

# STUDY ON NUTRITIONAL VALUES, TOTAL PHENOLIC AND FLAVONOID CONTENTS AND EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF FRUITS OF *Trapa natans* L. (Kywe-gaung-thee)

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## Abstract

In this research work, the fruits of *Trapa natans* L., Myanmar name Kywe-gaung-thee were selected for determination of some nutritional values, minerals contents, total phenolic content, total flavonoid content, antimicrobial activity and antioxidant activity. The fruits of *T. natans* (Kywe-gaung-thee) were collected from Patheingyi Township, Mandalay Region, Myanmar. Firstly, some nutritional values of fruits of *T. natans* such as moisture, ash, pH, protein, fiber and fat were determined by AOAC method. The mineral contents were also determined by EDXRF technique. Moreover, the total phenolic content of fresh fruit of *T. natans* was measured by Folin – Ciocalteu reagent and total flavonoid content was measured by AlCl<sub>3</sub> method with UV visible spectrophotometer. Furthermore, the antimicrobial activities of three solvent extracts of selected sample were tested by agar well diffusion method on six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli*. In addition, the antioxidant activity of the ethanolic extract of selected sample was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay method.

**Keywords** - *Trapa natans* L., nutritional values, total phenolic content, total flavonoid content, antimicrobial activity, antioxidant activity

## Introduction

*Trapa natans* L. is commonly known as "Water Chestnut". Water chestnut is one of the most popular vegetables used in Asia, due to its special feature and medicinal values which, it is found in Taiwan, China and parts of South East Asia (Chandana *et al.*, 2013). *T. natans* is an aquatic floating herb which belongs to the family Lythraceae. It has flaxuose stem, ascending in the water; the submerged parts are furnished with numerous opposite pairs of green root-like spreading pectinate organs. Leaves are alternate, crowded on the upper part of the stem, 3.8-5 cm long. Flowers are few, auxiliary, solitary, pure white (Adkar *et al.*, 2014).

The fruit is covered with a thick jet black outer cover shaped like a horn protruding from the head of the buffalo. The outer cover is hard, making it quite difficult to peel off to obtain the white meat (edible portion) inside (Gani *et al.*, 2015). It contains some vitamins like thiamine, riboflavin, nicotinic acid, vitamin C, vitamin A, D-amylase and considerable amount of phosphorylases. The water chestnut is used in many Ayurvedic preparations as nutrient, appetizer, astringent, diuretic, aphrodisiac, tonic, cooling and anti-diarrheal agent. It is also useful in lumbago sore throat, bilious affections, bronchitis, fatigue and inflammation. The whole herb has been reported for hepatoprotective activity, antimicrobial activity, antibacterial activity, antitumor activity, antioxidant activity and free radical scavenging activity (Chandana *et al.*, 2013).

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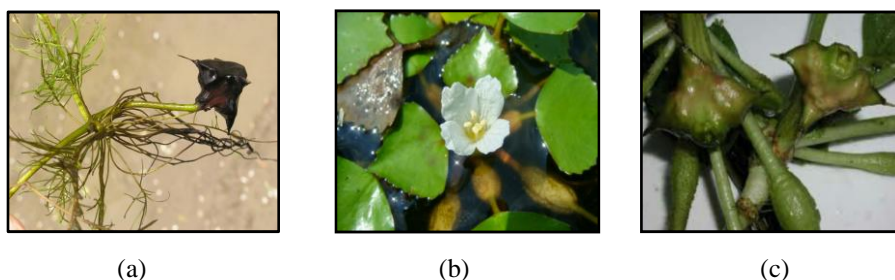
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In addition to this, the juice of the fruit has been used for diarrhea and dysentery. It is also said to have cancer-preventing properties. The antibiotic and antioxidant properties are important attributed to *T. natans* as a medicinal plant (Aidew, L. and Buragohain, A. K., 2014).

Fruits of *T. natans* are important sources of polyphenolic antioxidants that have high free radical scavenging properties being associated with protective effects against coronary heart disease, cancer diseases, neurodegenerative diseases and osteoporosis (Roni *et al.*, 2016).

In this research work, the fruits of *T. natans* (Kywe-gaung-thee) were selected for determination of nutritional values, mineral contents, total phenolic content, total flavonoid content, antimicrobial activity and antioxidant activity.

