EVALUATION OFTOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT ANDANTIOXIDANT ACTIVITY OF *PORTULACAOLERACEA* L. (MYET-HTAUK)

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This research is concerned with the phytochemical screening, nutritional values and antioxidant activities of *Portulacaoleracea* L. (Myet-htauk) plant. From the phytochemical investigation, Myet-htauk plant revealed the presence ofalkaloids, α-amino acids, carbonhydrates, glycosides, phenolic compounds, starch, saponins, flavonoids, steroids, terpenoids,reducing sugars but tannins andcyanogenic glycosides were absent.Nutritional values were determined by AOAC method. The whole plant of Myet-htauk contained higher amount of carbohydrate (36.98 %) and protein (23.9 %). The antioxidant activity of ethanol and watery extract was determined by DPPH assay method. Ethanol extract (IC_{50} value = 86.61 µg/mL) displayed higher antioxidant activity than watery extract (IC_{50} = 315.55 µg/mL). The total phenolic content was determined by the spectrophotometric method using Folin-Ciocalteu reagent. The total phenolic contents of ethanol and watery extracts were found to be 80.44 and 20.92 (µg GAE /mg), respectively. The total flavonoid content was measured by an aluminium chloride colorimetric method, the total flavonoidcontents of ethanol and watery extract were observed to be 164 and 34.9 (µg QE /mg). There was a strong correlation between antioxidant activity with total phenolic and total flavonoid content. In brief, all above scientific data indicated that the ethanol extract is more active than watery extract.

Keywords: Myet-htauk, antioxidant activity, DPPH, total phenolic content

INTRODUCTION

Portulacaoleracea L., commonly known as purslane, is a weed species belonging to the family Portulacaceae. This plant is very important because of its special medical function and all its therapeutic values,attributed to the presence of many biological active compounds which include flavonoids, alkaloids, coumarins, anthraquinone glycoside, cardiac glycoside, and high content of ω-3 fatty acids.It is known by the name 'purslane' in English, 'Rudravanti' in Hindi; 'Dahna' in Oriya and 'Nuner' in Kashmiri. (Loutfy, *et al*., 1984)

Purslanehas been recognized as the richest source of α- linolenic acid, essential omega-3 and 6 fatty acids, ascorbic acid, gluta-thione, α - tocopherol, and β-carotene. (Wenzel,*et al*., 1980)The stems and leaves are succulent and edible with a salty and acidic taste similar to spinach. Leaves and stems can be eaten cooked in soups and several dishes. Many varieties of purslane under many names grow in a wide range of climates and regions. It is an important component of green salad and its soft stem and leaves are used raw, alone, or with other greens. The plant is widely distributed in North Africa, Middle East, South Asia, Europe, America and Australia. It is native to India and Persia, while naturalized in America and used as a garden weed. (Anonymous, 2003)

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According to the literature,*Portulacaoleracea*possesses a wide spectrum of pharmacological properties, asneuroprotective, antimicrobial, antidiabetic, antioxidant, and antiinflammatory, anticancer, wound healing and antiulcerogenic; for these reasons, it is traditionallyand widely used for therapeutic purposes. (Chowdhary, *et al*., 2013)

In Rakhine State, Myet-htauk plant(Figure 1)is consumed as vegetableandhas been usedfor the treatment of dysentery, liver disease and boil.Natural foods certainly provide numerous health benefits. The present study was aimed to determine phytochemicals, nutritional and some biological properties such astotal phenolic contents, total flavonoids and antioxidant activity of *Portulacaoleracea* L. (Myet-htauk)grown in Rakhine State.

(a) *(b)*

Figure 1 Photographs of (a) plant (b) leaves and flowers of *Portulacaoleracea* L.

MATERIALS AND METHODS

Plant Materials

The aerial parts of*Portulacaoleracea* L.(Myet-htauk)used in this researchwas collected from Min-gan Quarter, SittwayTowship, Rakhine State, Myanmarin January 2019. The species was identified by the authorized botanist at Botany Department, Sittway University, Myanmar.The plant was washed thoroughly with water, chopped into small pieces and then dried under shade for a period of 15 days. The dried plant materials were ground into fine powder and stored in airtight bottles.

