Evaluation of Antimicrobial Activity and Antioxidant Capacity of *Peperomia pellucida* L. Kunth

Moh Moh Aye[1], Kyi Kyi Khine[2]

Abstract

One of the Myanmar traditional medicinal plants, *Peperomia pellucida* L. Kunth (Kyauk-thin-pone), was selected for this research work. The present work was designated to study the preliminary phytochemical investigation, nutritional values, antimicrobial, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities. Determination of nutritional values has also been carried out by AOAC method. The antimicrobial activity of pet-ether, $CHCl_3$, EtOH, EtOAc, MeOH and H_2O extracts was determined by agar well diffusion method against six species of microorganisms. EtOAc extract showed highest activity on *Escherichia coli* (20 mm) and pet-ether extract do not show antimicrobial activity on all tested microorganisms. The TPC of the ethanol and watery extracts were contained considerable equal amount (37.18 ± 0.01 µg GAE/mg and 38.48 ± 0.01 µg GAE/mg). The TFC of the ethanol extract contain higher contents (70.36 ± 0.01 µg GAE/mg) than watery extract (59.45 ± 0.01 µg GAE/mg). From the screening of free radical scavenging activity by DPPH assay, it was found that EtOH extract (IC_{50} = 90.63 µg/mL) showed the higher activity than H_2O extract (IC_{50} = 125.74µg/mL). Therefore, Kyauk-thin-pone plant may be used as natural antioxidant and antimicrobial agent.

Keywords: *Peperomia pellucida*, antimicrobial activity, total phenolic content, total flavonoid content, antioxidant activity

INTRODUCTION

Natural products from plants, animals and minerals are the basis for treating human diseases. Medicinal plants are presently in demand and their acceptance is increasing progressively. Without plants, humans and other living organisms cannot live in a way living should be (Fatemeh, *et al*., 2018). Medicinal plants constitute the basis of primary health care for a majority of the population and are a critical source of income for many rural people particularly in area near forests. They are a source of primary health care for more than 80% of the population in developing countries who are dependent on traditional systems of medicine (Pandey, 2017).*Peperomia pellucida* L. Kunth (Kyauk-thin-pone) plantwas selected for this research to investigate some biological activities. Scientifically which is known as *Peperomia pellucida* L. Kunth and its plant family is*Piperaceae*. Myanmar name is Kyauk-thin-pone. *Peperomia pellucida* is a slender herb (reaching 30-50 cm in length) with straight and succulent stem and is cosmopolitan in distribution. Leaves are opposite and alternate, up to 2.5 × 2 cm, ovate-deltoid, obtuse to acute at apex. Leaves are thin, fleshy, smooth, membranous when dry, 5-7 nerved from the base. Petiole is up to 1.5 cm long. Spikes are terminal and leaf-opposed, up to 5 cm long. Flowering occurs more or less throughout year. Fruits are ribbed and reticulate, minute in size and almost dry.Kyauk-thin-pone plant has been reported to possess antioxidant property, antitumor, antibacterial, analgesic and anti-inflammatory activities. Chemical constituents present in Kyauk-thin-pone are alkaloids, phenolic compounds, flavoids and essential oil.The present study deals with investigation of phytochemical, antimicrobial, total phenolic content andantioxidant activities from Kyauk-thin-pone plant.

[1] Dr, Lecturer, Department of Chemistry, Sittway University
[2] Dr, Associate Professor, Department of Chemistry, Sittway University

MATERIALS AND METHODS

Sample Collection

The samples was collected from Sittway Township, Rakhine State. After collection, the scientific name of *Peperomia pellucida* L. Kunth was identified by authorized botanist at the Department of Botany, Sittway University.The collected sample was clean and dried. The air dried sample was made up to powder in electric grinder and stored in air-tight container to prevent moisture changes and other contamination.

