Comparative Study of Phytochemical Constituents and Antioxidant and Anti-Proliferative Activities of Rhizomes of *Dioscorea alata* L. and Fruits of *Piper longum* L.

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Abstract

Medicinal plants have shown tremendous potentials for the development of the new drug molecules for various serious diseases. Our country, Myanmar has a rich source of indigenous medicinal plants, which have been used as folk medicines for a long history. In this study, *Dioscorea alata* L. and *Piper longum* L. have been chosen for study of antioxidant and anti-proliferative activities. The screening of phytochemical constituents was carried out by reported methods. The antioxidant activity of ethanol and watery extracts of the two selected plants was evaluated by 2, 2 – diphenyl – 1- picryl hydrazyl free radical scavenging assay observing that the IC_{50} values of ethanol and watery extracts of *D. alata* (7.28 µg/mL and 7.32 µg/mL) and that of ethanol and watery extracts of *P. longum* (43.52 µg/mL and 64.28 µg/mL), respectively. The selected plant, *D. alata* has higher potency on antioxidant activity than *P. longum*. The anti-proliferative activity or cytotoxicity of methanol extract of the rhizomes of *D. alata* and fruits of *P. longum* was evaluated by MTT assay using Hep G2 (human liver cancer cell).

Keywords: *Dioscorea alata* L., *Piper longum* L., phytochemical constituents, antioxidant activity, anti-proliferative activity

Introduction

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. They can be used not only for treatment of diseases but also as potential material for maintaining good health and conditions. In addition, the medicinal values of these plants depend on bioactive phytochemical constituents that produce definite physiological action on the human body. This study intended to show the scientific proof of Myanmar medicinal plants used as good remedies in the treatment of cancer. In this study, the two Myanmar indigenous medicinal plants, *Dioscorea alata* L. and *Piper longum* L**.** have been chosen to study bioactive phytoconstituents and also for their antioxidant and anti-proliferative activities.

Botanical Aspects of *Dioscorea alata* L. and *Piper longum* L.

***Dioscorea alata* L.**

Family	:	Dioscoreaceae
Genus	:	Dioscorea
Species	:	alata

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Botanical Name : *Dioscorea alata* L.
English Name : Water yam, greater yam
Myanmar Name : Myauk U
Common Names : White yam, Water yam, Greater yam, or simply yam
Part used : Rhizomes
Distribution : Southeast Asia, West India, West Africa, Myanmar

***Piper longum* L.**

Family : Piperaceae
Genus : Piper
Species : Longum
Botanical Name : *Piper longum* L.
English Name : Indian Long Pepper
Myanmar Name : Peik-chin
Common Names : Long pepper, Lendi Peepar, ant pipers
Part used : Fruits
Distribution : Myanmar, Indian, North Afria, Indonesia, Malaysia and Southeast Asia

Materials and Methods

Collection and Preparation of Plant Samples

The rhizome of *Dioscorea alata* L. was collected from Thapaung Township, Ayeyawady Region and the fruit of *Pipper longum* L. was collected from Natalin Township, Bago Region. They were identified at Botany Department, University of Yangon. The collected samples were washed with water, made into small pieces and allowed to air - dried at the room temperature and then grounded by grinding mill. The dried powdered samples were stored in the air-tight container for chemical and biological investigation.

Preliminary Phytochemical Investigation

Screening of preliminary phytochemical constituents was carried out on the dried powdered sample with a view to investigate the presence or absence of primary metabolites and secondary metabolites, such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, organic acids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids (M-Tin Wa, 1972). s

Investigation of Antioxidant Activity

Scavenging of DPPH (2,2-diphenyl-1-1-picryl hydrazyl) free radical is the basic of a common antioxidant assay. This assay is based on electron-transfer that produces a violet solution in ethanol. This free radical is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanol solution. This assay provides an easy and rapid way to evaluate antioxidant by spectrometry. Therefore, DPPH free radical scavenging assay has been chosen to evaluate the free radical scavenging effectiveness of phytochemicals present in crude extracts of *D. alata* and P. *longum* (Marinova and Batchvarov, 2011).

