INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES FROM THE LEAVES OF *CALOTROPIS GIGANTEA* (L.) W.P. AITON (MAYO GYI)

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Abstract

This research aims to evaluate the phytochemical tests, antioxidant, antimicrobial and cytotoxic activities from the leaves of *Calotropis gigantea* (L.) W.P. Aiton. The total phenol contents of watery extract (47.56 ± 0.1 mg GAE/g) and ethanol extract (64.18 ± 0.7 mg GAE/g) of the sample were tested by using Folin-Ciocalteu method. The total flavonoid contents of watery extract (33.49 ± 0.3 mg QE/g) and ethanol extract (35.47 ± 0.1 mg QE/g) of the sample were determined by aluminum chloride method. In addition, the tannin content (2.1 %) was determined by using titration method. From EDXRF investigation, *C. gigantean* sample contains high concentrations of organic compounds together with trace amounts of some elements. The antioxidant activity was done on the watery extract (IC_{50} = 42.37 µg/mL) and ethanol extract (IC_{50} = 33.67 µg/mL) by using DPPH assay. Then, antimicrobial activities of ethanol and watery extracts of the samples were investigated by agar well diffusion method using pathogenic microorganisms such as *Escherichia coli, Saccharomyces cerevisiae, Candida albicans, Agrobacterium tumefaciens and Salmonella typhi against test microorganisms* (13 - 15 mm inhibition zone diameters). Moreover, cytotoxicity of the ethanol extract (LD_{50} value of 1.76 µg/mL) was higher than watery extract (LD_{50} value of 8.57 µg/mL) of sample, determined by using brine shrimp lethality bioassay. According to these experimental results, the leaf of *C. gigantean* is safe and suitable to treat various diseases including cancer and aging.

Keywords: *C. gigantea*, antioxidant activity, cytotoxicity and antimicrobial activity

Introduction

Calotropis gigantea (L.) W.P. Aiton is usually called as "crown flower," and "giant milkweed". *C. gigantea* is commonly used in medicines such as asthma, colds, hacks and fever. *C. gigantea* is used to treat homeopathic, toothache, erasing, and disgorging. *C. gigantea* leaves has shown cytotoxic activity against human tumor containers. *Calotropin*, which was isolated from its roots, has antitumor activity, cytotoxic activity in leukemia and stomach cancer (Chakre, 2019).

Botanical Description

Botanical name	:	*Calotropis gigantea* (L.) W.P.Aiton
Genus	:	Calotropis
Species	:	*C. gigantea*
Family name	:	Apocynaceae
Myanmar name	:	Mayo Gyi
English name	:	Crown flower or giant milkweed
Parts used	:	Leaves
Medicinal uses	:	antitumor activity, cytotoxic activity, antibacterial activity, antioxidant activity, analgesic activity, anti-diarrhea activity, anti-inflammatory activity

Figure 1. *Calotropis gigantea* (L.) W.P. Aiton

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Materials and Methods

Sample Collection

The leaves of *C. gigantea* were collected from Yadanabon University Campus, Amarapura Township, Mandalay Region.

Phytochemical Screening

The phytochemical investigation of the sample was carried out by standard procedures (Harborne, 1984).

Determination of Mineral Elements

The elemental analysis of powder of *C. gigantea* leaves was determined by Energy Dispersive X-ray fluorescence (EDXRF) spectrometry using EDX-8000 instrument at Monywa University, Sagaing Region.

Determination of Total Phenol, Total Flavonoid and Total Tannin Contents of Ethanol and Watery Extracts from the Leaves of *C. gigantea*

The phenolic contents of watery and ethanol extracts of the leaves sample were determined by utilizing Folin-Ciocalteu reagent (FCR) method according to Song *et al.*, (2010). And the total flavonoid contents of watery and ethanol extracts were estimated by aluminium chloride method. Then, the whole tannin substance in tested sample was evaluated by titrimetric method of watery extract (Atanassova and Christova-Bagdassarian, 2009).

Screening of Antioxidant Activity of Ethanol and Watery Extracts from the Leaves of *C. gigantea*

Diphenyl-1-picrylhydrazyl (DPPH) may be a prevalent, fast, simple, and reasonable approach for the estimation of antioxidant properties that incorporates the utilization of the free radicals for surveying the potential of substances to serve as hydrogen suppliers or free-radical foragers (FRS). DPPH free radical scavenging activity was determined by UV-visible spectrophotometric method.

Screening of Antimicrobial Activity of Crude Extracts from the Leaves of *C. gigantea* by Agar Well Diffusion Method

The screening of antimicrobial activity of crude extracts such as ethanol and watery extracts of the leaves of *C. gigantea* were carried out by agar well diffusion method (Collins *et al*., 2004) at Department of Botany, Yadanabon University.

Determination of Cytotoxicity of Tested sample from the Leaves of *C. gigantea*

Brine shrimp lethality bioassay could be a quick and comprehensive bioassay for the bioactive compounds. The strategy utilizes *in vivo* lethality in a straight forward zoological living being (*brine nauplii*) as a convenient screen for screening and fractionation within the revealation of unused bioactive normal items (Hassan *et al*., 2015).

Results and Discussion

Phytochemical Constituents Present in the Leaves of *C. gigantea*

From the research work, the preliminary phytochemical tests, a variety of metabolites such as alkaloids, flavonoids, phenolic compounds, polyphenols, tannins, steroids, α-amino acids, carbohydrates, glycosides, terpenoids and saponins were found in tested sample.