Phytochemical Investigation

A phytochemical is a natural bioactive compound found in plant foods that works with nutrients and dietary fiber to protect against diseases. Phytochemical investigation was carried out to know the types of phytoorganic constituents present in Kyauk-thin-poneplant by test tube method (Robinson, 1983; M-Tin Wa, 1970; Vogel, 1996; Harborne, 1984; Marini-Bettolo, 1981).

Deterimnatoin of Nutritional Values

The nutritional values such as moisture, ash, protein, crude fiber, ether extract (crude fat) and carbohydrate of Kyauk-thin-pone plant were determined by AOAC methodsat Myanmar Food Processors and Exporters Association (MFPEA) in Lanmadaw Township, Yangon, Myanmar (Pearson, 1981, AOAC, 2000, Anderson, 1984).

Screening of Antimicrobial Activity

Antimicrobial activity was studied by using agar well diffusion method in various solvents system on six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* at Fermentation Laboratory, Pharmaceutical Research Department, Ministry of Industry, Yangon (Finegold, 1978).

Determination of Total Phenolic Contents (TPC)

Total phenolic contents (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent (Rekha *et al*., 2012). Crude extract (0.01 g) was dissolved in 20 mL methanol to obtainconcentration of 500 µg/mL. 5 mL of Folin-Ciocalteu reagent was added to 0.5 mL of the extract and incubated at room temperature for 30 min. Next, 4 mL of 1MNa_2CO_3 was added and kept at room temperature for 15 min and absorbance of reaction mixture was measured at 760 nm by a UV-visible spectrophometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Standard calibration curve of gallic acid was prepared by dilution of the stock solution (1000 µg/mL) to obtain various concentrations (100, 50, 25, 12.50, 6.25, 3.125) µg/mL.Gallic acid is used as the reference standard compound and total phenolic contents was estimated as microgram gallic acid equivalent per milligram (GAE).

Determination of Total Flavonoid Contents (TFC)

Aluminum chloride colorimetric method was used to determine the total flavonoid contents in the plant extracts. This method is based on the determination of the flavonoid-aluminum complex between flavonoid of the crude extract and aluminum chloride.Crude extract (0.03 g) was dissolved in 100 mL methanol to obtain concentration of 300 µg/mL. Briefly, 1 mL of extract in methanol (6.25 - 100 µg/mL) was mixed with 1mL aluminum chloride in ethanol (20 µg/mL) and a drop of acetic acid. The resulting mixture was then diluted with ethanol to 25 mL. The

absorption at 415 nm was read after 40 min. A blank sample was prepared in similar fashion omitting the extract. The calibration curve of quercetin was plotted using the same procedure and the amount of total flavonoids was calculated from linear regression equation obtained from the curve $y = 0.0023x + 0.0879$, $R^2 = 0.9993$ and expressed as quercetin equivalents (QE) per gram of the plant extract (Rekha *et al*., 2012).

Screening of Antioxidant Activity

Antioxidant activity of ethanol and watery extracts of Kyauk-thin-pone plantwas carried out by determination of DPPH (1, 1-Diphenyl-2-picryl hydrazyl) free radical scavenging property using UV spectroscopic method (Marinova and Batchvarov, 2011).

2 mg of each test sample and 10 mL of ethanol was thoroughly mixed and the mixture solution was filtered and the filtrate was used as a stock solution. Desired concentrations (5, 2.5, 1.25 and 0.625 μg/mL) of sample solutions were prepared from this stock solution by dilution with appropriate amount of ethanol.

Sample solution was prepared by thoroughly mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of test sample solution in the brown bottle. The control solution was also prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of ethanol. The solutions were then allowed to stand at room temperature for 30 min.Ascorbic acid (Vitamin C) synthetic antioxidnat was used as a standard and ethanol without sample was employed as control.After that, absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Decrease in absorbance indicates increases in radical scavenging activity. Absorbance measurements were done in three times for each sample solution and the mean value so obtained were used to calculate % inhibition of oxidation by using the following equation. Then IC_{50} (50 % inhibition concentration) was determined by using linear regressive e:

$$\text{\% Inhibition of oxidation} = \frac{A_{DPPH} - (A_{sample} - A_{blank})}{A_{DPPH}} \times 100$$

A_{DPPH} = Absorbance of DPPH solution

A_{sample} = Absorbance of sample + DPPH solution

A_{blank} = Absorbance of solvent

RESULTS AND DISCUSSION

Phytochemical Investigation

From the results of phytochemical investigation, Kyauk-thin-pone plants showed the presence of alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, steroid, terpenoids, glycoside, starch and α-amino acids but tannins, cyanogenic glycosides and reducing sugar were absent.