### Botanical Description of *Trapa natans* L.



**Figure 1** (a) Plant, (b) flower and (c) fruits of *Trapa natans* L.

Family name	: Lythraceae (Loosetrife family)
Genus	: <i>Trapa</i>
Species	: <i>natans</i>
Scientific name	: <i>Trapa natans</i> L.
Myanmar name	: Kywe-gaung-thee
English name	: Water Chestnut, Water Caltrop (Takano, A. and Kadono, Y., 2005)
Part used	: Fruit
Medicinal uses	: Tonic, debility, emaciation, asthma, cracked heels, diarrhea, dysentery, bilious affections, piles, leucorrhoea and menorrhagia. (Sofowora, A., 1993)

## Material and Methods

### Sample Collection

*Trapa natans* L. fruits were collected from Pathein-gyi Township, Mandalay Region, Myanmar. Fresh fruits were washed thoroughly under running tap water, and fruit coat was removed. The fleshy, white edible part was gently rinsed with distilled water. After then, the sample was crushed with mortar and pestle into pieces.

### Determination of Nutritional Values of Fruits of *T. natans*

Nutritional values of *T. natans* were measured by AOAC (Association of Official Analytical Chemists) method.

### **Determination of Mineral Contents from Fruits of *T. natans***

Mineral contents in fruits of *T. natans* were determined by EDXRF method, at the Department of Chemistry, Monywa University.

### **Quantitative Determination of Total Phenolic and Flavonoid Content of *T. natans***

#### **Preparation of fresh fruit juice solution of *T. natans***

The fresh fruit sample (100 g) was blended. The blended sample was homogenized with about 350 mL of distilled water. 393 mL of sample solution were obtained. Then the solution was filtered and the filtrate was centrifuged two times and the clear solution was used for the measurement of total phenolic and flavonoid content.

#### **Preparation of standard gallic acid stock solution**

10 mg of the standard gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of this solution was made up to 10 mL with distilled water (Slinkard and Singleton, 1977).

#### **Construction of calibration curve of standard gallic acid**

The prepared gallic acid solution was taken by micro pipette into a series of test tubes 20  $\mu\text{L}$ , 40  $\mu\text{L}$ , 60  $\mu\text{L}$ , 80  $\mu\text{L}$  and 100  $\mu\text{L}$ , respectively. The volume was made up to 1.6 mL with distilled water in each tube. And then 100  $\mu\text{L}$  of Folin - Ciocalteu reagent and 300  $\mu\text{L}$  of saturated  $\text{Na}_2\text{CO}_3$  (20 %) solution were added. After each standard solution was heated in the water bath at 40 °C for 30 min and then cooled at room temperature. The absorbance values of prepared standard gallic acid solutions were measured by UV visible spectrophotometer at 765 nm with respect to the blank solution. The calibration curve for standard gallic acid is shown in Figure 4.

#### **Determination of total phenolic content of *T. natans***

The total phenolic content of fresh fruit juice was measured by using the Folin-Ciocalteu method. Firstly, 40  $\mu\text{L}$  of expressed juice was taken in a test tube. It was made up to 1.6 mL with distilled water. 100  $\mu\text{L}$  of Folin-Ciocalteu reagent was mixed and then 300  $\mu\text{L}$  of saturated  $\text{Na}_2\text{CO}_3$  (20 %) solution was added.

The mixture was heated in a water bath at 40 °C for 30 min and then cooled at room temperature. The absorbance of this prepared sample solution was measured at 765 nm using a UV spectrophotometer. The assay was carried out in triplicate. The results are shown in Table 4. The total phenolic content of *T. natans* juice was expressed as mg gallic acid equivalent per 100 g fresh weight (Slinkard and Singleton, 1977).

#### **Construction of calibration curve of standard quercetin**

10 mg of the standard quercetin was taken in a test tube. 100 mL of MeOH was added to the standard compound. The stock solution was obtained. It was diluted with MeOH in various ratios to obtain four different of concentrations, such as 25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , 75  $\mu\text{g/mL}$ , and 100  $\mu\text{g/mL}$  respectively. Then, 4.0 mL of solution was prepared for each concentration. 0.5 mL of each standard quercetin solution was taken in 5 mL test tube and 1.5 mL methanol, 0.1 mL of

10 % aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added separately to each tube.