Elemental Analysis of the Leaves of *C. gigantea*

According to EDXRF analysis, some mineral elements required for human beings such as Ca, K, Cu, S, P, Fe, Mn, Sr were observed to be present in plant sample.

Total Phenol, Total Flavonoid, and Total Tannin Contents of the Crude Extracts of *C. gigantea*

In the quantitative analyses of some phytochemical constituents, the total phenol contents of ethanol and watery extracts of *C. gigantea* leaves were found to be 64.18 ± 0.7 and 47.56 ± 0.1 mg GAE/g crude extract, respectively.

In addition, the total flavonoid contents of ethanol and watery extracts of the *C. gigantea* leaves were observed to be 35.47 ± 0.1 and 33.49 ± 0.3 mg QE/g crude extract, respectively. The total phenol and flavonoid contents of ethanol extracts were found to be greater than watery extracts of the sample. Total tannin content was 2.1 % in the tested sample.

Antioxidant Activity of the Leaves of *C. gigantea*

The antioxidant activity was determined in terms of radical scavenging ability of the EtOH and watery extracts of the sample by using the stable radical DPPH assay. These results are shown in Table 1. From these observations, ethanol extract of the *C. gigantea* leaves (IC_{50}= 33.67 μg/mL) possessed the better radical scavenging property than the watery extract (42.37 μg/mL).

Table 1. Percent Radical Scavenging Activity of Crude Extracts of *C. gigantea*

Tested samples	% RSA (mean ±SD) in different concentrations (μg/mL)					IC_{50} (μg/mL)
	6.25	12.5	25	50	100	
ethanol extract	29.97±0.2	36.42±0.2	44.35±0.2	62.22±0.1	94.16±0.1	33.67
watery extract	25.97 ± 0.2	30.57 ±0.6	41.92 ± 0.6	56.54±0.1	83.89±0.1	42.37
gallic acid (standard)	46.95±0.6	51.78±0.1	55.40±0.2	65.96±0.1	82.98±0.1	10.4

Antimicrobial Activity of Crude Extracts of the Leaves of *C. gigantea*

The antimicrobial activity of ethanol and watery extracts of selected sample was investigated by agar well diffusion against eight microorganisms. The results are described in Table 2.

According to experimental results, ethanol extract of sample showed low activity on four tested organisms and medium activity on *Escherichia coli* (13-15 mm inhibition zone diameters) and then, no activity on *Bacillus subtilis*, *Micrococcus luteus*, and *Pseudomonas fluorescens*.

Furthermore, watery extract of sample responded low activity on *E. coli* and no activity on other organisms. Ethanol extract of sample has higher antimicrobial activity than watery extract of sample. So, the selected sample, *C. gigantea* leaves may be used to treat diseases caused by harmful microorganisms.

Table 2. Antimicrobial Activity of the Ethanol and Watery Extracts of *C. gigantea*

Micro organisms	Inhibition zone diameter (mm)	
	Ethanol extract	Watery extract
A. tumefaciens (NITE 09678)	13	-
B. subtilis (IFO 90571)	-	-
C. albicans (NITE 09542)	13	-
E. coli (AHU 0255)	15	13
M. luteus (NITE 83297)	-	-
P. fluorescens (IFO 94307)	-	-
S. cerevisiae (NITE 52847)	13	-
S. typhi (AHU 9743)	13	-

Agar well – 10 mm; 10 mm–14 mm (low activity); 15 mm–19 mm (medium activity); above 20 mm (high activity); No activity (-),

Cytotoxicity of the Ethanol and Watery Extracts of the Leaves of *C. gigantea*

Brine shrimp cytotoxicity bioassay showed that the ethanol extract of sample was more harmful to brine shrimp than watery extract. The LD_{50} values of ethanol and watery extracts were 1.76 and 8.57 µg/mL, respectively. These results are indicated in Table 3.

Table 3. Cytotoxicity of Ethanol and Watery Extracts of the Leaves of *C. gigantea*

Sample	Dead % in different concentrations (µg/mL) of samples				LD_{50} (µg/mL)
	1	10	100	1000	
ethanol extract	44.7 ± 0.7	73.1± 0.1	85.5± 0.8	87.9± 0.4	1.76
watery extract	36.4 ±0.7	51.9± 0.7	77.6± 0.5	80.5 ± 0.4	8.57
*caffeine	0±0	0±0	9.6 ± 0.9	12.7 ± 0.4	>1000
** $K_2Cr_2O_7$	48.6 ± 0.2	73.1 ± 0.5	74.7 ± 0.1	100 ± 0.1	1.58

*Standard caffeine = negative control
**Standard $K_2Cr_2O_7$ = positive control

Conclusion

According to the preliminary phytochemical tests, different types of metabolites such as alkaloids, flavonoids, phenolic compounds, polyphenols, tannins, steroids, α-amino acids, carbohydrates, glycosides, terpenoids and saponins, were detected and starch, reducing sugar and protein were not detected in the leaves of *C. gigantea*. The total phenol contents and total flavonoid contents were higher in ethanol extract than the watery extract. The ethanol extract had more prominent antioxidant activity than that of the watery extract. Ethanol extract showed medium antimicrobial activity. The leaves of *C. gigantea* may possess strong anticancer activity due to their high cytotoxic effect. Therefore, the tested plant might be valuable in the formulation for the treatment of antitumor.

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