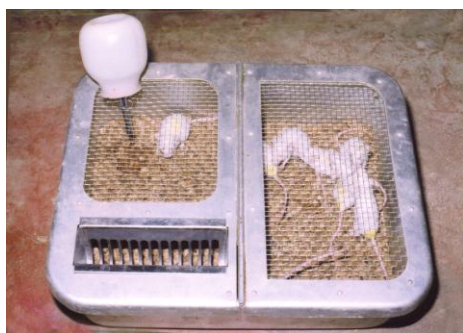


Figure 1. Albino mice (one-group contain 10 mice)



Figure 2. Administration of the extracts of the mice

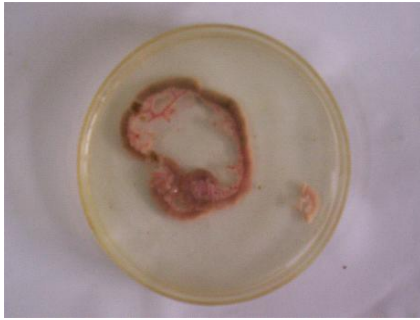


Figure 3. Isolated rat's intestine

Figure 4. Apparatus set used (2 channel recorder GEMIN 7070, organ bath, water jacket, O<sub>2</sub> cylinder & pipe)

## Results

### Diagnostic Characters of *Atalantia monophylla* Correa.

Large shrubs or treelets, about 2.1 m high; stems-woody, cylindrical, armed, much-branched with single, stout, sharp spines at the nodes; leaves simple, alternate, ovate lanceolate or elliptic, petiole very short, wingless petioles, base broadly obtuse, margin entire or slightly undulate, the tip emarginate, gland dotted; inflorescence terminal and axillary, fasciculated corymbose, racemes; flower very fragrant, bracteate, pedicellate, bisexual, complete, regular actinomorphic tetramerous, hypogynous; sepals (4), sepaloid, gamosepalous, the tube campanulate, scarious, persistent, inferior; petals (4), petaloid (white), obovate, adnate to the staminal tube, glabrous, inferior; stamens (8), monadelphous, the filaments united into a tube, basifixed, introrse, staminal tube small, inferior; ovaries 4 celled, syncarpous, tetralocular, axile placentation, the style one terminal, the stigma capitate, superior. The fruits are hesperidium, globoid, indehiscent, yellowish-green when ripe, locules filled with numerous pulp-vesicles, essential oil present. The seeds have one or two ovules in each locule, one to five seeded, often some of the locules are seed-free trigonous, green in colour, oblong in shape. The flowering and fruiting period is from October to January.

Figure 5. Habit of *Atalantia monophylla* Correa.

Figure 6. Leaves



Figure 7. Inflorescences

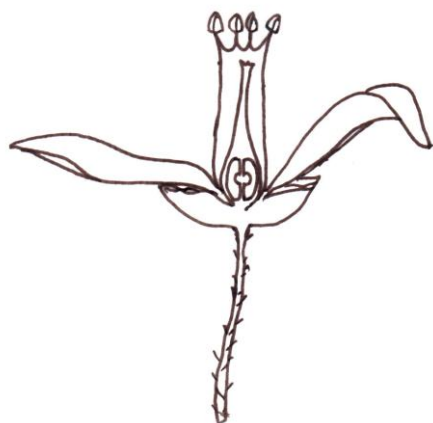


Figure 8. L.S of flower (x 4)



Figure 9. Fruits



Figure 10. Roots

### Acute Toxicity of the Aqueous and 50% Ethanolic Extracts of Taw-shauk Roots

The acute toxicity test was done according to the method described by Litchfield and Wilcoxon (1949). It showed that no lethality of the mice was observed up to two weeks, even with the maximal given dose (12 g/kg) of both aqueous and 50% ethanolic extracts. The mice were found to be alive and healthy during the observation period of two weeks. Therefore, it was observed that the extracts were free from acute toxic or harmful effects. If the amount of extract is more than 12 g, the extract was poorly soluble in distilled water and it limits further increase in dose administered to find the lethal dose in this study. The oral route of administration was chosen because it is the intended route for use in human subjects. The results were shown in Table 1.

