BIOACTIVITY STUDY OF *CLEOME BURMANNI* L.MERR. (TAW-HINGALA) AND *ELEUSINE INDICA* L.GAERTN.(SINNGO-MYET)

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Abstract

In the present investigation, two medicinal plants, *Cleome burmanni* L. (Taw-hingala) and *Eleusine indica* L. (Sinngo-inyet) were selected for some bioactivity studies. Antioxidant activity of crude extracts was investigated by using DPPH radical scavenging activity assay. According to the observed data, ethanol extract of Sinngo-myet (IC_{50} = 86.14 µg/mL) showed similar activity with that of Taw-hingala (IC_{50} = 86.18 µg/mL). The cytotoxicity of 70 % ethanol and watery extracts of Taw-hingala and Sinngo-myet was studied by brine shrimp cytotoxicity bioassay. Among the four crude extracts (ethanol and watery extracts from two samples), only ethanol extract of Taw-hingala showed strong cytotoxic effect on brine shrimp at LD_{50}= 1.50 µg/mL but the other crude extracts did not exhibit their cytotoxic effect up to the optimum dose of 1000 µg/mL. Antitumor activity of ethanol and watery extracts of Taw-hingala and Sinngo-myet was also tested on tumor produced bacterium using PCG (Potato Grown Gall) test. From this experiment, the text extracts from all samples were significantly found to inhibit the formation of tumor in the dose of 0.062 g/disc. From the result of screening of antiproliferative activity, it was observed that methanol extracts of the whole plant of Taw-hingala and Sinngo-myet showed mild antiproliferative activity of IC_{50} value at > 100 µg/mL for lung cancer, cervix cancer, breast cancer, normal human fibroblast, liver cancer, pancreatic cancer and pancreas ductal adenocarcinoma respectively.

Keywords*:* *Marne burmanni* L. Merr and *Eleusine indica* L. Gaertn., antioxidant activity, cytotoxicity, antitumor activity, antiproliferative activity

Introduction

Traditional medicinal plants namely *Cleome burmanni* L. Merr. (Taw-hingala) and *Eleusine indica* L. Gaertn. (Sinngo-myet) (Figure 1) were selected in this study. The genus *Cleome* (Capparaceae) is one such genus

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reportedly used in traditional systems of medicine (Bose *et al.,* 2007). *Cleome burmanni* is the most commonly occurring species of *Cleome.* Many species of *Cleome* such as *C.viscosa, C. gynandra, C.chelidonii,* found growing as roadside weeds are reportedly used in traditional systems of medicines. Reports on the phytochemical analysis and medicinal value of this plant are scare or almost absent. However, a preliminary phytochemical screening has shown that many phytochemicals is present in the various extracts of this plant (Sofowora, 1993). *Eleusine indica* (Sinngo-myet) belongs to the family Poaceae. This plant is a common herbage with long, narrow leaves and tubular culm, including cereals, bamboo, sugarcane, fodder grass, goose grass, wire grass etc. Family Poaceae is the largest of the world flora and contain a very wide range of chemical constituent. However, a large proportion of chemical work has been devoted (Sindhia and Bairwa, 2010). There was no scientific information antitumor activity and antiproliferative activity concerning these two plants having in Myanmar. This study intended to illustrate the scientific proof of Myanmar medicinal plants used as good remedies in the treatment of tumor and cancer.

(a) **(b)**

Figure 1: Photographs of (a) Taw-hingala (THG) (b) Sinngo-myet (SNM)

Brine shrimp toxicity test

Artemia found favour as a "standard" organism in toxicological assay, despite the recognition that it is too robust organism to be a sensitive indicator species in pollution research. *Artemia,* the brine shrimp, has extensive use a test organism and in some circumstances is an acceptable alternative to the toxicity testing of mammals in the laboratory. The fact that millions of brine shrimp are also easily reared has been an important help assessing the effects of environment on the brine shrimp under well controlled experimental condition (Lieberman, 1999).

Brine shrimp

Brine shrimp is a small fairly shrimp that lives in vine pool and is used as food for aquarium fish (Figure 2).The scientific classification of brine are as follows:

Scientific	:	*Artemia salina*
Family name	:	Artemiidae
Genus	:	*Artemia*
Marketing name	:	Sea-Monkeys
Species	:	*salina*
Common name	:	Brine shrimp

Figure 2: Image of brine shrimp *(Artemia salina)* (X 200)

Materials and Methods

Screening of Antioxidant Activity by DPPH Assay

DPPH (2, 2- diphenyl-1-picryl hydrazyl) free radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system. (Lee *et al,* 2003) In this experiment, the antioxidant activity of ethanol and watery extracts of two selected plant samples was studied by DPPI-1 free radical scavenging assay.

