Evaluation of Phytochemical Constituents and Some Bioactivities from Flowers of *MelastomaMalabathricum*L. (Nyaung-Ye-O-Pan)

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Abstract

The present study concerned with the screening of some pharmacological activities such as cytotoxicity, antitumor and antiproliferative activities from flowers of *Melastomamalabathricum* L. (Nyaung-ye-o-pan, NYOP). The preliminary phytochemical results showed that the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids in flowers of NYOP. Cytotoxicity of watery and ethanol extracts of NYOP flowers were investigated against *Artemiasalina*(Brine Shrimp). All of these extracts showed cytotoxic effect in the range of LD_{50} 2.29 to 2.80 μg/mL. Antitumor activity was also investigated by using PCG (Potato Crown Gall) test. In this antitumor activity, various crude extracts of NYOP flowers in different concentration (0.05, 0.1, 1.5 g/mL) showed the inhibition of tumor formation. Moreover, antiproliferative activity of human cancer cell lines, A 549 (Lung cancer) and MCF 7 (Breast cancer), HeLa (Cervix cancer), WI 38 (Fibroblast cancer), Hep G2 (Liver cancer) was screened by using Cell Counting Kit 8 (CCK 8 assay.) According to the experimental results, ethanol extracts of NYOP flowers were not found any potency in antiproliferative activity against A 549, MCF 7,WI 38, HeLa and Hep G2 human cancer cell lines. But watery extract of NYOP flowers was found to exhibit against MCF 7.

Keywords: *Melastomamalabathricum*L., phytochemicals, cytotoxicity, antitumor and antiproliferative activity

Introduction

Plants are used not only for food but also in traditional herbal medicine. Medicinal plants have long played in the treatment of diseases all over the world. Medicinal plants are a source for a wide variety of natural antioxidants and are used for the treatment of diseases throughout the world. Medicinal plants have also been a reliable source for preparation of new drugs. Nowadays, researchers more than before are dependent on medicinal plants for discovery of new drugs with fewer side effects (Rafieian-kopaei, 2012).

In this study, our attention has been focused on *Melastomamalabathricum* L. that belongs to the family Melastomataceae and it is called Nyaung-ye-o-pan (NYOP) in Myanmar. It is native to India, China, Japan, Cambodia, Myanmar, Malaysia, Nepal, Philippines, Thailand and Vietnam (Nadkarni, 2000).It is a medicinal plant with a height of 2-3 ft. It is ever green erect shrub, much branches shrub. The stem of the plant having4-sided to substerte, generally bristle, covered with small rough scales and reddish in color. The leaves of the plant are long, narrow and pointed at the both ends. The flowers grow in 5 to 10 clusters and 5 petals. On rare occasions, *M. malabathricum* consists of 3 varieties, dark-purple petals, light pink-magenta petals

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and (the rare variety) white petals (Susanti*et al*., 2007). The fruits are classified as berries and when they are ripe, they break open irregularly to reveal the soft, dark purple, sweet but rather astringent-tasting pulp and numerous orange seeds. The seeds are tasteless and can be eaten and they stain the tongue black (Koya, 2008). Other medicinally usesare to treat ulcers, gastric ulcer, scar, pimple and black spot at skin (Lohezic-Le Devehat*et al*., 2002). The flowers of*M. malabathricum* are also used as a nervous sedative and for hemorrhoidal bleeding. The leaves and flowers are useful for the treatment of cholera, diarrhoea, prolong fever, dysentery, leucohorrea, wounds and skin diseases and for the preparation of gargle (Perry, 1980).

Materials and Methods

Plant materials

The flowers of NYOP were collected from Hlar-ka-myin Village, Hpa-an Township in Kayin State. The plant was identifiedby authorized botanist, at Botany Department, Hpa-an University, Myanmar. After cleaning and drying at room temperature, each of the dried samples was ground into powder and stored separately in air-tight containers.Each powder sample was used for screening of phytochemical constituents and some biological activities. The various crude extracts of NYOP flowers were used for some pharmacological activities such as cytotoxicity, antitumor and antiproliferative activities.

Chemicals

Ferric chloride,potassium iodide, picric acid, sodium hydroxide, ninhydrin, α-napthol, sulphuric acid,lead acetate, acetic anhydride, iodine, bromocresol green, 1% gelatin, ethanol, magnesium ribbon, hydrochloric acid, chloroform, petroleum ether, potassium dichromate, caffeine, sodium chloride, sodium hypochlorite, dimethyl sulphoxide, agar powder, potassium iodide, phosphate buffer saline, fetal bovine serum, trypsin, non-essential amino acid, sodium pyruvate and CCK-8 kit/water soluble tetrazolium salt were used.

Instruments

96 well plate, aluminum foil, a stirrer, an autoclave, eppendrof tubes, an incubator, haemacytometer, microscope, vibrator, multipipette (1000 µL and 200 µL) UV- spectrophotometer (UV- 7504 KWF, China), shaker and electric balance.