These tubes were left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The calibration curve was plotted by using the resulted absorbance data of standard quercetin solutions at concentrations 25 µg/ mL to 100 µg/ mL in methanol.

#### **Determination of total flavonoid content of *T. natans***

The total flavonoid content of fresh fruit juice was measured by aluminium chloride (AlCl<sub>3</sub>) method using quercetin as a standard. Firstly, 0.5 mL of fresh fruits juice was taken in test tube and 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1M potassium acetate and 4.3 mL distilled water were added into it. This tube was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The assay was performed in triplicate. The results are shown in Table 4. The total flavonoid content of fresh fruit juice was expressed as mg quercetin equivalent (QE)/100g fresh weight (Bag *et al.*, 2015).

#### **Determination of Antimicrobial Activities of Fruits of *T. natans***

Antimicrobial activities of various solvent extracts of *T. natans* were investigated by employing agar well diffusion method at Pharmaceutical Research Department (PRD) in Yangon. The solvents used were n-hexane, ethyl acetate and ethanol. Tested organisms are *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E. coli*.

#### **Determination of Antioxidant Activity of Ethanol Extract of Fruits of *T. natans***

The antioxidant activity of ethanol extract of fruits of *T. natans* (Kywe-Gaung-Thee) was determined by DPPH (1, 1- Diphenyl - 2 -picryl - hydrazyl) Radical Scavenging Assay method.

Sample solution was prepared by thoroughly mixing 2 mL of 60 µM DPPH solution and 2 mL of test sample solution. The solution was then allowed to stand at room temperature for 15 min. Control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol. The solution was then allowed to stand at room temperature for 15 minutes. Blank solution was prepared by mixing 1.5 mL of test sample solution and 1.5 mL of 95 % ethanol. The solution was then allowed to stand at room temperature for 15 min. Absorbance values of these solutions were measured at 517 nm by using UV-visible spectrophotometer. Experiment was done in triplicate for the ethanol extract of fruits of *T. natans*. Percent inhibition was calculated by using the following equation.

$$\% \text{ inhibition} = \frac{\text{DPPH}_{\text{alone}} - (\text{Sample} - \text{Blank})}{\text{DPPH}_{\text{alone}}} \times 100$$

Where, % inhibition = percent inhibition of test sample  
 DPPH alone = absorbance of DPPH solution  
 Sample = absorbance of sample solution  
 Blank = absorbance of blank solution

Finally, IC<sub>50</sub> (50 % inhibition concentration) was determined by using liner regressive excel program. Ascorbic acid was used a standard for comparison purpose.

### Results and Discussion

In this section, the results obtained from the experimental works such as nutritional values, mineral contents, total phenolic content, total flavonoid content, antimicrobial activity and antioxidant activity of the fruits of *T. natans* are discussed.

#### Nutritional Values of Fruits of *T. natans*

Some nutritional compositions of *T. natans* such as moisture, ash, pH, fat, fiber and protein content were determined by AOAC method and observed data are listed in Table 1.

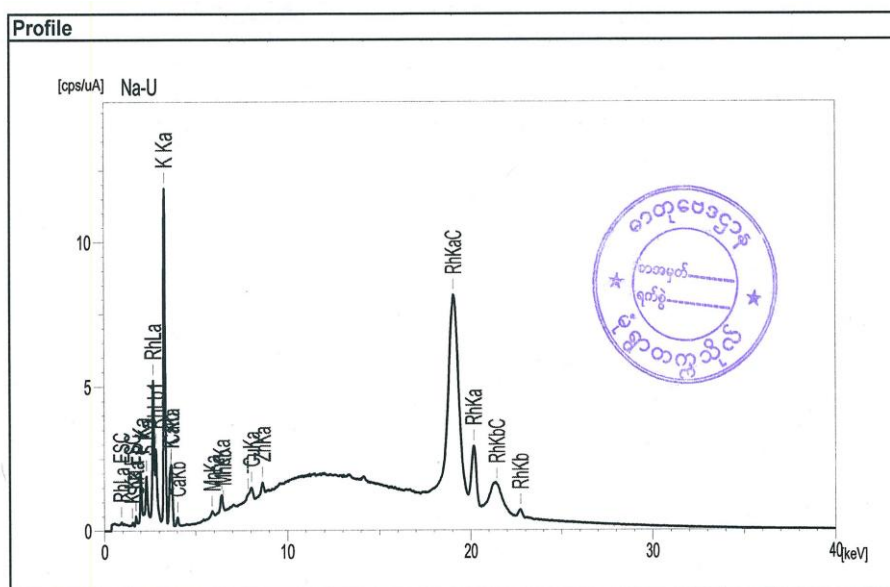
**Table 1 Results of Nutritional Values of Fruits of *T. natans***

