Screening on the Phytochemical Constituents, Total Phenol Contents and Antioxidant Potency of Flowers and Pseudostems of *Aeginetia indica* Linn. (Kauk-hlaing-ti)

Nwe Ni Hlaing[1], Aye Aye Nwe[2]

Abstract

This research work deals with the study of phytochemical constituents, total phenol contents and antioxidant potency of Flowers (F) and Pseudostems (P) of *Aeginetia indica* L.(Kauk- hlaing -ti). The phytochemical investigation indicated that alkaloids, carbohydrates, flavonoids , glycosides, organic acids, phenolic compounds , reducing sugars, saponins, steroids and terpenoids are present while starch and tannins are absent in both the samples. In addition, α-aminoacids are present in the flowers while they are absent in the pseudostems. The nutritional value of flowers and pseudostems of *A.indica* were determined by AOAC method. The total phenol contents of various crude extracts were determined by UV-visible spectrophotometric using Folin-Ciocalteu reagent (FCR) method and garlic acid was used to construct standard calibration curve. Antioxidant activity of flowers and pseudostems of *A.indica* were also investigated by using DPPH assay method. The IC_{50} values of crude extracts were observed to be (31.66 μgmL^{-1}) for F-EtOH, (82.63 μgmL^{-1}) for F-H_2O, (72.74 μgmL^{-1}) for P-EtOH and (114.59 μgmL^{-1}) for P-H_2O respectively. Among the crude extracts, the order of radical scavenging activity were observed as F-EtOH > P-EtOH > F-H_2O >P-H_2O. All extracts showed mild activity when compared to the standard antioxidant vitamin C (21.79 μgmL^{-1}).

Keywords **:** *Aeginetia indica* L . , nutritional value, total phenol content¸ antioxidant activity

1. INTRODUCTION

Aeginetia indica L. is root parasite, producing numerous tubercles or swellings. Flowers are solitary and leafless. Scapes are solitary or several, very slender, from 15.40 cm long. Corolla is tube broad and incurved. Calyx is ovoid, 1.5 to 3 centimeters long, purplish with longitudinal yellow stripes. Capsules are ovoid or rounded. Seeds are yellowish (Chai *et al.,* 1992). *Aeginetia indica* L. grows in India, Bangladesh, Cambodia, Thailand, Malaysia, Indonesia and in Myanmar.

1.1 Botanical Aspects of *Aeginetia indica* Linn. (Kauk-hlaing-ti)

Botanical name	:	*Aeginetia indica Linn.*
Family	:	Scrophulariaceae
Genus	:	*Aeginetia*
Species	:	*indica*
Myanmar name	:	Kauk-hlaing-ti
Plant part use	:	Flowers and Pseudostems

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

Figure 1. Photograph of *Aeginetia indica* Linn. (Kauk-hlaing-ti)

1.2 Medicinal uses of *Aeginetia indica* Linn.

Aeginetia indica L. is a root parasite that grows on bamboo used extensively in Thai traditional medicine. *Aeginetia indica* L. induces potent antitumor immunity in tumor-bearing mice and may be a useful immunotherapeutic agent for patients with malignant diseases. Study the effect of *Aeginetia indica* L. in the treatment of renal cancer. *Aeginetia indica* L. extract showed an inhibitory effect on tumor cell-induced metastasis. Experimental results suggest that the extract has a synergistic effect on apoptosis induced by chemotherapeutic agents and an inhibitory effect on cell adhesion, migration, and invasion. It provides evidence as a potential of *Aeginetia indica* L. extracts are novel alternatives in the treatment of human renal cancer (Chai *et al.*, (1992).

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

Aeginetia indica L. samples were collected from Tha-hton Township, Thayettaw village, Mon State in August 2017. These samples were cleaned by washing with water, then cut into small pieces and air dried at room temperature. The dried samples were ground into powder in an electric blender and separately stored in airtight container.