Nutritional Values

The nutritional values of moisture, fiber, ash, protein, carbohydrate and energy content were found as shown in Table 1. As a result, the nutritional parameter of carbohydrate was rich and protein was present as major nutrient in Kyauk-thin-pone plant.

Table 1 Results of Nutritional Values from Kyauk-thin-pone Plant

No.	Types of Nutrients	Observed values
1	Moisture (%)	16.41
2	Ash (%)	21.33
3	Protein (%)	17.24
4	Crude fiber (%)	13.14
5	Fat (%)	3.16
6	Carbohydrate (%)	43.72
7	Energy value (kcal/100g)	272.28

Antimicrobial Activity

Screening of antimicrobial activity of various crude extracts such as pet-ether, $CHCl_3$, EtOAc, EtOH, MeOH and H_2O extracts from Kyauk-thin-pone plant was investigated by employing agar well diffusion method. In this study, the samples were tested on six species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species. The inhibition zone diameters of all crude extracts against six microorganisms tested are shown in Table 2.

According to these results, EtOAc extract showed highest activity on *Escherichia coli* (20 mm) and *Pseudomonas aeruginosa* (18 mm) and then *Bacillus subtilis* (13 mm). EtOH, MeOH, $CHCl_3$ and H_2O extracts exhibited lower antimicrobial activity (11-13 mm) than EtOAc extract on all tested organisms. PE extract do not showed antimicrobial activity on all tested microorganisms.

Table 2 Results of Antimicrobial Activity of Various Crude Extracts

No.	Type of Organisms	Diameter of Inhibition Zone (mm)					
		PE	$CHCl_3$	MeOH	EtOAc	EtOH	H_2O
1	*B.subtilis*	-	-	12	13	11	11
2	*S.aureus*	-	11	12	11	11	11
3	*P. aeruginosa*	-	-	-	18	-	-
4	*B. pumilus*	-	11	-	-	-	-
5	*Candida albicans*	-	-	-	12	-	-
6	*E.coli*	-	-	-	20	13	11

Agar well diameter = 10 mm, 10 mm ~14 mm = (+), 15 mm ~ 19 mm = (++), 20 mm ~ above = (+++), Not detected =(-)

Total Phenolic Contents

Total phenolic contents in the Kyauk-thin-pone plant extracts using the Folin-Ciocalteu's reagent is expressed as microgram of gallic acid equivalent per milligram of crude extract (µg GAE/mg). Gallic acid standard curve gave a straight line. The total phenolic contents of EtOH and watery extracts were found to be (37.18 ± 0.01)and (38.48 ± 0.00) µg GAE/mg respectively. Thus, The TPC of the ethanol and watery extracts were contained considerable equal amount. The results are recorded in Tables3, 4 and Figures 2, 3.

Total Flavonoid Contents (TFC)

Spectrophotometric method using Aluminum Chloride Colorimetric (ACC) methodwas used to determine the TFC. The standard quercetin solutionwas prepared by dilution with the different concentration of 100, 50, 25, 12.5 and 6.25 µg/mL.The standard quercetingave a straight line.The amount of total flavonoid contents was calculated from linear regression equation obtained from the curve y = 0.0023x + 0.0879, R^2 = 0.9993and expressed as µgQE/mg.The total flavonoids contents of EtOH and watery extracts were found to be(115.34 ± 0.01) µg QE/mg and (70.00 ± 0.00) µg QE/mg respectively. Thus, EtOH extract was more effective than watery extract.The results are recorded in Table 6, 7 and Figure 4, 5.

