INVESTIGATION ON SOME PHYTOCONSTITUENTS AND BIOACTIVITY SCREENING OF LEAVES OF *Calotropis gigantea* R. Br. (Mayo)

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Abstract

In the present research, the leaf of *Calotropis gigantea* R.Br. (Mayo), Family- Apocynaceae, was chosen for investigation of some phytoconstituents and bioactivity studies such as acute toxicity, antitumor, and larvicidal activities. Acute toxicity of 95 % ethanol extract was studied with the dosage of 2000 mg/kg, 5000 mg/kg body weight in albino mice and no lethality was observed up to fourteen days after administration. The antitumor activities of PE, 95 % EtOH, EtOAc and H_2O extracts were screened on *Agrobacterium tumefaciens* by Potato Disc Assay method and all extracts exhibited antitumor activity after 5 days and 7 days observation. The larvicidal activities ($LC_{50\ and}\ LC_{90}$) of PE, 95 % EtOH, EtOAc and H_2O extracts were investigated in the range of 0.0125 to 0.2 g /100 mL by *Aedes* larvaemethod. The lowest knockdown of *Aedes* larvae was found at the concentration of 0.0125 g /100 mL of H_2O extract. The highest mortality rate (95.60 %) of *Aedes* larvae at the concentration of 0.2 g / 100 mL was found in the EtOAc extract. The lowest mortality rate (8.80 %) of H_2O extract was observed at concentration of 0.0125 g /100 mL. Among the tested four crude extracts, EtOAc extract showed the highest lethal activity (LC_{50} = 0.0235 g / 100mL and LC_{90} = 0.1224 g /100mL).

Keywords: acute toxicity, antitumor activities, *Calotropis gigantea* R.Br., larvicidal activities

Introduction

Calotropis gigantea R.Br. (Mayo), a member of the Apocynaceae family, is a well-known plant throughout the tropical world and they are native to the tropical and subtropical parts of Asia and Africa. Calotropis species are considered common weeds in some parts of the world. Flowers of these plants are fragrant and are often used in making floral tassels in some mainland Southeast Asian cultures. Fibers of these plants are called madar or mader (Bhagavathy and Jancy, 2015). In Myanmar, the plant is distributed in

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various regions and used as remedy for various medicinal purposes. Myint Myint Khine (2007) reported for her PhD dissertation that leaves of *C. procera* (Mayo) possessed antimicrobial activity. The importance of medicinal plants to the economy of low-income countries remains critical and strategic because medicines are keys to a health population that drives and sustain the economy. In this research work, the leaves of *C. gigantea* (Mayo) was chosen for determination of some phytochemical constituents, and screening of some bioactivities.

Botanical Aspect of *Calotropis gigantea* R.Br.

Family	: Apocynaceae
Sub-family	: Asclepiadaceae
Botanical name	: *Calotropis gigan*
Myanmar name	: Mayo
English name	: Swallow-Wort,]
Synonyms	: *Asclepias gigantea* L.
Part used	: Leaves

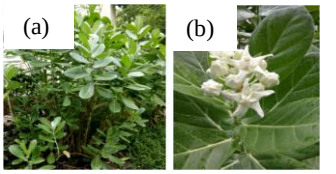

Image of *C.gigantea*
(a) plant (b) flower

Local Uses of *Calotropis gigantea* R.Br. (Mayo)

Traditional practice in Myanmar especially in Ayeyarwady Region, leave of *C.gigantea* is being used mainly by mixing leave with latex in the controlling of snake bite, treatment of paralysis, chronic sinusitis, swellings, and intermittent fevers, cold and asthma. Moreover, after boiling the whole parts of the plants, the obtained watery extract was used to relief arthritic. For low-income countries to attain any appreciable level of self-reliance regarding availability of safe and effective pharmaceuticals use for the management of endemic disease conditions, it was considered a priority because of the importance of self-reliance in the current and perhaps future economic. Today, traditional medical practice has been recognized by the world health organization (WHO) as a building block of primary healthcare. But, safety should be the overriding criterion in the selection of herbal remedies for use in healthcare (Patli and Saini, 2012).