2.2 Some physicochemical analysis of *Aeginetia indica* Linn.

Analysis of the proximate components was done by using the standard official methods of the Association of Analytical chemists. The moisture content was

determined by oven drying method and the ash content was estimated by incinerating 2 grams of the dry powder in muffle furnace at 550 °C for 2 hours. Crude protein was estimated by determining the percent nitrogen content using the macro-Kjeldahl method and multiplying by a factor of 6.25. Crude fats was determined by Soxhlet extraction from 5 g of the sample with n-hexane for 8 hours. Crude fibre was determined by treating 5 grams of powdered sample with 1.25 % H_2SO_4 and 1.25 % NaOH. Total carbohydrates were obtained by subtracting the value of moisture, crude protein, crude fibre, crude fat and ash from 100 %. The total energy value was equal to the addition of fat, protein and carbohydrate, each gram of fat give 9 k cal, protein and carbohydrate give 4 k cal energy (AOAC, 2000).

2.3 Preparation of crude extracts

The dried powdered sample *Aeginetia indica* Linn. was extracted with pet-ether, ethyl acetate, ethanol and water by using maceration method. After 72 h of extraction, each extract was filtered through Whatman's filter paper no.1 separately. Each filtrate was evaporated to dryness on boiling water bath and the crude extracts were obtained.

2.4 Phytochemical screening

Phytochemical examinations of all the extracts were carried out by using the following tested and reagents; alkaloids with Mayer's and Dragendroff's tests, glycosides with Keller Killani test, saponin with foam test, phytosterols with Libermann Burchard's test, phenols with Ferric chloride test, tannins with Gelatin test, and flavonoids with alkaline reagent test (Tiwari *et al.,* 2011).

2.5 Total Phenol Contents of Flowers and Pseudostems of *Aeginetia indica* Linn.

The total phenolic content in water and ethanol extract of *Aeginetia indica* was estimated by Folin- Ciocalteu method.(Rekha *et al*.,2012).Each sample solution 90.5ml) was added into 5ml of FC reagent.(FCR:H_2O;1:2) and incubated for 5 min. To each tube,4ml of 1MNa_2CO_3 was added and the tubes were kept at room temperature for 15 min and the absorbance of reaction mixture was read at λ_{max}765nm. The blank solution was prepared as above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as μg garlic acid equivalents per milligram (μg GAE/ mg)of extracts.(Rebeca., 2003).

2.6 Screening of antioxidant activity

The antioxidant activity of each extract of the sample was estimated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Maisarah *et al.,* 2013).

2.6.1 DPPH radical scavenging assay procedure

The control solution was prepared by mixing 1.5 mL of 60 M DPPH solution and 1.5 mL of EtOH using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 60 M DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm by using UV 7504 spectrophotometer. Absorbance measurements were done in triplicate for each concentration and the mean values so obtained were used to calculate percent inhibition of oxidation by the following equation:

$$\% \text{ RSA} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where, % RSA = percent of radical scavenging activity

$A_{Control}$ = absorbance of control solution

A_{Sample} = absorbance of tested sample solution

IC_{50} value was calculated by linear regressive excel program.

3. RESULTS AND DISCUSSION

Physicochemical properties were determined by the standard official methods of the Association of Analytical Chemists are described in Table 1. According the following results, the presence of the important nutrients like fat, fiber, protein, carbohydrate and the physical properties like ash and moisture mean the selected samples could be used as a nutritionally valuable and health ingredients to improve medicine. The calculated energy value of flowers was found to be higher than that of pseudostems.

Table1. Nutritional Values of Flowers and Pseudostems *Aeginetia indica* Linn.

No	Parameter	Content(%)	
		Flowers	**Pseusostems**
1	Moisture	12.17	11.06
2	Ash	5.15	7.17
3	Fiber	14.92	17.85
4	Fat	2.73	0.60
5	Protein	4.52	3.41
6	Carbohydrate	60.51	59.91
7	Energy value (kcal/100g)	284.69	258.68

3.2 Phytochemical Investigation of *Aeginetia indica* Linn.

Phytochemical investigation was carried out to understand the types of phytoorganic constituents present in selected *Aeginetia indica* L. These results are summarized in Table 2.

Table 2. Results of Phytochemical Investigation of Flowers and Pseudostems *Aeginetia indica* Linn.