Preliminary Phytochemical Tests

Preliminary detection of phytochemical compounds present in Myet-htaukpowder sample was carried out according to the phytochemical methods (Evans and Furlong, 2003; Marini-Bettolo*et al*., 1981; M-Tin Wa, 1972; Robinson, 1983; Treaseand Evans, 1978; Harborne, 1993).

Determination of Nutritional Values

The determination of moisture content, ash content, protein content, fat content, fiber content and carbohydrate contents were carried out at Livestock and Irrigation Small Scale Industries Department, Ministry of Agriculture, Yangon. The procedures were performed by the standard methods (AOAC, 2000).

Preparation of Plant Extracts

The dried powdered sample (100g) was immersedin 500 mLof 95% ethanol for 7 days at room temperature with frequent agitation.The extracts were filtered and this procedure was carried out for three times. The total combined filtrate was concentrated by distilling and evaporated to obtain ethanol extract.Aqueous extract was also prepared by boiling 30 g of sample with 100 mL of distilled water for one hour and filtered. It was repeated for three times and the filtrates were combined followed by removal of the water to give aqueous extract, which was stored at 4 °C in an airtight container until further use.

Screening of Antioxidant Activity

Antioxidant activity of aqueous and ethanol extract of Myet-htauk was determined by DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). The control solution was prepared by mixing 1.5 mL of0.002 % DPPH solution and 1.5 mL of ethanolin the brown bottle. The sample solution was also prepared by mixing thoroughly1.5 mL of 0.002 %DPPH solutions and 1.5 mL of test sample solution. Similarly the blank solution was preparedbymixing 1.5 mL of test sample solution and 1.5 mL of ethanol.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 byusing UV- visible spectrophotometer.In this study, six different concentrations (12.5, 25, 50, 100, 200, 400 µg/mL) of each extract were prepared by serial dilution. Ascorbic acid was used as standard. The absorbance measurements were done in triplicate for each solution and then mean values were obtained by calculating percent inhibition of oxidation by the following equation. From the average value of % inhibition, 50% inhibition concentration (IC_{50}) were calculated by linear regressive excel program.

$$\% \text{ inhibition} = \frac{A_{DPPH} - (A_{test\ sample} - A_{blank}) \times 100}{A_{DPPH}}$$

Determination of Total Phenolic Contents

The total phenolic content of crude ethanol and watery extracts of Myet-htaukwas evaluated with Folin-Ciocalteu method. Sample containing polyphenols are reduced by theFolin-Ciocalteu reagent there by producing bluecolored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve.To prepare a calibration curve, 0.5 mL of(3.125, 6.25, 12.5, 25, 50, 100 µg/mL) aqueous gallic acid solutionswas mixed with 0.5mL methanol and 5 mL of Folin-Ciocalteu reagent (diluted ten-fold) in a beaker and incubated at 37ºCfor 30 min. After incubation for 5 min, 4 mL of 1M Na_2CO_3 was added to each sample solution. The sample was kept for 15 min at room temperature. The absorbance was measured

at 760 nm by a UV-visible spectrophotometer. The calibration curve was constructed by putting the value of absorbance vs concentration. A similar procedure was adopted for the extract as above described in preparation of calibration curve. All determinations were performed in triplicate.Total phenolic content was expressed as microgram ofgallic acid equivalent (GAE) per mg of extract (Singleton,*et al*., 1999andMaizura, *et al*., 2011).

Determination of Total Flavonoids Contents

The total flavonoid content of ethanol and watery extractsof Myet-htaukwas estimated by Aluminum Chloride Colorimetric Assay.(Lee*et al*., 2015)Quercetin (10 mg) was dissolved in 100 mL of methanol to obtained concentration 100µg/mL. This solution was twofold diluted with methanol to get various concentration (3.125, 6.25, 12.5, 25, 50, 100 µg/mL), respectively. 0.5 mL ofdifferent concentrations of standard quercetinor plantextracts solution was mixed with 1.5 mL of methanol, 0.1 mL of 1 % aluminium chloride solution, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The resultant mixture was mixed well and allowed to stand for 40 minutes at room temperature. Absorbance of the resulting solution was measured against reagent blank at 415 nm using spectrometricially. The experiment was done in triplicate. The concentrations of quercetin equivalent (QE) in the plant extracts were calculated by using the linear regression equation from standard calibration curve of quercetin. Total flavonoid contents (TFC) in the plant sample was expressed as microgram ofquercetin equivalent per milligram dry plant extract (µg QE/mg extract) (Maizura, *et al*., 2011).