Medicinal Uses of Various Parts of *Calotropis gigantea* R.Br. (Mayo)

The *C. gigantea* plant is used for skin diseases, boils and sores and as a tonic and purgative in small doses, and as an emetic in larger doses. The powdered root bark is used to cure dysentery, elephantiasis, and leprosy. The stem bark is diaphoretic and expectorant, and is used for dysentery, spleen complaints, convulsions, lumbago, scabies, ringworm, pneumatisms and tumors and also as an antiseptic, vermifuge, emetic and purgative, as well as for poisoning arrows. The powdered flowers are given for coughs, colds and asthma. The crushed and warmed leaves are applied on burns, headaches and rheumatic pains, and as a tincture for intermittent fever (Gaur *et al*., 2013). Milky white latex is sticky and the plant is popular amongst the common population because of this peculiarity. This is mildly poisonous and is considered as one of the plant toxins in Ayurveda. Though toxic, this latex can be purified and put to use as a very effective antidote as well as herbal medicine.

This paper focuses on the investigation of phytochemical constituents present in *C. gigantea* leaves and some of its biological activities such as acute toxicity, antitumor activity and larvicidal activity.

Materials and Methods

Sampling of Plant Material and Identification

The leaves of *C. gigantea* were collected from Ngawon Kyung Tha Street, Pathein Township, Ayeyarwady Region, Myanmar, during June to August, 2017. The collected leaves sample was identified as *Calotropis gigantea* R.Br. (Mayo) according to the authorized botanist from Department of Botany, Pathein University. The collected fresh leaves samples were washed and air dried at room temperature for two weeks and the dry leaves were ground into powder and then stored in air tight container.

Preliminary Phytochemical Investigation of the Leaves of *C. gigantea*

In order to classify the types of organic constituents present in samples, preliminary phytochemical tests on leaves samples were carried out according to the appropriate reported methods.

Preparation of Crude Extracts by Direct Extraction Method for Screening of Some Biological Activities

Each dried powdered sample (50 g) was extracted with 150 mL of pet-ether (60-80 °C) for 6 h by using Soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give the respective PE crude extract. Ethyl acetate, 95 % ethanol, and watery extracts were also prepared by similar manner mentioned in above procedure. Each extract was dried at normal pressure on a water bath and stored under refrigerator for screening some bioactivities.

Screening of Some Bioactivities of the Crude Extracts of *C. gigantea*
(a)Determination of acute toxicity of the leaves of *C. gigantea*

To determine the consequent of the plant and to determine the nature and degree of toxicity produced by these extracts and to find out the medium lethal doses (LD_{50}) of the extracts, acute toxicity test was done. Usually the acute lethality of a compound is determined on the basis of deaths occurring in 24 h but the survivors should be observed for at least seven days in order to detect delayed effects. In this study, acute toxicity effect of ethanol extract of *C.gigantea* leaves (two doses) were determined on albino mice at Laboratory Animal Services Division, Department of Medical Research (DMR), Yangon.

Acute toxicity of different doses of ethanol extracts of *C.gigantea* (Mayo) leaves was evaluated by the methods of OECD Guidelines for the Testing of Chemicals 423 (OECD, 1998; OECD, 2000). According to the test description, total number of 18 adult female albino mice, weighing (25-30g) were selected and divided into three groups. Each group contained six animals. They were maintained in accordance with the recommendation of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N. 85-23, revised 1996) for studies involving experimental animals. They had free access to feed and clean drinking water during the three days acclimatization period and throughout the experimental period. They were fasted for 18 h before giving the extracts. Group (A) mice were orally administrated with 95 % EtOH extract of *C.gigantea* (Mayo) leaves 2000 mg/kg dose. Group (B) mice were given orally with 95 % EtOH extract of *C.gigantea* (Mayo) leaves 5000

mg/kg dose. Group (C) mice performed as a control group and they were treated with clean water and normal animal food. All groups of mice were kept in the three mouse cages in the separated room at the room temperature of 26 º C. After administration of extract, each group of animals was observed first 6 h continuously for mortality and behavior changes. Then the animals each was checked in 24 h for fourteen days (Table 1).