The control solution, prepared by mixing 1.5 mL of 0.002 % DPPH solution and1.5 mL of ethanol, and the sample solution, prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution, were incubated at room temperature and shaken on shaker for 30 min. After incubation, the absorbance of different concentrations (0.625, 1.25, 2.5, 5, 10, 20 g/mL) of tested sample was measured at 517 nm. Absorbance measurements were used to calculate percentage of radical scavenging activity (% RSA) by the following equation:

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

where,

% RSA = % radical scavenging activity of test sample
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 (g/mL) that result in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

Determination of Cytotoxicity or Anti-Proliferative Activity by MTT Assay

The cytotoxicity or anti-proliferative activity of methanol extract of rhizomes of *D. alata* and fruits of *P. longum* was determined against human liver cancer cell line, Hep- G2 by 3- (4, 5- dimethylthiazoyl - 2)- 2, 5- diphenyl tetrazolium bromide (MTT) assay at the College of Pharmacy and Natural Products Research Institute, Seoul National University, Seoul (Bahuguna *et al*., 2017).

Cytotoxic activity of crude extract was screened in 1 x 10^4 cells/well seeded in a 96 well plate (30 mm) and incubated in CO_2 incubator for 24 h for attachment. Different concentrations of MeOH extract (0 – 300 µg mL^{-1}) solutions were freshly prepared in DMSO and treated with the prepared cell medium and then kept for 24 h. Thereafter, 10 µL of MTT solution (5 mg mL^{-1}) were added to each well and mixed thoroughly to dissolve the dye crystals, and then incubated in darkness at 37 ºC for 4 h. The culture medium was discarded. The formazan crystals were solubilized by adding 100 µL DMSO per well and then mixed by gently shaking for 10 min. The amount of MTT – formazan is proportional to the number of living cells and the absorbance was measured in a microplate reader at 595 nm. The fractional absorbance was calculated by the following equation:

$$\% \text{ Cell survival} = \quad \text{x } 100$$

The 50% inhibition concentration (IC_{50}) was determined as the lowest concentration which reduced cell growth by 50% in treated compared to untreated culture.

Results and Discussion

Phytochemical Constituents of *Dioscorea alata* L. and *Piper longum* L.

According to the experimental results, alkaloids, α-amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, steroids, organic acids and terpenoids were found to be present in *D. alata* but tannins, reducing sugars and cyanogenic glycosides were absent in this sample. And then, another selected plant, *P. longum* contained all tested phytoconstituents; alkaloids, α-amino acids, carbohydrates, glycosides, starch, saponin, steroids, organic acids, tannins, reducing sugar, phenolic, flavonoids, cyanogenic glycosides and polyterpenes.

Therefore, this study may provide the valuable scientific base for the use of herbs in the traditional medicine.

Antioxidant Activity of Crude Extracts of *Dioscorea alata* L. and *Piper longum* L.

In determining the antioxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a stable free radical from DPPH tends to capture hydrogen from the antioxidant. Due to its free radical, the ethanolic DPPH solution is violet and absorbance is measured at 517 nm. The colour changes upon neutralization of this free radical from violet to pale yellow by daylight. The decolouration of the initial colour is proportional to the test substances having antiradicalizing power.

It was found that the larger % RSA indicates the higher antioxidant activity. In contrast, the lower IC_{50} value indicates the more effective antioxidant activity. The antioxidant activity is expressed as % radical scavenging activity (% RSA). The results of % RSA of two crude extracts of the rhizome of *D. alata* and fruit of *P. longum* are tabulated in Table 1 and 2 and Figure 3 and 4. Furthermore, their respective IC_{50} values are shown in Table 3.

According to the results, the ethanol extract of *D. alata* (IC_{50} = 7.28 µg/mL) was found to be more potent antioxidant power than other extracts; watery extract of *D. alata* (7.32 µg/mL), ethanol extract of *P. longum* (IC_{50} = 43.52 µg/mL) and watery extract of *P. longum* (64.28 µg/mL). Therefore, the antioxidant potency of ethanol and water extracts of *P. longum* were concluded to be weak by compared with the potency of ethanol and water extracts of *D. alata*.