Antioxdant Activity

The antioxidant activity of ethanol and watery extracts of Kyauk-thin-pone plantwas studied by DPPH free radical scavenging assay. The resultant average % inhibition property values in different concentrations (400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) for all samples is tabulated in Table 8 and Figure 7and 8. From these figures, it can be seen that as the concentration of the samples increased, the respective % inhibition also increased.The antioxidative potential of sample can be determined by IC_{50} (50% inhibition concentration). These IC_{50} value for each extract was determined by linear regressive excel program and can also be obtained from the plot of % inhibition *vs* concentration of the samples. The IC_{50}values were found to be 90.625 µg/mL for EtOH extract and 125.74 µg/mL for watery extract. Since the lower the IC_{50}values, the higher the free radical scavenging activity, i.e., the higher the antioxidative property. Watery extract has the higher IC_{50}than that of EtOH extract. Therefore, the antioxidant potential of ethanol extract was found to be higher thanthat of watery extract. In addition, it was found that all of these extracts have the

Table 4 Absorbance of Various Concentrations of Standard Gallic Acid

Concentration (µg/mL)	Absorbance at 760 nm
3.125	0.201
6.25	0.221
12.50	0.237
25.00	0.284
50.00	0.402
100.00	0.598

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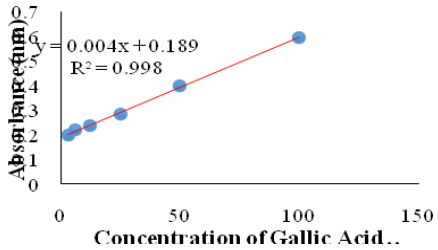
Table 5 Results of Total Phenolic Contents

Extracts	TPC (µg GAE/mg ± SD)
EtOH	37.18 ± 0.01
Watery	38.48 ± 0.01



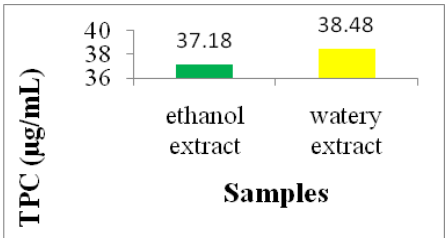


Table 5 Absorbance of Standard Compound Quercetin at λ_{max} 415 nm

No	Concentration of Quercetin (µg/mL)	Absorbance at 415 nm
1	3.125	0.092
2	6.25	0.104
3	12.50	0.118
4	25.00	0.145
5	50.00	0.198
6	100.00	0.314

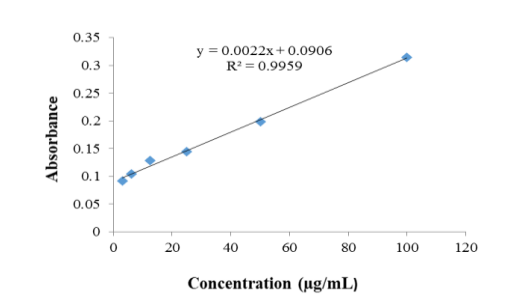


Figure 4 Standard calibration curve of quercetin concentration vs absorbance

Table 6 Results of Total Flavonoid Contents

Extracts	TFC (µgQE/mg ± SD)
EtOH	70.36 ± 0.01
Watery	59.45 ± 0.01

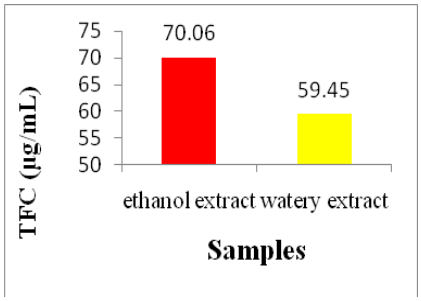


Figure 5 A bar graph of total flavonoid contents of ethanol and watery extracts

Table 8 Average % Inhibition of Oxidation and IC_{50} Values of Watery and Ethanol Extracts and Standard Ascorbic Acid