DPPH free radical scavenging activity was determined by UV-visible spectrophotometric method according to the procedure described by Marini-Bettolo *et al*.,(1981).The control solution was prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm by using UV-visible spectrophotometer. The percent radical scavenging activity was calculated by the following equation.

$$\% \text{ RSA} = [(A_{DPPH} - A_{Sample}) A_{Blank} / A_{DPPH}] \times 100$$

where,

% RSA = % radical scavenging activity

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50% reduction of initial absorbance of DPPH solution. IC_{50} (50% inhibitory concentration) values were calculated by linear regressive excel program.

Investigation of Cytotoxicity by Brine Shrimp Lethality Bioassay

Test solution (1 mL) was mixed with 9 mL of artificial sea water and placed in the chamber of ice cup. Alive brine shrimp (10 napulii) was taken with Pasteur pipette and placed into each chamber which was kept at room temperature for about 24 h. After 24 h incubation, the number of survival brine shrimp was counted and 50% lethality dose (LD_{50}) was calculated (Dockery and Tomkins, 2000). The control solution was prepared as the above procedure by using distilled water instead of sample solution.

Screening of Antitumor Activity

In this section, antitumor activity screening of 70 % ethanol, ethyl acetate, methanol extracts of the whole plant of Taw-hingala and Sinngo-myet were carried out by Potato Crown Gall (PCG) test (or) Potato Disc Assay (PDA) method, at Pharmaceutical Research Department, Ministry of Industry, Yangon.

Fresh, disease-free potatoes were purchased from a local market. Tubers of moderate size were surface-sterilized by immersion in 0.1 % sodium hypochlorite for 20 min. Ends were removed and the potatoes were soaked in 0.1% sodium hypochlorite for 10 min. A core of the tissue was extracted from each tuber with a surface-sterilized 1.0 cm cork borer. Pieces of 2 cm were removed from each end and discarded. The remainder of the cylinder was cut into 0.5 cm thick disc with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL distilled water, autoclaved for 20 min at 121 °C, 20 mL poured into each Petri dish. Each plate contained four potato discs and 4 plates were used for each sample dilution.

Sample (0.031, 0.062, 0.125, 0.250, 0.500 and 1 g) of crude extract were respectively dissolved in DMSO (2 mL) and filtered through millipore filters (0.22 mL) into sterilized tube. This solution (0.5 mL) was added to sterilize distilled water (1.5 mL) and broth culture of *A. tumefaciens* in PBS (2mL) was added. Controls were made in this way; DMSO (0.5 mL) and sterilized distilled water(1.5 mL) were added to the tube containing 2 mL of broth culture of *A. tumefaciens.* 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 plates were sealed with tape to minimize moisture loss and incubated at room temperature for three days at 27-30 °C. Tumors were observed on potato discs after 3 days under stereo-microscope followed by staining with Lugol's iodine (10 % K1 and 5 % 1_2) after 30 min and compared with control. The antitumor activity was examined by observation of tumor produced or not.

Investigation of Antiproliferative Activity

Antiproliferative activity of Taw-hingala and Sinngo-myet were studied *in vitro* using cancer cell lines at Division of Natural Product Chemistry, Institute of Natural Medicine, University of Toyama, Japan.

The *in vitro* antiproliferative activity of the methanol crude extract was determined by the procedure described. Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and incubated in the respective medium at 37 °C under 5 % CO_2 and 95 % air for 24 hr (Dahab and Afifi, 2007).

After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the tested samples wereadded. After 72 h incubation, the cells were washed with PBS and 100μg/mL of medium containing10 % WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured. The concentrations of the serial dilutions of the tested samples were 100, 10, 1μg/mL for crude extract and 10, 1, 0.1 mM for positive control. Cell viability was calculated from the mean values of the data from three wells using the equation below and antiproliferative activity was expressed as the IC_{50} (50 % inhibitory concentration) value 5-fluorouracil was used as positive control.

$$(\%)\ \text{Cell viability} = 100 \times \frac{\left\{\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})}\right\}}{\left\{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}\right\}}$$

Results and Discussion

Cytotoxicity

The cytotoxicity of ethanol and watery crude extracts was expressed in terms of mean ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose). Among the four crude extracts (watery and ethanol crude extract of two samples), only ethanol extract of Taw-hingala showed strong cytotoxic effect on brine shrimp at LD_{50} = 1.50 μg/mL, but the other crude extract did not exhibit cytotoxic effect to brine shrimp up to optimum dose of 1000 μg/mL (Table 1). The LD_{50} values of standard $K_2Cr_2O_7$ and caffeine are 43.741 μg/mL, and 1000 μg/mL, respectively.