Table 1. Radical Scavenging Activity (%RSA) of Ethanol and Watery Crude Extracts of *D. alata* and Ascorbic acid

Sample	%RSA ± SD at Different Concentrations (µg/mL)					
	0.625	1.25	2.5	5	10	20
EtOH	13.46	18.77	26.93	40.61	61.12	90.91
	0.00	0.01	0.01	0.03	0.01	0.00
H_2O	18.60	22.44	28.93	41.92	59.25	65.64
	0	0.01	0.00	0	0.00	0.02

Figure 1. A plot of % RSA vs. concentrations of ethanol and watery extracts of the rhizomes of *D. alata*

Table 2. Radical Scavenging Activity (%RSA) of Watery and Ethanol Crude Extracts of *P. longum*

Sample	%RSA ± SD at Different Concentrations (µg/mL)					
	6.25	12.5	25	50	100	200
EtOH	22.62	26.74	37.72	54.29	67.48	75.53
	0.00	0.00	0.01	0.00	0.00	0.00
H_2O	20.64	23.75	34.22	45.87	60.33	65.78
	0.00	0.00	0.01	0.01	0.01	0.00

Figure 2. A plot of % RSA vs. concentrations of ethanol and watery extracts of the fruit of *P. longum*

Table 3. IC_{50} Values of Methanol Extracts of the rhizome of *D. alata* and fruit of *P. longum*

Samples	extract	IC_{50} (g/mL)
D. alata	95 % ethanol	7.28
	Watery	7.32
P. longum	95 % ethanol	43.52
	Watery	64.28

Anti-proliferative Activity of *Dioscorea alata* L. and *Piper longum* L.

The methanol extracts of the rhizome of *D. alata* and *P. longum* were used to evaluate the anti-proliferative activity or cytotoxicity by MTT assay using Hep G2 (human liver cancer cell). This activity is measured in terms of cells viability and IC_{50} value (inhibitory concentration at which 50 % cells are died). DMSO is used as a standard. The methanol extract of *P. longum* (IC_{50} = 87.7 μg/mL) was found to possess more anti-proliferative potency than that of *D. alata* (IC_{50} = 195.21 μg/mL) against Hep G2 (human liver cancer cell). Above this concentration, the cell mortality rate is increased and below this, the cell survival rate is higher. However, the methanol extracts of both selected plants have very weak anti-proliferative activity by comparing with the IC_{50} value. If the crude extract has IC_{50} less than 20 μg/mL, it is considered as an active constituent against cancer cells which is followed by the standard National Cancer Institute (NCI) criteria (Vinod *et al*, 2014). The results are shown in Table 4.

Table 4. Anti-proliferative Activity of Methanol Extracts against Human Liver Cancer Cell Lines (Hep G2)

Sample	Cell viability ± SD at different concentrations (g/mL)			IC_{50}(μg/mL)
	50	100	200	
MeOH –extract (*D. alata*)	0.8435 ± 0.0479	0.6808 ± 0.0549	0.4908 ± 0.0416	195.21
MeOH – extract (*P. longum*)	0.7113 ± 0.0453	0.4312 ± 0.0638	0.3082 ± 0.0501	87.7

Conclusion

This study may provide the results of phytochemical constituents which are responsible for antioxidant and anti-proliferative activities. According to the results, both crude extracts of *D. alata* contribute to higher in free radical scavenging activity than that of *P. longum.* However, the methanol extract of *P. longum* was found to possess higher anti-proliferative activity against Hep G2 than that of *D. alata.* Therefore, the selected traditional medicinal plants, *D. alata* and *P. longum* may be used in the prevention of free radical reactions and cell proliferation as necessary.

Acknowledgements

The authors would like to thank to Dr Ni Ni Than (Professor and Head), Department of Chemistry, University of Yangon, for her kind encouragement.

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