Table 1. Results of Acute Toxicity Tests of Aqueous and 50% Ethanol Extracts of *Atalantia monophylla* Correa. Root on Albino Mice

Groups	Drugs administered	Dose (g/kg)	Tested animals	Ratio of dead/ test animal
1	Distilled water	10	10	0/10
2	Aqueous extract	3	10	0/10
3	Aqueous extract	6	10	0/10
4	Aqueous extract	12	10	0/10
5	50% ethanol extract	3	10	0/10
6	50% ethanol extract	6	10	0/10
7	50% ethanol extract	12	10	0/10

### Antispasmodic Activity Taw-shauk Roots

The relaxation effects on smooth muscle of isolated rat intestines were also studied *in vitro* by Magnu's method using various concentrations of 95% ethanolic extracts (2, 3, 4, 5, 6 mg/mL bc). From these result, it was observed that the relaxation effects were increased with increasing the concentrations of sample tested.

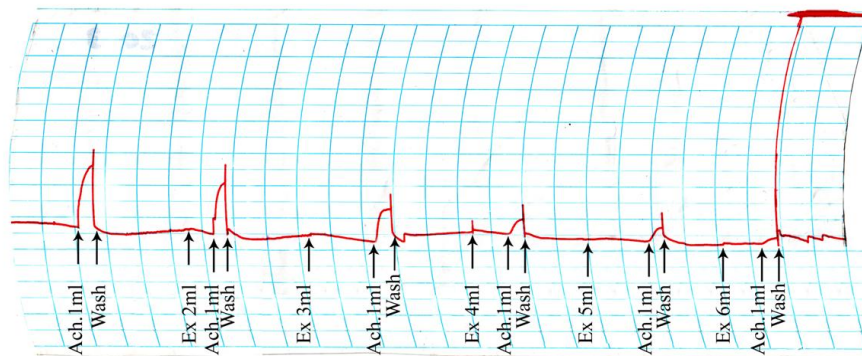


Figure 11. Tracing sample showed effect of Ethanol extract of *Atalantia monophylla* Correa. root on contraction induced by Acetylcholine *in vitro* experiment

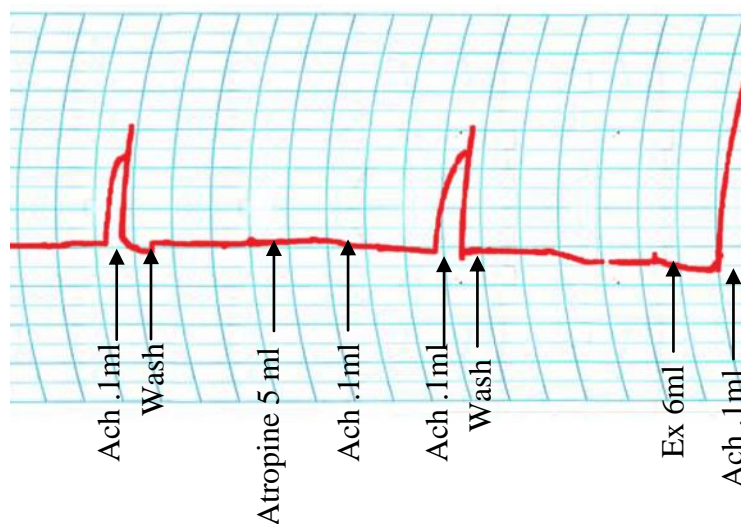


Figure 12. Tracing sample showed effect of atropine on intestine relaxant activity *in vitro* of ethanolic extract on acetylcholine induced intestine contraction in isolated rat intestine.