These results revealed that the two selected plants were used to prepare one of the traditional medical formulation and were used to folk medicine as anticancer. The reported active (cytotoxic) plant in the study are worth of further pharmacological and medical studies in order to define what kind of antitumor activity they have and to isolate the natural active constituents, which are responsible for the activity.

Table 1: Cytotoxicity of Different Doses of Watery and Ethanol Crude Extracts of Taw-hangala and Sinngo-myet on *Artemia salina* (Brine Shrimp)

Tasted	Sample	No. of Dead Brine Shrimp (Mean± SEM) in various concentration (µg/mL)				
		1	**10**	**100**	**1000**	**LD_{50}µg/mL**
1	Watery Extract	1.50 ± 0.86	2.00 ± 1.15	2.50 ± 1.44	4.00 ± 2.31	> 1000
2	EtOH Extract	2.50 ± 1.44	3.50 ± 2.02	4.50 ± 2.60	5.50 ± 3.17	1.50
3	Watery Extract	1.50 ± 0.86	2.00 ± 1.15	3.00 ± 1.73	4.00 ± 2.31	> 1000
4	EtOH Extract	1.00 ± 0.57	2.00 ± 1.15	2.50 ± 1.44	3.50 ± 2.02	> 1000
5	$K_2Cr_2O_7$	0.67 ± 0.66	2.00 ± 2.00	10.00 ± 0.00	10.00 ± 0.00	43.74
6	Caffeine	0	2.33 ± 1.20	3.00 ± 0.58	5.00 ± 1.16	1000

Antioxidant Activity

From antioxidant screening test, 70 % ethanol and watery extracts of Taw-hingala showed mild antioxidant activity and their IC_{50} values were found to be 86.18 and 34.05 µg/mL respectively (Figure 3). In addition, 70 % ethanol and watery extracts of Sinngo-myet also showed mild antioxidant activity with IC_{50} values of 100.61 and 86.14 µg/mL respectively. The lower the IC_{50} values, the higher the antioxidant activities was found. Therefore, 70 % ethanol extracts are more potent than watery extracts in both samples and all of the extracts showed lower antioxidant activity than standard vitamin C (IC_{50}= 11.4 µg/mL)

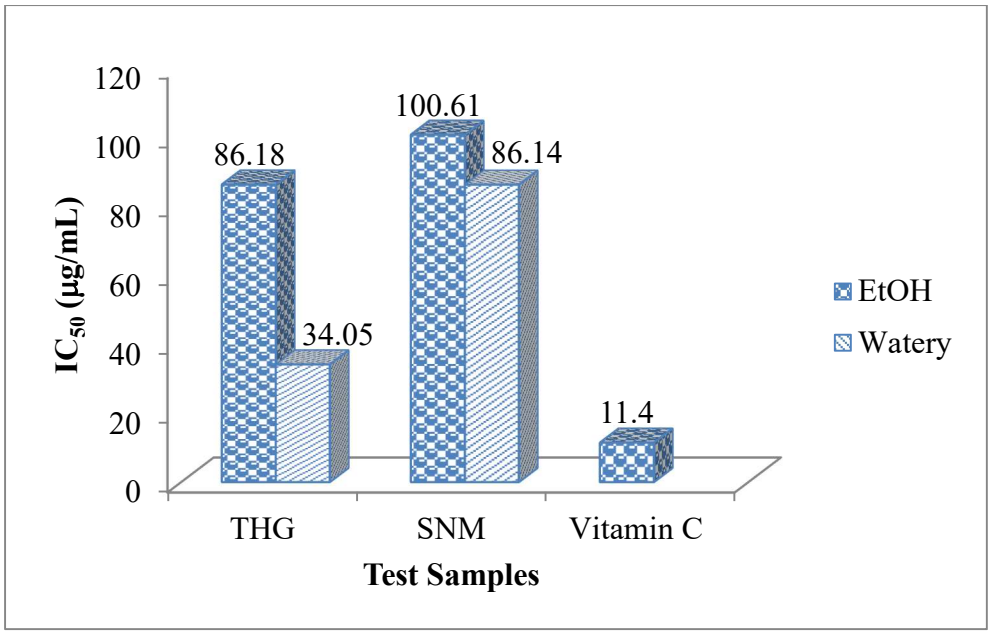

Figure 3: IC_{50} values of crude extract of Taw-hingala and Sinngo-myet

Antitumor Activity

The antitumor activity of watery and ethanol extracts of both plant samples were investigated by using PCG test with the isolated bacterium *A. tuniefaciens.* From this experiment, it was found that watery and 70 % ethanol extracts of both plants sample were effective in preventing the tumor formation with the doses of 1 to 0.062 g/disc in *vitro* potato disc assays (Table 2).