No.	Parameter	Observed data	Analytical method	Apparatus used
1	Moisture (%)	5.3	Gravimetric method	Temperature controlled oven
2	Ash (%)	1.7	Loss of weight in ignition	Temperature controlled oven, Muffle furnace
3	pH	6.8	-	pH meter
4	Fat (%)	2.1	Petroleum ether extraction	Soxhlet extractor (SOX-412)
5	Fiber (%)	2.3	Enzymatic gravimetric method	Autocleaved and microwave heated
6	Protein (%)	1.24	Macro kjeldahl method	Protein analyzer

According to this table, the results obtained in nutritional values are moisture 5.3 %, ash 1.7 %, pH - 6.8, fat 2.1 %, fiber 2.3 % and protein 1.24 %. All of the resulting data were obtained by triplicate measurements. Mean value was described for each.

#### Mineral Contents in Fruits of *T. natans*

Figure 2 and Table 2 shows that potassium was the highest amount in the sample. Decreasing order of mineral content is K > P > S > Si > Ca > Fe > Cu > Mn > Zn. Therefore fruits of *T. natans* contain the essential minerals for human health. There is no toxic material contained in the selected sample.



**Figure 2** EDXRF spectrum of dried fruits of *T. natans*

**Table 2** The Results of Mineral Contents in Fruits of *T. natans*

No.	Elements	Symbols	Relative Abundances (%)
1.	Potassium	K	0.672
2.	Phosphorus	P	0.308
3.	Sulphur	S	0.143
4.	Silicon	Si	0.114
5.	Calcium	Ca	0.068
6.	Iron	Fe	0.003
7.	Copper	Cu	0.002
8.	Manganese	Mn	0.002
9.	Zinc	Zn	0.001

### Total Phenolic Content of Fruits of *T. natans*

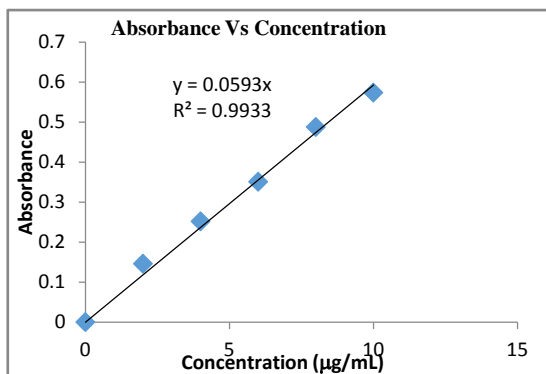
Determination of total phenolic content was carried out by Folin-Ciocalteu reagent by using UV spectrophotometer at 765 nm.

The standard gallic acid solutions at concentration 2 to 10  $\mu\text{g/mL}$  in distilled water were measured to know their absorbance by PD-303 UV visible spectrophotometer. The calibration curve was plotted against by using the resulting data of standard gallic acid solution as shown in Figure 4.

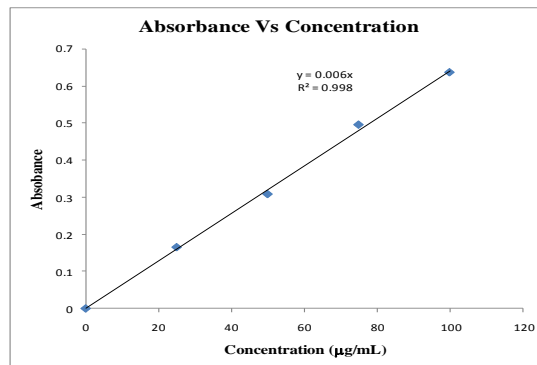
The total phenolic content was determined by Folin-Ciocalteu reagent using spectrophotometer. The total phenolic content of fruits of *T. natans* was calculated from the standard curve prepared from different concentrations of gallic acid in which the simple spectrophotometric procedure was followed for the working standard. The total phenolic content of fruits of *T. natans* was found to be  $70.24 \pm 1.68$  mg/100 g fresh weight.