The experiment revealed that 95% EtOH extracts of Taw-shauk root at dose 6 mg/mL bc was able to completely block the contraction induced by Ach (0.1 mg) while the action of Ach (0.1 mg) was also completely blocked by atropine (5 mg) (Figure 12). So the mechanism of action was antimuscurinic activity. Therefore, 95% EtOH of Taw-shauk plants have the relaxation effect on intestine smooth muscle in dose response manner with atropine like activity.

Table 2. Effect (Height of Contraction) of 95% Ethanolic Extract of *Atalantia monophylla* Correa. Root on Acetylcholine induced Contraction in Isolated Rat Intestine

No. of experiments	Height of contraction (cm)					
	Ach 0.1ml/bc	Ach +2mg/mlbc	Ach +3mg/mlbc	Ach +4mg/mlbc	Ach +5mg/mlbc	Ach +6mg/mlbc
1	2.6	0.9	0.8	0.7	0.3	0.4
2	4.5	2.4	1.6	0.9	0.5	0.5
3	1.9	1.5	1.0	0.4	0.4	0.1
4	2.1	1.0	0.7	0.4	0.3	0.2
5	2.0	1.9	1.5	1.2	1.0	0.9
6	1.7	1.2	0.8	0.7	0.6	0.2
7	1.0	0.6	0.3	0.2	0.1	0
SUM	15.8	10.5	6.7	4.5	3.2	2.3
MEAN	2.257143	1.5	0.957143	0.642857	0.457143	0.328571
SEM	0.415679	0.232993	0.173009	0.1288836	0.108797	0.114879

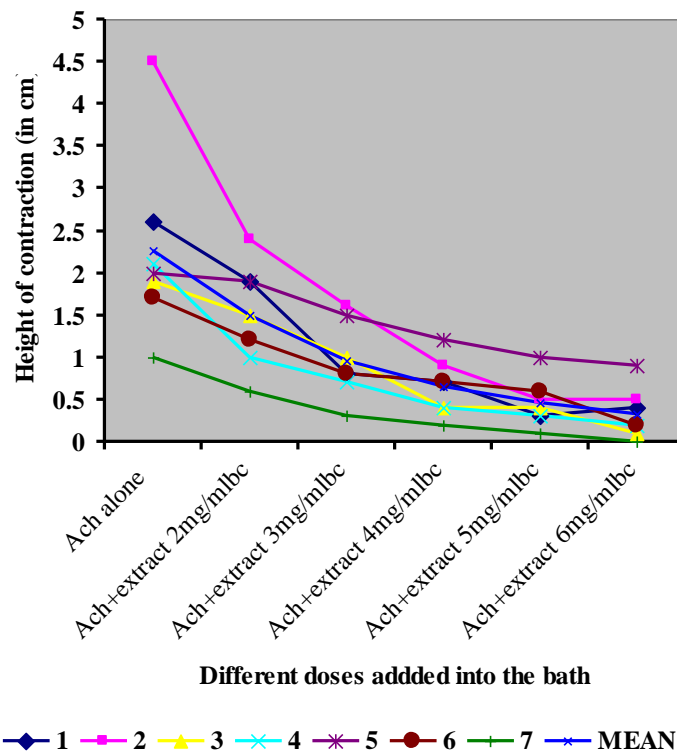


Figure 13. The comparative effects (height of contraction) of ethanolic extract of *Atalantia monophylla* Correa. root on acetylcholine induced isolated contractile smooth muscle of rat intestine  
 Each point represents the height of each observations and the longitudinal lines for each experiment

Table 3. Effect (Width of Contraction) of 95% Ethanolic Extract of *Atalantia monophylla* Correa. Root on Acetylcholine induced Contraction in Isolated Rat Intestine