(b) Screening of antitumor behaviors of crude extracts by potato crown gall test or potato disc assay method

The antitumor activity screening of different crude extracts such as pet-ether, ethyl acetate, 95% ethanol, and watery extracts of leaves of *C. gigantea* was carried out against *Agrobacterium tumefacien* by Potato Crown Gall test or Potato Disc Assay method at the Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar (Moh Moh Aye, 2009). Fresh, disease free potato tubers were obtained from local market and transferred within 48 h to the laboratory. Tubers of moderate sizes were surface-sterilized by immersion in 50 % sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 1.5 cm wide cork borer. And, 2 cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 0.5 cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20 mL poured into each petri dish). Each plate contained three discs. The procedure was done in the clean bench in the sterile room. 50 mg, 100 mg and 150 mg of each extract was separately dissolved in 2 mL of dimethyl sulphoxide (DMSO); the solution was filtered through millipore filters (0.22 μm) into a sterile tube. 0.5 mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens* strain (48 h culture containing $3\text{-}5\times10^{9}$cells/mL) were added aseptically. Controls were made as mentioned above procedure.

Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. The process of cutting the potatoes and incubation must be conducted within

30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for 12 days. After incubation, Lugol's solution (I_2-KI) was added and the tumors were counted with a microscope and compared with control. The antitumor activity was examined by observation of tumor produced or not.

(c) Determination of larvicidal activity of the leaves of *C. gigantea* (Mayo)

Based on preliminary tests, dilutions of crude samples were prepared with same type of purified water. Different periods. Dead larvae were identified when the larvae failed to move after probing with a needle in the cervical region. Mortality rate was recorded after 24 h daily. The lethal concentrations of LC_{50} and LC_{90} values were calculated after 24 h using dose-effect probity.

Results and Discussion

The results of preliminary photochemical analysis of the leaves extract of *C. gigantea* showed that the leaves contain alkaloids, steroids, saponins, glycosides, tannins and α-amino acids. But reducing sugars, flavonoids, starch, phenolic compounds, carbohydrates, terpenoids and cyanogenic glycosides were found to be absent in the leaves of Mayo.

Acute Toxicity

Acute toxicity screening of 95% EtOH extract *C. gigantea* leaves, was done with the dosage of 2000 mg/kg and 5000 mg/kg body weight in each group of albino mice. The condition of mice groups were recorded after fourteen days administration (Table 1). The results show that no lethality of the mice was observed up to fourteen days administration. Each group of animals were also observed still alive and did not show any visible symptoms of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death.

Table 1: Acute Toxic Effect of Ethanol Extract of *C. gigantea* (Mayo) Leaves on Albino Mice Model after Two Weeks Administration

No.	Group	Extract Administration	Dosage (mg/kg)	No. of death	% of death after 14 days
1	Group A	95% EtOH	2000	Nil	0
2	Group B	95% EtOH	5000	Nil	0
3	Group C	No administration	Nil	Nil	0

Screening of Antitumor Behaviours of Some Crude Extracts from the Leaves of Mayo

The antitumor behaviours screening of different crude extracts **(**PE, EtOAC, EtOH and watery extracts) of the leaves of Mayo were carried out against *Agrobacterium tumefaciens* by Potato Crown Gall test or Potato Disc Assay method (Table 2 and Figures 2 and 3). All of the tested crude extracts of the leaves of Mayo were found to exhibit antitumor activity against *A. tumefaciens.*

Table 2: Comparsion among Antitumor Behavioues of Different Concentrations of Crude Extracts from the Leaves of Mayo

Extracts	Concentration (mg mL^{-1})	Antitumor Activity	
		Days-5	Days-7
PE	25	+	+
	50	+	+
	75	+	+
EtOAc	25	+	+
	50	+	+
	75	+	+
95% EtOH	25	+	+
	50	+	+
	75	+	+
H_2O	25	+	+
	50	+	+
	75	+	+