RESULTS AND DISCUSSION

The phytochemical screening of *Portulacaoleracea* L. (Myet-htauk)revealed the presence of terpenoids, alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, steroids, terpenoids, glycosides, starch, α- amino acidsandreducing sugars but tannins and cyanogenic glycosides were absent.

The nutritional values of Myet-htaukwere determined by AOAC method.The determination of nutrient values showed that carbohydrate (36.98%) and protein (23.9 %)are present as major nutrient than others such as crude fiber (7.32%),fat (5.83 %), moisture (5.97%) and ash (3.41 %),respectively (Table 1).

The antioxidant activity of ethanol and aqueous extracts of Myet-htauk was studied by DPPH free radical scavenging assay. The ethanol extract (IC_{50}=86.61 µg/mL) is more effectivethan watery extract (IC_{50}=315.55µg/mL). The lower the IC_{50} value indicated the more effective antioxidant activity(Tables 2 and 3 and Figures 2 and 3).

Folin-Ciocalteu colorimetric method was used in determining the total phenolic content of Myet-htauk. A standard curve of solutions with known concentrations of gallic acid was used to estimate the concentrations of phenolic component of the sample extracts. Knowing the concentrations of the sample extracts with the use of the standard curves (Table 4 and Figure 4), total phenolic content in terms of gallic acid equivalents (GAE) was calculated as shown in Table 6 and Figure 6. The ethanol extracts(80.44µg GAE/ mg) contained higher amounts of the phenolics component as compared to the watery extracts(20.92µg GAE/ mg) because of their higher solubility in ethanol. Phenolic substances are responsible for the

antioxidant activity of plant materials which is gaining much interest these days because of their known health benefits.

As a basis quantitative determination, flavonoid contents of EtOH and watery extracts of Myet-htauk were determined using aluminium chloride in a colorimetric method. The results were derived from the calibration curve ($y = 0.0022x+0.0906$, $R^2 = 0.9959$) of quercetin (3.125-100 µg/mL) (Table 5 and Figure 5). Ethanol and watery extracts of Myet-htauk was found to be (164.00 µg QE/ mg) and (34.90 µg QE/ mg). In this experiment, ethanol extract possessed high flavonoid content than watery extract(Figure6 and Tables 6).

In brief, all the above scientific data may contribute to the utilization of the leaves and stems of Myet-htaukplant in Myanmar traditional medicinefor the treatment ofskin diseases, burns, wound infection, food poisoningand diarrhea.

Table 1Nutitional Values of *Portulaca oleracea* L. (Myet-htauk) plant

Sr No.	Type of nutrient	Contents (%)
1	Moisture	5.97
2	Ash	3.41
3	Protein	23.9
4	Crude fiber	7.32
5	Crude fat	5.83
6	Carbohydrate	36.98
	Energy value (kcal/100 g)	295.99

Table 2 Percent Oxidative Inhibition and IC_{50} Values of Ethanol and Watery Extracts of *Portulacaoleracea* L. (Myet-htauk)

Extracts	% Inhibition (mean ±SD) in different concentration (µg/ mL)						IC_{50} (µg/mL)
	12.5	25	50	100	200	400	
Watery	7.00± 6.54	7.52± 6.17	17.12± 0.39	27.63± 2.23	41.89± 3.53	55.9± 5.25	315.55
Ethanol	14.57± 1.04	21.68± 3.45	34.19± 2.70	55.78± 2.35	82.43± 0.15	95.20± 0.39	86.61

Table 3 Percent Oxidative Inhibition and IC_{50} Values of Standard Ascorbic acid

Standard	% Inhibition (mean ±SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	0.2	0.8	4	20	100	
Ascorbic acid	16.34± 2.3	39.20± 1.4	70.52± 2.6	88.09± 1.2	95.95± 3.7	1.9