No. of experiments	Width of contraction in cm					
	Ach 0.1ml/bc	Ach +2mg/mlbc	Ach +3mg/mlbc	Ach +4mg/mlbc	Ach +5mg/mlbc	Ach +6mg/mlbc
1	0.4	0.4	0.4	0.4	0.3	0.3
2	0.3	0.3	0.5	0.5	0.4	0.3
3	0.5	0.4	0.5	0.4	0.4	0.4
4	0.5	0.6	0.5	0.5	0.4	0.3
5	0.3	0.5	0.5	0.4	0.4	0.3
6	0.6	0.6	0.5	0.4	0.4	0.4
7	0.5	0.5	0.3	0.3	0.2	0
SUM	3.1	5.3	6.2	6.9	7.5	8
MEAN	0.442857	0.471429	0.457143	0.414286	0.357143	0.285714
SEM	0.042857	0.042056	0.029738	0.026082	0.029738	0.050843

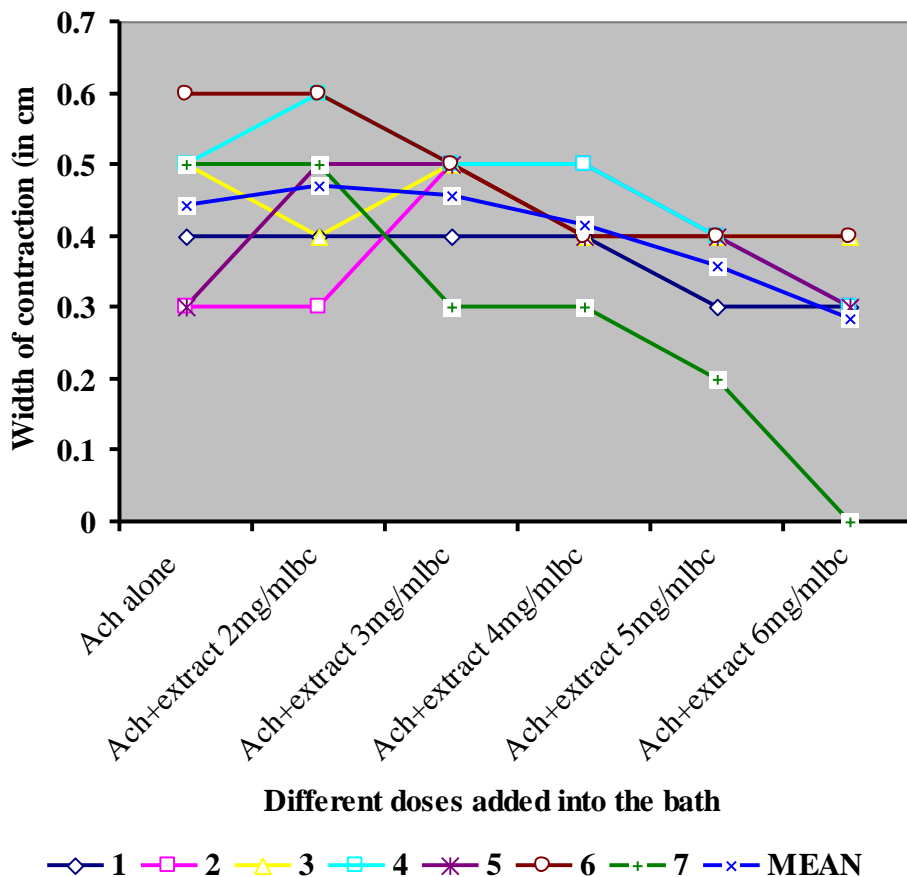


Figure 14. The comparative effects (width of contraction) of ethanolic extract of *Atalantia monophylla* Correa. root on acetylcholine induced isolated contractile smooth muscle of rat intestine  
 Each point represents the width of each observations and the longitudinal lines for each experiment