(+) = Exhibit antitumor activity (–) = No antitumor activity

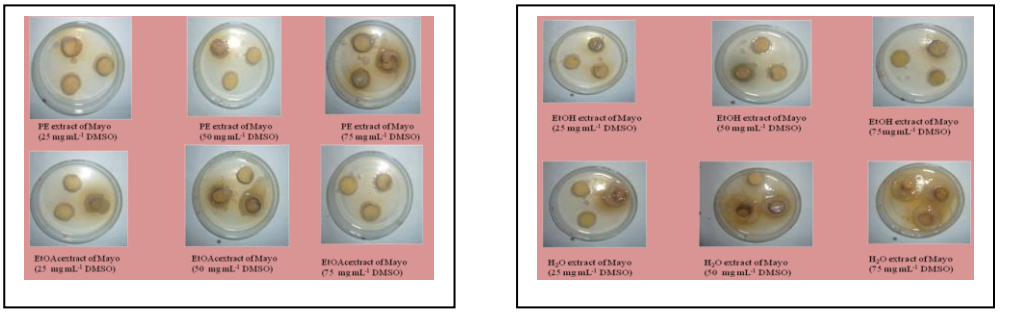

Figure 2: Antitumor behaviours of different concentrations of PE and EtOAc, EtOH and H_2O extracts of Mayo leaves on days 5

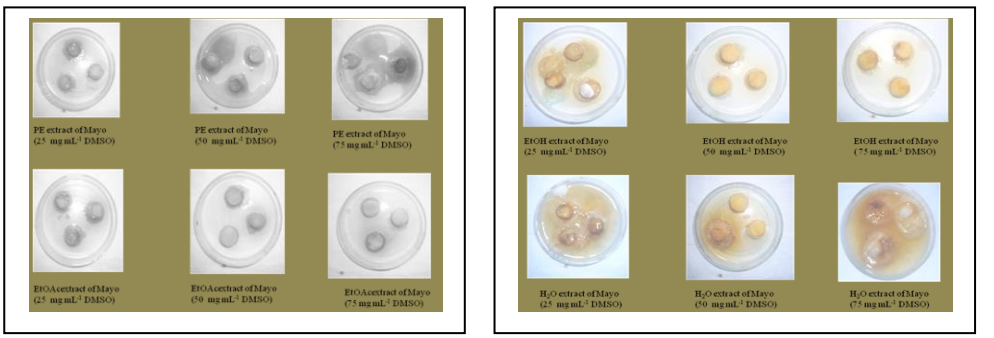

Figure 3: Antitumor behaviours of different concentrations of PE and EtOAc, EtOH and H_2O extracts of Mayo leaves on days 7

Larvicidal Activity of the Leaves of *C. gigantea* (Mayo)

According to the larvicidal activity study, the highest knockdown effect at the concentration of 0.2 g /100 mL was found to be 83.20 % in EtOH extract, followed by 82.80 % in EtOAc and 81.60% in PE extracts. The lowest effect was found as 67.20 % in H_2O extract was shown in Table 3. Moreover, the lowest knockdown of *Aedes* larvae was found at the concentration of 0.0125 g /100 mL of H_2O extract. The highest mortality rate of *Aedes* larvae at the concentration of 0.2 g /100 mL was found to be 95.60 % in EtOAc extract and followed by 94.00 % mortality in EtOH and PE extracts. In 0.1 g /100 mL concentrations of EtOH, EtOAc and PE extracts, mortalities were found as 86.80 %, 85.60 % and 80.40 % respectively as shown in Table 4.