**Total Flavonoid Content in *T. natans***

The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Figure 5. The results of total flavonoid content are described in Table 4.



**Figure 4** Absorbance Vs concentration curve for standard gallic acid solution



**Figure 5** Absorbance Vs concentration curve for standard quercetin solution

**Table 3** The Total Phenolic and Flavonoid Contents of Fruits of *T. natans*

Name of Sample	Phenolic Content	Flavonoid Content
	(mg/100g)	(mg/100g)
	Mean ± Standard Deviation	Mean ± Standard Deviation
Fresh Fruits of <i>Trapa natans</i> L.	70.24 ± 1.68	13.04 ± 0.19

In accordance with these results, the total phenolic and flavonoid content of these fresh fruit solutions were found to be 70.24 ± 1.68 mg/100 g and 13.04 ± 0.19 mg/100 g fresh weight respectively.

**Antimicrobial Activities of Fruit of *T. natans***

Antimicrobial activities of the fruit of *T. natans* (Kywe-gaung-thee) were tested by agar well diffusion methods on six selected organisms (Figure 4). The results are shown in Table 4.

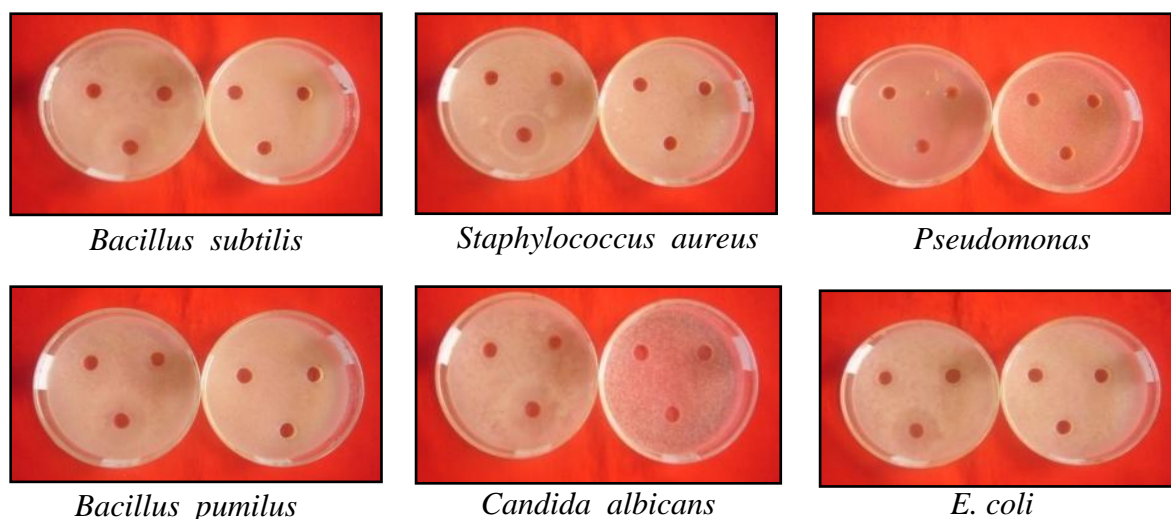
**Table 4** Results of Antimicrobial Activities of Fruit of *T. natans*

Extracts	Diameter of Inhibition Zone (mm)					
	I	II	III	IV	V	VI
n-hexane	13 (+)	13 (+)	12 (+)	14 (+)	13 (+)	14 (+)
EtOAc	15 (++)	25 (+++)	28 (+++)	25 (+++)	22 (+++)	26 (+++)
EtOH	20 (+++)	17 (++)	16 (++)	17 (++)	17 (++)	15 (++)

Agar well – 10 mm

Micro-organisms

- 10 mm ~ 14 mm (+)      I = *Bacillus subtilis*                      IV = *Bacillus pumilus*
- 15 mm ~ 19 mm (++)      II = *Staphylococcus aureus*                      V = *Candida albicans*
- 20 mm above      (+++)      III = *Pseudomonas aeruginosa*                      VI = *E. coli*



**Figure 3** Antimicrobial activities of various solvents (n-hexane, ethanol, ethylacetate) extracts of *T. natans* and control solutions