Phytochemical Investigation

The dried powdered samples were used to chemical tests for the presence of phytochemical using standard procedure (Tin Wa, 1972, Trease and Evans, 1980, Shriner *et al*., 1980, Harbone, 1984, Marini-Bettole*et al*., 1981, Robinson, 1983, Vogel, 1966).

Screening of Cytotoxicity

The screening of cytoxicityof crude extracts such as watery and EtOHextracts of NYOP flowers were carried out by Brineshrimp Lethality Assay according to the procedure described by Dockery and Tomkins, 2000. The brine shrimp (*Artemiasalina*) was used for this assay. Brine shrimp cysts (0.5 g) were added to the 1 L of artificial sea water. The container was placed near a lamp and supplied O_2 for 24 hours. After 27 hours incubation, hatching of brine shrimp cysts occurred and the

alived brine shrimp (napulii) were ready to be used for cytotoxicity testat Department of Chemistry, University of Yangon, Myanmar.

Screening of Antitumor Activity

The antitumor activity was screened by Potato Crown Gall (PCG) or Potato Disc assay (PDA) method (Coker *et al*., 2003). In this study, watery and EtOH extracts of NYOP flowers were also used.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 an additional 10 min. A core of the tissue was extracted from each and discarded. The remainder of the cylinder was cut into 1.0 cm thick discs with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL deionized distilled water, autoclaved for 20 min at 121 ºC, 20 mL poured into each Petri dish). Each plate contained four potato discs and 4 plates, were used for each of the sample solution.

Sample (0.05, 0.10, 0.15 g) was individually dissolved in DMSO (1 mL) and filtered through Millipore filters (0.22 µm) into sterile tube. This solution (0.5 mL) was added to sterile distilled water (1.5 mL), and broth culture of *A. tumefaciens* in PBS (2 mL) was added. Controls were made in this way; DMSO (0.5 mL) and sterile distilled water (1.5 mL) were added to the tube containing 2 mL of broth culture of A. tumefaciens. By using a sterile disposal pipette, 1 drop (0.05 mL) each from these tubes was used to inoculate each potato disc by spreading it over the disc surface. After inoculation, Petri dishes were sealed by film and incubated at 27~30 ºC for 3 days. Observation was made on appearance of tumors on potato discs after 3 days under stero-microscope followed by staining with Lugol's iodine (10 % KI and 5 % I2) after 30 min and compared with control. The antitumor activity was examined by observation of tumor produced or not on the potato discs.

Screening of Antiproliferative Activity

Antiproliferative activity of ethanol and watery extracts of NYOP flowers were investigated in *vitro* by using cancer cell lines at Division of Natural Product Chemistry, Institute of Natural Medicine, and University of Toyama, Japan. Five cancer cell lines such as A 549 (Lung cancer), MCF 7 (Breast cancer), WI 38 (Fibroblast cancer), Hep G2 (Liver cancer) HeLa (Cervix cancer) were used in this study. α-Minimum essential medium with L-glutamine and phenol red (α-MEM, Wako) were used for cell cultures. All media were supplemented with 10 % fetal bovine serum (FBS, sigma) and 1 % antiobioticantimycotic solution (Sigma). For MCF 7 cell, 1 % 0.1 M non-essential amino acid (NEAA, Gibco) and 1 % 1 mM sodium pyruvate (Gibco) were also supplemented. The*vitro*antiproliferative activity of the crude extracts was determined by the procedure described by Win *et al.* (2015). Briefly, each cell line was seeded in 96-well plates (2 × 103 per well) and incubated in the respective medium at 37°C under 5 % CO_2 and 95 % air for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the tested samples were added. After 72 h incubation, the cells were washed with PBS and 100 µL of medium containing 10 % 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 crude extracts were 200, 100, 10 µg/ mL and 10, 1, 0.1 mM for positive control were prepared by serial dilution. 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 (5FU) was used as positive control.

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

Results and Discussion

Preliminary Phytochemical Investigation

According to the phytochemical test results, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids were present in flowers of NYOP. However, cyanogenicglycosides were absent in this sample. From these results,it can be seen that the NYOP flower samples might contain potent bioactive secondary metabolites.

Cytotoxicity of the Watery and EtOH extracts of NYOP flowers

The cytotoxicity of watery and ethanol extracts from the NYOP flowers was evaluated by brine shrimp cytotoxicity bioassay. The cytotoxicity of crude extracts was expressed in term of mean ± SEM (standard error mean) and LD_{50} (50% Lethality Dose) and the results are shown in Table 1. In this experiment, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well-known toxic in this assay and caffeine is a natural product. According to these results, all of the tested samples have cytotoxic effect.