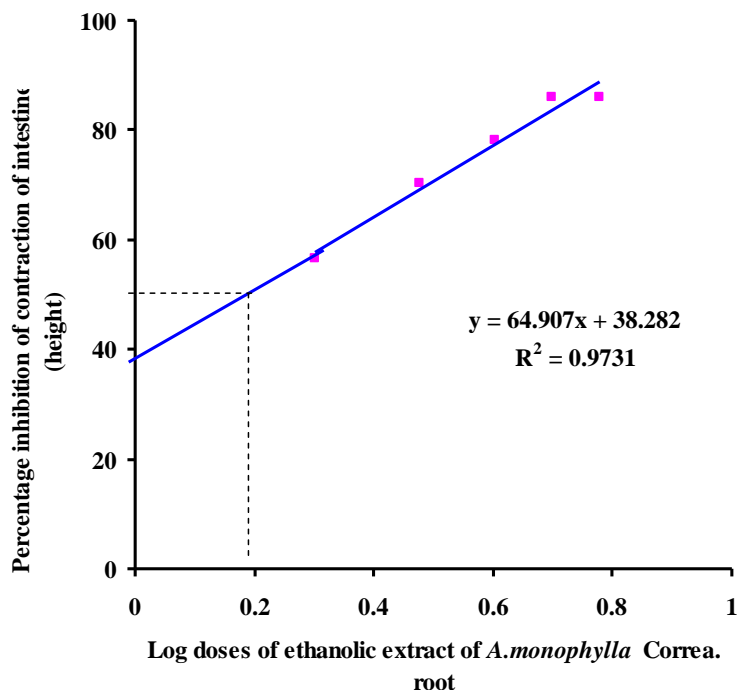


Figure 15. Dose-response relationship of ethanolic extract of *Atalantia monophylla* Correa. root on acetylcholine induced isolated contractile smooth muscle of rat intestine. Each point represents the mean of observations and the blue trend line indicates regression line. ED<sub>50</sub> was 1.52 mg/mL bc.

### Discussion

The plants *Atalantia monophylla* Correa. were collected from wildy grown in hilly regions of Monywa District in Sagaing Region during October to January.

This plant belongs to the family Rutaceae. It is large shrubs or treelets, stout, sharp spines at the nodes. The leaves are simple, alternate, wingless petiole, the tipe emarginates these characters are in agreement with those mentioned by (Hooker, 1875; Brandis, 1971).

The inflorescence is fasciculated corymbose, racemes. The flowers are white, actinomorphic and fragrant. The sepals are synsepalous, campanulate, irregularly lobed and perristent. The petals are free usually four in number and are white in colour. The stamen are (8), monadelphous, ovary-4 celled, syncarpous. The fruits are appearing like small green orange, essential oil present, the seeds are one or two ovules in each locule these characters are in agreement with those described by Cooke (1958); Hooker (1875); Kirtikar & Basu (1933) and Skurz (1974).

Acute toxicity study of aqueous and 95% ethanol extracts of Taw-shauk root on albino mice was observed that even with the maximal permissible dose (12 g/kg), the mice were found to be alive and healthy during the observation period of 14 days. The oral route of administration was chosen because it is the intended route to use in man. So, Taw-shauk root can be stated as free of acute toxic effect on albino mice and the median lethal dose (LD<sub>50</sub>) of the extracts of Taw-shauk root were supposed to be greater than 12 g/kg body weight.

Table 4. The Percent Inhibition Effect of Height of Contraction of 95% Ethanolic Extract of *Atalantia monophylla* Correa. Root on Acetylcholine induced Contraction in Isolated Rat Intestine

No. of experiments	Percent inhibition of height of contraction				
	Ach +2mg/mlbc	Ach +3mg/mlbc	Ach +4mg/mlbc	Ach +5mg/mlbc	Ach +6mg/mlbc
1	0	0	0	25	25
2	0	-66.6667	-66.6667	-33.3333	0
3	20	0	20	20	20
4	-20	0	0	20	40
5	-66.6667	-66.6667	-33.3333	-33.3333	0
6	0	16.66667	33.33333	33.33333	33.33333
7	0	40	40	60	100
SUM	-64.6667	-73.6667	-2.66667	96.66667	224.3333
MEAN	-9.52381	-10.9524	-0.95238	13.09524	31.19048
SEM	10.47619	15.3739	14.3596	13.05477	12.8417