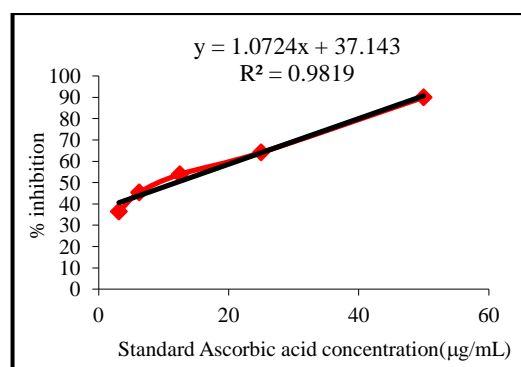
According to these results, ethanol extract of fruit of *T. naptans* responds high activity on *Bacillus subtilis* and medium activities on remaining five organisms and ethylacetate extract gives high activities on all tested organisms except *Bacillus subtilis*. n-Hexane extract of selected sample has low activities on all tested organisms. Therefore, polar solvent extracts of *T. naptans* responds more antimicrobial activities than non-polar solvent extract.

#### Antioxidant Activity of Fruits of *T. natans*

The result of antioxidant activity using DPPH assay in standard ascorbic acid is shown in Table 5 and Figure 6.  $IC_{50}$  value was calculated by using linear regressive equation.

**Table 5** Percent Oxidative Inhibition of Various Concentration of Standard Ascorbic Acid

Ascorbic acid concentration ( $\mu\text{g/mL}$ )	% inhibition	$IC_{50}$ ( $\mu\text{g/mL}$ )
50	89.936	
25	64.103	
12.5	53.840	11.98
6.25	45.350	
3.125	36.378	



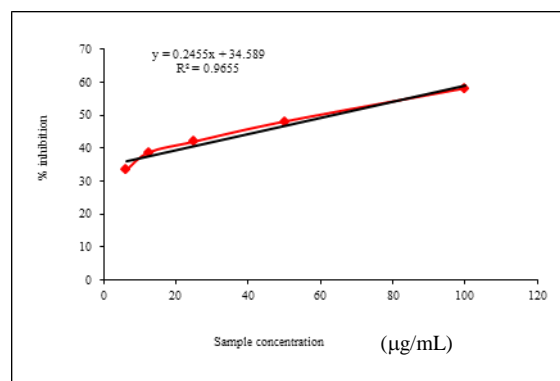
**Figure 6** % Inhibition Vs different concentration of standard ascorbic acid

The antioxidant activity of ethanolic extract of the fruit of *T. natans* is shown in Table 6 and Figure 7.  $IC_{50}$  value of selected sample was also calculated by using linear regressive equation.



**Table 6 Percent Oxidative Inhibition and IC<sub>50</sub> Value of *T. natans***

Sample concentration (µg/mL)	% inhibition	IC <sub>50</sub> (µg/mL)
100	58.228	62.80
50	48.101	
25	42.025	
12.5	38.734	
6.25	33.418	

**Figure 7** % Inhibition Vs different concentration of sample solution

The IC<sub>50</sub> values of ethanolic extract of fruit of *T. natans* is 62.80 µg/mL which gives significant activity compared to standard ascorbic acid, IC<sub>50</sub> 11.98 µg/mL.

### Conclusion

In this research work, the edible parts of the fruits of *T. natans* (Water chestnut), Myanmar named Kywe-gaung-thee were selected for chemical investigations. According to the results of present studies, the fruits of *T. natans* were found to contain good nutritional values, nontoxic mineral contents and high antimicrobial activities. Especially, the fruit of *T. natans* was found to be the rich source of phenolic compounds which support the preventing cancer and other serious diseases. The relative property of phenolic compounds is antioxidant activity. Hence, the ethanolic extract of *T. natans* gave significant antioxidant activity compared to the standard ascorbic acid. The fruit of *T. natans* is not popular fruit in Myanmar. But it has very good potency for human health. Moreover, it is cheaper than other popular fruits. Therefore, the fruit of *T. natans* should be edible for health.

### Acknowledgements

We are extremely grateful to Dr Thida Win, Rector, University of Mandalay and Professor Dr Yee Yee Myint, Head of Department of Chemistry, University of Mandalay, Myanmar for their permitting, understanding, kind helps and good guidance on our research studies. We also thank to Dr Than Than Aye, Professor and Head (Rtd.) and Dr Kyae Mon Lwin, Professor, Head of Department of Chemistry, Kyaukse University for their permitting and supporting the some research facilities throughout our research works.

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