Table 1.Cytotoxicity of Watery and Ethanol Crude Extracts fromNYOP Flowers

Crude extracts	% of Dead brine shrimp (Mean±SEM) in various concentrations(μg/mL)				LD_{50} (μg/mL)
	1	10	100	1000	
Watery	46.47± 0.58	63.33± 0.58	83.33± 0.58	100.00± 0	2.80
EtOH	43.57± 0.53	70.00± 0.58	90.00 ± 0.58	100.00 ± 0.58	2.29
*Caffeine	0.00± 0.00	0.00± 0.00	9.58± 0.57	12.73± 0.57	>1000
*$K_2Cr_2O_7$	36.57± 0.57	53.33± 0.58	66.67± 0.57	76.67± 0.57	8.21

*Used as cytotoxic standard

Antitumor Activity of the Watery and EtOH extracts of NYOP flowers

Antitumor activity of watery and ethanol extracts of NYOP was investigated by potato crown gall (PCG) assay as it is a precious information that showed antitumor activity of the tested samples by their inhibition of the crown gall formation that was induced in wounded potato tissues by *Agrobacterium tumefaciens*. The antitumor results of tested samples were described in Table 2.According to these results, it was found that both of watery and ethanol extracts of NYOP flowers inhibited tumor formation with the dose of 0.05, 0.10 and 0.15 g/mL.

Table 2. Results of Antitumor Activity of Watery and Ethanol Crude Extracts from NYOP Flowers

Crude Extracts	Concentration of sample (g/mL)	Tumor*
Control	0.00	+
	0.05	-
Watery	0.05	-
	0.10	-
	0.15	-
EtOH	0.05	-
	0.10	-
	0.15	-

*(+) Tumor appeared, *(-) No Tumor appeared

Antiproliferative activity of the Watery and EtOH extracts of NYOP flowers

Antiproliferative activity of watery and ethanol extracts of NYOP flowers was evaluated in *vitro* by using CCK 8 assay. Forantiproliferative activities, ethanol and watery extracts from the NYOP flowers was tested by using five human cancer cell lines such as A 549 (Lung cancer), MCF 7 (Breast cancer), WI 38 (Fibroblast cancer), Hep G2 (Liver cancer) HeLa (Cervix cancer). Antiproliferative activity was expressed as the IC_{50} (50 % inhibitory concentration) value. 5-Fluorouracil was used as positive control. The antiproliferative activity of crude extracts is summarized in Table 3. According to the experimental results, ethanol extracts of NYOP flowers were not found any potency in antiproliferative activity against A 549, MCF 7,WI 38, HeLaand Hep G2 human cancer cell lines. But watery extract of NYOP flowers was found to exhibit against MCF 7 (IC_{50} = 58.20 µg/mL).

Table 3. Results of Antiproliferative Activity of Watery and Ethanol Crude Extracts from NYOP Flowers

Tested samples	IC_{50} (µg/mL) of various samples against tested cell lines				
	A 549	MCF 7	WI 38	HeLa	Hep G2
Watery	>100	58.20	>100	>100	>100
EtOH	>100	>100	>100	>100	>100
*5FU	10.2	11.5	5.6	6.93	9.3

*5 FU = 5 fluorouracil

Conclusion

From the overall assessment concerning with the investigation of phyotochemicals, and biological activities from the flowers of *M.malabathricum*(Nyaung-ye-o-pan, NYOP), the following inferences could be deduced.The preliminary phytochemical screening in this study revealed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenol compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids in flowers of NYOP. But cyanogenic glycosides were absent in this sample.According to brine shrimp lethality bioassay, the LD_{50} values of watery and ethanol extract of flowers of NYOP were found to be 2.80 and 2.29 µg/mL respectively. From these results, the effect of cytotoxicity was found in the flowers of NYOP. Thisindicated the presence of potent cytotoxic and probably antitumor components of these samples.

The antitumor activity of watery and ethanol extracts from the flowers of NYOP were screened by Potato Crown Gall (PCG) assay. According to this assay, all the extracts showed significant tumor inhibition. Therefore, the flowers of NYOP possessed effective antitumor agent. For antiproliferative activity, five cancer cell lines such as A 549 (Lung cancer cell line), MCF 7 (Breast cancer cell line), WI 38 (Fibroblast cancer cell line) HeLa (Cervix cancer cell line) and Hep G2 (Liver cancer celline) were used for this assay. Among these five cancer cell lines, the watery extracts of NYOP flowers showed antiproliferative activity especially for breast cancer cell lines. The watery extracts of NYOP flowers exhibited significant inhibitory effects by comparing standard 5 FU but the ethanol extracts did not show antiproliferative activity.

Acknowledgements

I would like to acknowledge to Professor Dr. Ni Ni Than, (Professor and Head), Chemistry Department, University of Yangon, for their kind encouragement and supervisions, and DrKhin Chaw Win, Lecturer, Chemistry Department, University of Yangon, for close supervisions, invaluable suggestions, helpful advice, patient guidance and encouragement.

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