Table 2: Results of Antitumor Activity Screening on 70 % EtOH and Watery Extractsof Taw-hingala and Sinngo-myet

No	Test samples	Concentrations (g/disc)	Tumor Inhibition
1	EtOH (THG)	1	-
		0.500	-
		0.250	-
		0.125	-
		0.062	-
		0.031	+
2	Watery (THG)	1	-
		0.500	-
		0.250	-
		0.125	-
		0.062	-
		0.031	+
3	EtOH (SNM)	1	-
		0.500	-
		0.250	-
		0.125	-
		0.062	-
		0.031	+
4	Watery (SNM)	1	-
		0.500	-
		0.250	-
		0.125	-
		0.062	-
		0.031	+
5	Standard compound (Taxol)	0	+

(-) = tumor appear

(+) = no tumor appear

Screening of Antiproliferative Activity on Cell Lines

Antiproliferative activity is the activity relating to a substance used to prevent or retard the spread of cells, especially malignant cells, into surrounding tissues. Antiproliferative activity were studied *in vitro* using human cancer cell lines. Screening of antiproliferative activity of methanol extracts of Taw-hingala and Sinngo-myet was done by using seven human cancer cell lines. The cell lines used were A 549 (human lung cancer), Hela (human cervix cancer), MCF 7 (human breast cancer), and WI-38 (normal human fibroblast), HePG2 (human liver cancer). PSN 1 (human pancreatic cancer) and PANC1 (pancreas ductal adenocarcinoma).

From the results, it was observed that methanol extracts of the whole plant of Taw-hingala and Sinngo-myet showed mild antiproliferative activity at IC_{50} value = > 100 μg/mL for lung cancer, cervix cancer, breast cancer, normal human fibroblast, liver cancer, pancreatic cancer and pancreas ductal adenocarcinoma (Table 3).

Table 3: Antiproliferative Activity of Methanol Crude Extracts of Taw-hingala and Sinngo-myet against Various Types of Cancer Cell Lines

Samples	IC_{50} (μg/mL) of Various Samples against Tested Cell Lines						
	A549	**Hela**	**MCF7**	**WI-38**	**HePG2**	**PSN1**	**PANC1**
THG (Methanol extract)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
SNM (Methanol extract)	> 100	> 100	> 100	> 100	> 100	> 100	> 100

A 549 = human lung cancer
Hela = human cervix cancer
MCF 7 = human breast cancer
WI-38 = normal human fibroblast
HePG2 = human liver cancer
PSN1 = human pancreatic cancer
PANC1 = pancreas ductal adenocarcinoma

Conclusion

In the present investigation, antioxidant activities of 70% ethanol and watery extracts of Taw-hingala and Sinngo-myet were determined by DPPH assay method using UV spectrophotometer. According tothe observed data, ethanol extract of Sinngo-myet (IC_{50} = 86.14 μg/mL) showed similar activity with watery extract of Taw-hingala(IC_{50} = 86.18μg/mL).

The cytotoxicity of 70 % ethanol and watery extracts of Taw-hingala and Sinngo-myet was studied by brine shrimp cytotoxicity bioassay. Among the four crude extracts(watery and ethanol extract from two samples), only ethanol extract of Taw-hingala showed strong cytotoxic effect on brine shrimp at LD_{50}= 1.50 μg/mLbut the other crude extracts did not exhibit their cytotoxic effect up to the optimum dose of 1000μg/mL.

Antitumor activity of ethanol and watery extracts of Taw-hingala and Sinngo-myet was also tested on tumor produced bacterium using PCG (Potato Grown Gall) test and all of the test extracts from all samples were significantly found to inhibit the formation of tumor in the dose of 0.062 g/disc. From the screening of antiproliferative activity, it was observed that all of methanol extracts of the whole plant of Taw-hingala and Sinngo-myet showed mild antiproliferative activity of IC_{50} value at > 100 μg/mL for lung cancer, cervix cancer, breast cancer, normal human fibroblast, liver cancer, pancreatic cancer and pancreas ductal adenocarcinoma respectively.

Acknowledgement

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

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