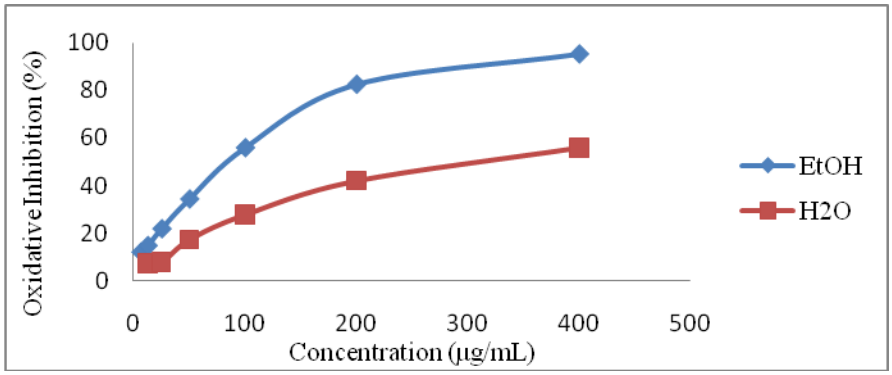

Figure 2 Plot of % oxidative inhibitionvsconcentrations (µg/mL) of watery and ethanol extracts of Myet-htauk

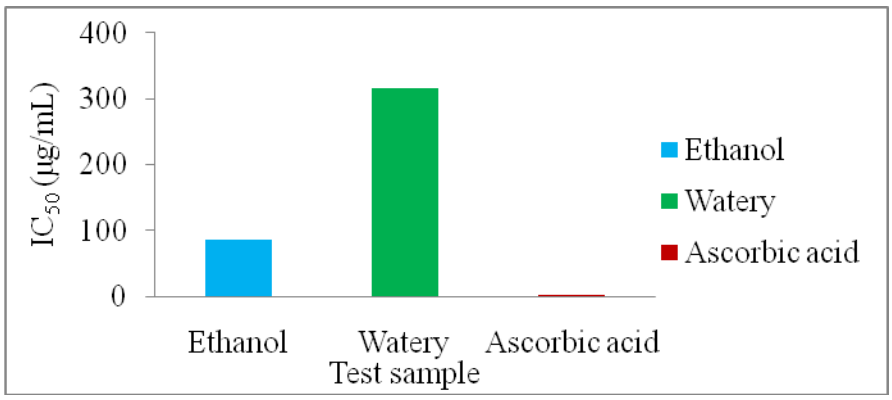

Figure 3 Bar graph of IC_{50} values of watery and ethanol extracts of Myet-htaukcomparedwith standard ascorbic acid

Table 4 Absorbance of StandardGallic Acid at λ_{max} 760 nm

No	Concentration of Gallic Acid (µg/mL)	Absorbance at 760 nm
1	3.125	0.201
2	6.25	0.221
3	12.5	0.237
4	25	0.284
5	50	0.402
6	100	0.598

Table 5 Absorbance of Standard 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	12.5	0.128
4	25	0.145
5	50	0.198
6	100	0.314