Extracts	Average % Inhibition (mean ± SD) in different concentration (µg/mL)							IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	400	
Watery	10.38 ± 0.40	15.54 ± 0.23	21.18 ± 0.47	29.31 ± 0.35	46.98 ± 0.23	58.71 ± 0.47	60.82 ± 0.56	125.74
Ethanol	25.19 ± 0.43	27.49 ± 0.16	33.25 ± 0.59	40.64 ± 0.34	52.16 ± 0.33	61.8 ± 0.33	64.61 ± 0.16	90.625

Samples	Average % Inhibition (mean ± SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	0.16	0.8	4.0	20.0	100	
Standard ascorbic acid	16.34 ± 2.33	39.20 ± 1.41	70.52 ± 2.59	88.09 ± 1.18	95.95 ± 3.70	1.9

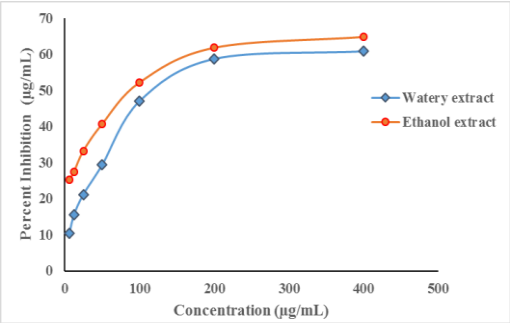


Figure 6 Percent inhibition of oxidation *vs* concentration of ethanol and watery extracts

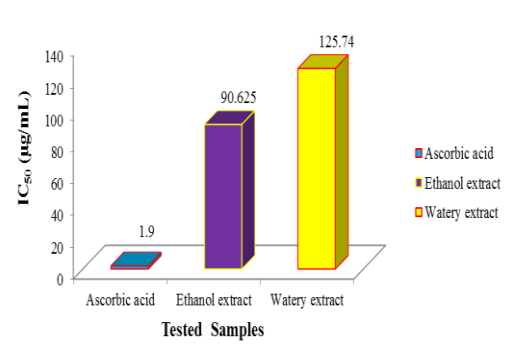


Figure 7 Bar graph of IC_{50} values of ethanol and watery extracts compared with standard ascorbic acid

CONCLUSION

The preliminary phytochemical tests on Kyauk-thin-pone plantrevealed the presence of alkaloids, α-amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, steroids and terpenoids. tannins, cyanogenic glycosides and reducing sugar were absent in the sample.Nutritional values were found to be 16.41% of moisture, 21.33 % of ash, 17.24 % of protein, 13.14 % of crude fiber, 3.16 % of fat, 43.72 % of carbohydrate and 272.28 kcal/100g of energy, based of dried sample. According to the results of antimicrobial activity, EtOAC extract showed highest acitivity on *E. coli* (20 mm) and *P. aeruginosa* (18 mm) and then *B. subtilis* (13 mm). EtOH, MeOH, $CHCl_3$ and H_2O extracts exhibited lower antimicrobial activity (11 – 13 mm) on all tested microorganisms. The total phenolic contents of EtOH and watery extracts were found to be (37.18 ± 0.01) and (38.48 ± 0.01) µg GAE/mg respectively. Thus, ethanol and watery extracts contain considerable equal amount of TPC.The total flavonoids contents of EtOH and watery extracts were observed to be (70.36 ± 0.01) µg QE/mg and (59.45 ± 0.01) µg QE/mg respectively. From the screening of free radical scavenging activity by DPPH assay, it was found that EtOH extract (IC_{50} = 90.625 µg/mL) showed the higher antioxidant activity than H_2O extract (IC_{50} = 125.74 µg/mL). The present research is therefore, EtOH and watery extracts of Kyauk-thin-pone plantmay be useful for the cure ofbacteria infections and oxidative stress related diseases.

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