No	Test	Extract	Test Reagents	Observation	F	S
1	Alkaloids	1% HCl	(i) Dragendorff' reagent	Orange ppt	+	+
			(ii) Sodium picrate	yellow ppt.	+	+
			(iii) Wagner's reagent	brown ppt.	+	+
			(iv) Mayer's reagent	white ppt.	+	+
2	α-Amino acids	H_2O	Ninhydrin reagent	purple ppt.	+	-
3	Carbohydrates	H_2O	10% α- napthol and conc:H_2SO_4	red ring	+	+
4	Flavonoids	EtOH	Mg turning and conc: HCl	pink colour	+	+
5	Glycosides	H_2O	10% lead acetate	white ppt.	+	+
6	Organic acids	H_2O	Bromocresol green	yellow colour	+	+
7	Phenolic compounds	EtOH	5% Fe Cl_3	deep blue	+	+
8	Reducing sugars	H_2O	Benedict's Solution	brick red colour	+	+

9. Saponins	H_2O	Distilled water	frothing	+	+
10. Starch	H_2O	Iodine	bluish black	-	-
11. Steroids	PE	Acetic anhydride & conc: H_2SO_4	blue	+	+
12.Terpenoids	$CHCl_3$	Acetic anhydride & conc: H_2SO_4	pink colour	+	+
13.Tannins	H_2O	1% Gelatin	white ppt.	-	-

(+) Present, (-) Absent , ppt . = Precipitate, F = Flowers, S = Pseudostem

3.3 Total Phenol Contents of Flowers and Pseudostems of *Aeginetia indica* Linn.

In the present study, the total phenolic content in water and ethanol extract of *Aeginetia indica* was estimated by Folin- Ciocalteu method.Gallic acid was use to contrast standard calibration curve. According to the results, TPC (µg GAE/ mg) was found to be the highest in watery extract of pseudostems (26.15 ± 2.38) and lowest in ethanol extract of flowers (13.59 ± 2.39).The highest total phenolic content was observed in watery extract than ethanol extract of both samples. This mean that phenolic compound were more soluble in water than in ethanol. The results were shown in Table 3 and Figure 2.

Table 3. Total Phenol Contents of Flowers and Pseudostems of *Aeginetia indica* Linn.

Samples	TPC (µg GAE/ mg ± SD)	
	Water	Ethanol
Flowers	27.25±2.38	14.75±2.39
Pseudostems	32.21±9.08	27.62±9.67

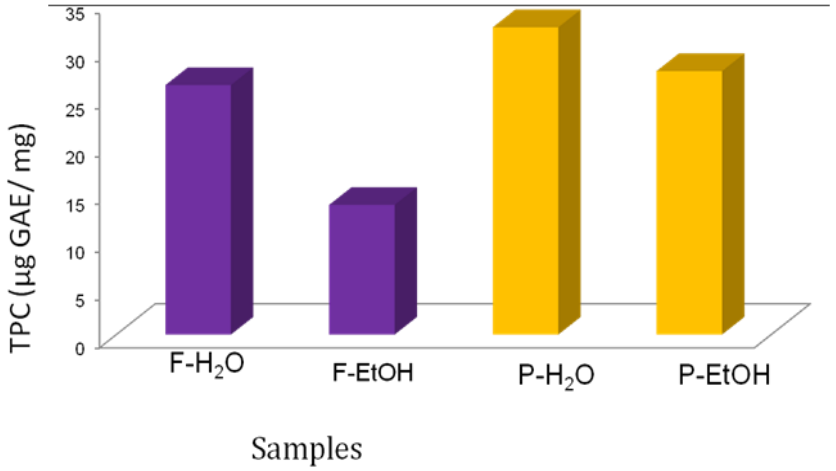

Figure 2 . A bar graph of total phenol content of ethanol and water crude extracts of flowers and pseudostems of *Aeginetia indica* Linn.

3.4 Antioxidant Activity of Flowers and Pseudostems *Aeginetia indica* Linn.

Antioxidant compounds in food play an important role is a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. The antioxidants activity of *Aeginetia indica* extracts were estimated by measuring the DPPH radical scavenging activity of different concentration of extracts. Determination of absorbance was carried out at wavelength 517 nm and is violet in color. The color changes upon neutralization of this free radical from violet to pale yellow by daylight (Moon *et al.*, 2009). The radical scavenging activity of crude extracts are expressed in term of % RSA and IC_{50} (50 % inhibitory concentration) values were calculated by linear regressive excel program. The results are shown in Table 4 and Figure 3.