Table 3: Knockdown Effect (within 60 min) of Different Dilutions of *C.gigantea* on the Instars *Aedes aegypti* Larvae

Concentration (g / 100mL)	Number of Knockdown and % Knockdown effect of different extracts			
	EtOH	PE	H_2O	EtOAc
0.20	208 (83.20)	204 (81.60)	168 (67.20)	207 (82.80)
0.10	172 (68.80)	168 (67.20)	146 (58.40)	172 (68.80)
0.05	137 (54.80)	119 (47.60)	76 (30.40)	187 (74.80)
0.025	103 (41.20)	67 (26.80)	48 (19.20)	118 (47.20)
0.0125	35 (14.00)	34 (13.60)	18 (7.20)	51 (20.40)
Control	0 (0)	0 (0)	0 (0)	0 (0)

Total Larvae-250

Table 4:Mortality Effect (within 24 h) of Different Dilutions of *C. gigantea* Leaves Extracts against 3rd and 4th Instars *Aedes aegypti* Larvae

Concentration (g/100mL)	Number of Mortality and % Mortality			
	95% EtOH	PE	H_2O	EtOAc
0.2	235 (94.00)	235 (94.00)	186 (74.40)	239 (95.60)
0.1	217 (86.80)	201 (80.40)	139 (55.60)	214 (85.60)
0.05	168 (67.20)	154 (61.60)	96 (38.40)	177 (70.80)
0.025	117 (46.80)	106 (42.40)	54 (21.60)	139 (55.60)
0.0125	83 (33.20)	86 (34.40)	22 (8.80)	75 (30.00)
Control	0 (0)	0 (0)	0 (0)	0 (0)

Total Larvae-250

Although mortality of H_2O extract was 55.60 %, the lowest mortality rates of all extracts were observed at the concentration of 0.0125 g /100mL. The doses of 50 % mortality (LC50) and 90 % mortality (LC90) values of all extracts against 3th and 4th instars *Aedes* larvae are shown in Table 5. The lowest dose for 50% mortality was found 0.0235g of EtOAc extract concentration followed by 0.0249 g / 100 mL and 0.0277 g / 100 mL of EtOH and PE extract respectively. H_2O extract found in highest amount of dose 0.0791g / 100 mL concentration was needed for 50% mortality of 3rd and 4th instars *Aedes* larvae. The lowest dose (highest efficiency) for 90% mortality was 0.1224 g / 100 mL of EtOAC extract concentration followed by 0.1386 g / 100 mL of 95% EtOH in the highest amount of dose and it was 0.4771 g/ 100 mL concentration for 90% mortality of 3rd and 4th instars *Aedes* larvae in 100 mL water.

Table 5: Lethal Concentration (LC) Values of *C. gigantea* Leaves Extracts against 3rd and 4th Instars *Aedes aegypti* Larvae

Lethal Concentration	*C.gigantea* Leaves Extracts (g / 100 mL)			
	95% EtOH	PE	H_2O	EtOAc
LC_{50}	0.0249	0.0277	0.0791	0.0235
LC_{90}	0.1386	0.1661	0.4771	0.1224
Chi Square X^2	3.5203	10.7585	0.5489	2.2458
Df	4	4	4	4
P value	0.05	0.05	0.05	0.05

Conclusion

The present study on the leaves of *C. gigantea* provides the following information. The preliminary phytochemical investigation revealed the presence of alkaloids, steroids, saponins, glycosides, tannins and α-amino acids in the leaves of *C. gigantea*. But, reducing sugars, flavonoids, starch, phenolic compounds, carbohydrates, terpenoids and cyanogenic glycosides were found to be absent in the leaves of *C. gigantea*. The constituents such as alkaloids and steroids present in the sample may contribute to possess bioactivities such as antimicrobial, antioxidant, anticancer, antitumor,

antipyretic, and antiulcer properties in Mayo. According to the screening of acute toxicity activity of the 95% EtOH extract of leaves of Mayo, the results showed that no lethality of the mice was observed up to fourteen days after administration. Each group of animals was also observed still alive and did not show any visible symptoms. And then, leaves extract of *C. gigantea* may have good larvicidal activity. Moreover, due to its antitumor activity, leaves of *C. gigantea* may be used to prevent the diseases related to tumor and cancer. From the acute toxicity test on leaves of Mayo, leaf extracts were found to be free from acute toxic. This study provides the health at affordable cost and *C.gigantea* leaves have valuable medicinal properties and may be used safely.

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