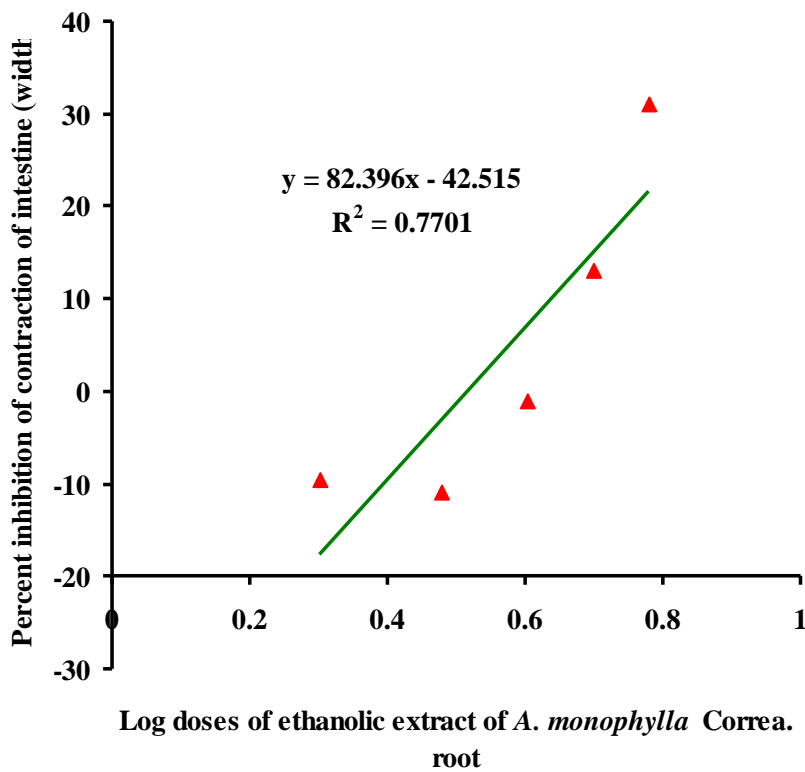


Figure 16. Dose-response relationship of ethanolic extract of *Atalantia monophylla* Correa. root on acetylcholine induced isolated contractile smooth muscle of rat intestine. Each points represents the mean of observations and the green trend line indicate regression line

Antispasmodic activity test results revealed that the relaxation effects were increased with increasing the concentration of sample tested. In addition, 95% ethanolic extract (6 mg) of Taw-shauk root sample could completely blocked the contraction induced by Ach (0.1 mg) while the action of Ach (0.1 mg) was completely blocked by atropine (5 mg). So the mechanism of action was antimuscurinic activity. It may be inferred that 95% EtOH extract of Taw-shauk root have the relaxation effect on intestine smooth muscle in dose dependent manner with atropine like activity.

### Conclusion

Taw-shauk wildly grown in hilly regions of Monywa District in Sagaing division was identified as *Atalantia monophylla* Correa. on the basis of morphological and microscopical study. The plant belongs to the family Rutaceae. The Rutaceae are distinguished by the presence of translucent pellucid dots in the foliage and stamen usually opposite the petals. The development of oil glands producing an aromatic oil is a characteristics feature of the family. Prismatic crystals are abundantly found in leaves and starch grains are found in T. S. of young roots and mature roots. The presence of oils, crystals and starch grains are thought to be medicinally important.

Aqueous and 50% EtOH extracts of *A. monophylla* Correa. were found to be free from acute toxic effect. Therefore, the plant may be used safety. In addition, it may be inferred that 95% EtOH extract of *A. monophylla* Correa. has invaluable medicinal purpose for the treatment of spasm related diseases such as intestinal colic, inflammation of intestine, urinary colic for relieve pain and colic due to its antispasmodic activity.

### Acknowledgements

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