Among the crude extracts, the IC_{50} values of the ethanol and watery extracts of pseudostems (EtOH = 72.46 μg/mL, H_2O = 114.59 μg/mL) are higher than that of the flowers (EtOH = 31.66 μg/mL, H_2O = 82.63 μg/mL) respectively. The IC_{50} values of standard ascorbic acid were determined to be 21.79 μg/mL, which indicated that the antioxidant activity of water and ethanol crude extracts of flowers and pseudostems are very much weaker than those of standard ascorbic acid.

Table 4. Percent Oxidative Inhibition of Different Concentrations an IC_{50} Values of Flowers and Pseudostems of *Aeginetia indica* Linn. Crude Extracts and Standard Vitamin C

Test samples	Percent Oxidative Inhibition in Different Concentrations (μg/mL)						IC_{50} (μg/mL)
	6.25	12.5	25	50	100	200	
F-H_2O	5.52±0.94	11.07±7.62	17.89±2.72	33.57±7.58	52.21±7.37	64.94±0.36	82.63
F-EtOH	26.01±2.03	33.76±0.95	43.90±2.06	66.78±7.32	89.85±0.98	92.06±2.10	31.66
P-H_2O	10.95±1.74	19.45±0.66	27.12±2.51	33.42±0.01	45.20±1.77	79.72±4.49	114.59
P-EtOH	13.90±1.64	28.36±1.95	39.01±1.35	45.87±1.77	53.42±0.15	65.97±2.64	72.74
Standard ascorbic acid	40.77±2.18	46.15±0.48	51.34±0.15	54.03±0.51	58.84±0.31	63.26±0.31	21.79

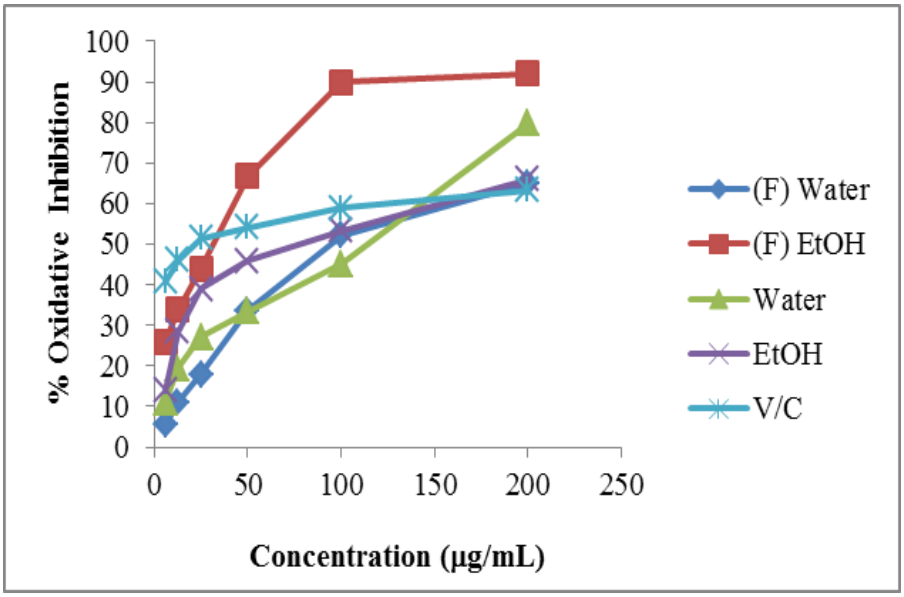

Figure 3. Plot of % oxidative inhibition Vs concentration of watery and ethanol extracts of both samples and standard ascorbic acid

4. Conclusion

From the overall assessment of the present research work, the following inferences can be drawn. The preliminary phytochemicals investigated by test tube method resulted that except starch and tannins, others bioactive phytochemicals constituents are present in both samples. The nutritional values determined by AOAC method indicated that the flowers and pseudostems of *Aeginetia indica* Linn. are rich source of fiber and carbohdrate. Moreover results of total phenol content and antioxidant study suggest that *Aeginetia indica* Linn. might be considered as good source of natural oxidant that could help in the prevention of diseases related to radical mechanisms. In conclusion, it can be deduced that flowers and pseudostems of *Aeginetia indica* Linn. can be used as food source as well as phytotheraputic agent.

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