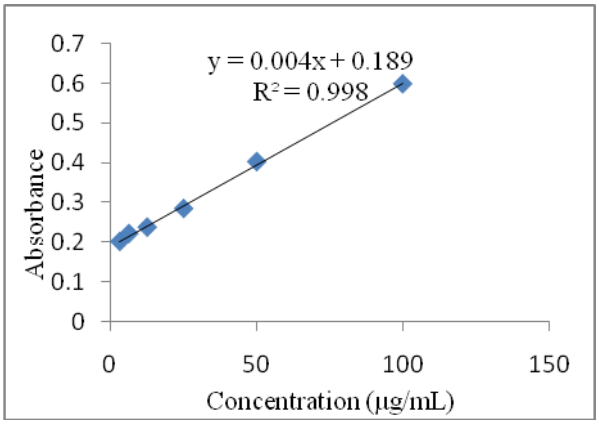

Figure 4 Standard calibration curve for gallic acid

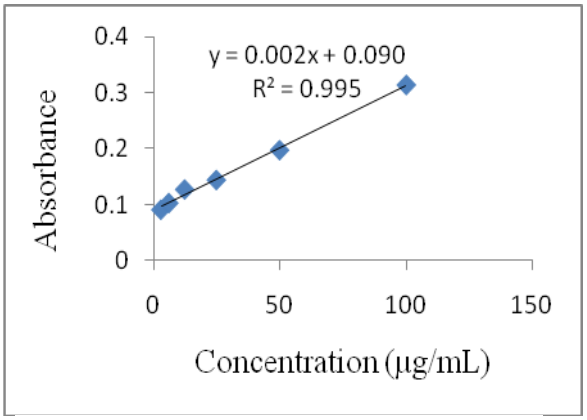

Figure 5 Standard calibration curve for quercetin

Table 6Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Crude Extracts

No	Extracts	TPC (GAEµg/mg of extract ± SD)	TFC (µg QE/mg of extract ± SD)
1	Ethanol	80.44 ±0.002	164.00 ± 0.024
2	Watery	20.92 ± 0.002	34.90 ± 0.001

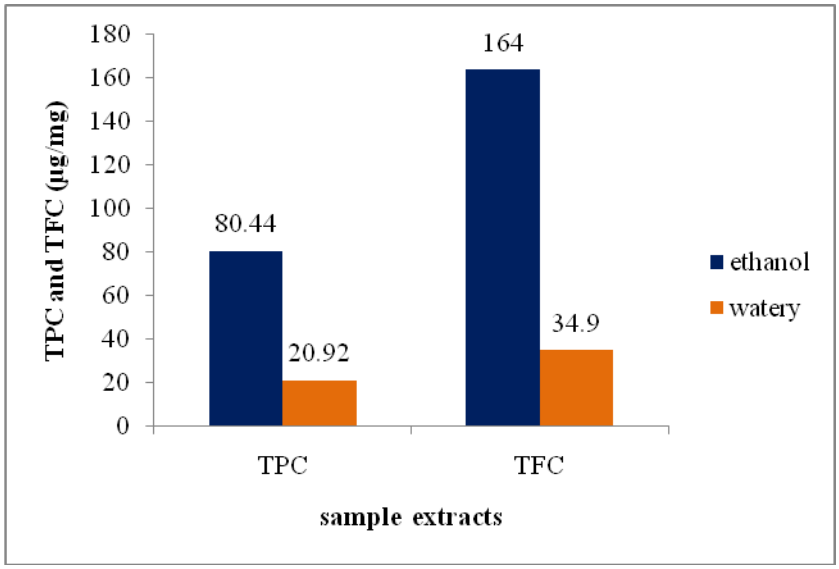

Figure 6 A bar graph of total phenolic content and total flavonoid contents of ethanol and watery extracts of Myet-htauk

CONCLUSION

The following inferences could be deducedfrom the overall assessment of the chemical investigation on aerial part of *Portulacaoleracea* L. (Myet-htauk). Preliminary phytochemical investigation of Myet-htaukrevealed that alkaloids, terpenoids,flavonoids, carbohydrate, phenolic compounds, saponins, steroids, terpenoids, glycosides, starch, reducing sugars and α- amino acidswere present while tannins and and cyanogenic glycosides were absent. From the results of nutritional values determination, Myet-htaukhas higher carbohydrates,proteinand fiber content than others nutrients.

From the results of the antioxidant activity of Myet-htaukby DPPH assay, it was found that ethanol extract (IC_{50}= 86.61 µg/mL) showed the highest activity thanwatery extract (IC_{50}= 315.55 µg/mL). The total phenolic content of ethanol and watery extracts of Myet-htaukwas found to be (80.44 µg GAE/ mg) and (20.92 µg GAE/ mg), respectively.The ethanol extract (164µg QE/mg)of Myet-htaukalso have higher total flavonoid content thanwatery extracts (34.9µg QE/mg). In this experiment, ethanol extract contained higher amount of phenolic and flavonoid components as compared to the water extract because of their higher solubility in ethanol.

In this study, the assessment of antioxidant activity indicates that Myet-htauk plant with higher phenolic and flavonoid contents could be a significant source of natural antioxidants.

Portulacaoleracea L. (Myet-htauk) plant is commonly available and the aerial part of the plant is known for its edible property.

ACKNOWLEDGEMENTS

We wish to express our warmest thanks to Dr Win Naing, Rector, Sittway University, for his kind permission to submit this paper. We would like to express our profound gratitude to Professor & Head,DrTetTun, Department of Chemistry, Sittway University, for his kind provision of the research facilities. We would like to theDepartment of Botany, Dagon University, for inviting us to present research paper.

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