NUTRITIONAL VALUES, TOTAL PHENOLIC, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF RED AND WHITE FLOWER PETALS OF *SESBANIA GRANDIFLORA* (L.) PERS.

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Abstract

The objectives of this research work were to evaluate the nutritional values, phytochemicals, total phenolic, total flavonoid and antioxidant activity of red and white flower petals of *Sesbania grandiflora* (L.) Pers. Red and white flower petals possess nutritional values of moisture (6.13 and 5.28 %), ash (4.37 and 5.10 %), fiber (1.40 and 3.60 %), protein (5.99 and 8.32 %), fat (0.83 and 1.53 %), carbohydrates (81.28 and 76.17 %) and energy value (357 and 352 kcal/100 g), respectively. Both varieties of flower samples contain plenty of phytochemicals such as alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, glycosides, α-amino acids, starch, reducing sugars, carbohydrates, saponin and tannins. The total phenolic content was determined by Folin-Ciocalteu assay and expressed as gallic acid equivalent. Total phenolic content of red flower petals was found to contain 185.15 μg GAE/mg of ethanol extract and 82.08 μg GAE/mg of aqueous extract. Total phenolic content of white flower petal was found to contain 134.38 μg GAE/mg of ethanol extract and 55.92 μg GAE/mg of aqueous extract. Total flavonoid content was determined by aluminium chloride colourimetric method and expressed as quercetin equivalent. It was observed that red flowers contain total flavonoid content of 356.85 μg QE/mg of ethanol extract and 285.33 μg QE/mg of aqueous extract, and white flowers contain 45.33 μg QE/mg of ethanol extract and 22.30 μg QE/mg of aqueous extract. Antioxidant activity of each extract was evaluated by DPPH assay. In DPPH assay, IC_{50} values were used to determine the antioxidant potential of the sample. Among the extracts ethanolic extracts of both red and white flower varieties exhibited higher DPPH radical scavenging activity with IC_{50} value of 213.67 μg/mL and 322.31 μg/mL, respectively. *S. grandiflora* can be regarded as promising candidates for natural plant sources of antioxidant with high values.

Keywords: *Sesbania grandiflora*, nutritional values, phytochemicals, phenolic, flavonoid, antioxidant activity

Introduction

Sesbania grandiflora (L.) Pers. is a popular Myanmar medicinal plant which belongs to family Leguminosae. It is small, erect, fast-growing and sparsely branched tree that reaches 15 m in height. All *Sesbania* species have pinnately compound leaves where each leaf is divided into multiple leaflets. The leaves can be up to 30 cm long with 5-15 paired leaflets that are oblong to elliptic in shape and about 3 cm in length. The flowers of *S. grandiflora* are large and 7-9 cm long. Two varieties of *S. grandiflora* are recognized as red flower variety and white flower variety (Bahera *et al*., 2012). In Myanmar, red flower variety is called *Pauk-pan-ni* and white flower variety is called *Pauk-pan-byu.* The photographs of *S. grandiflora* plants and flowers are shown in Figures 1 and 2.

The bioactive chemical constituents of *S. grandiflora* are leucocyanidin and cyanidin present in seeds. Oleanolic acid and its methyl ester and kaempferol-3-rutinoside are present in flowers. The bark contains tannins and gum. Saponin and sesbanimide are observed in seeds (Outtara, 2011; Vijay *et al*., 2009).

All parts of *S. grandiflora* are used for medicine as well as vegetables in Southern Asia and India. In Folk Medicine it is reported to be aperient, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic. It is also used for the treatment of bruises, catarrh, dysentery, fevers, headaches,

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smallpox, sores, sore throat and stomatitis. It also possesses anxiolytic, anticonvulsive and hepatoprotective properties (Alli and Salwau, 2011; Mbatchou, 2011; Vijay *et al*., 2009). The present study evaluates the nutritional values, phytochemicals, total phenolic, total flavonoids and antioxidant activity of red and white flowers petals of *S. grandiflora* (*Pauk-pan-ni* and *Pauk-pan-byu*).


Figure 1 Photographs of *Sesbania grandiflora* plants and flowers (red variety)


Figure 2 Photographs of *Sesbania grandiflora* plant and flowers (white variety)

Materials and Methods

Collection and Preparation of Plant Materials

The red and white flowers of *S. grandiflora* (*Pauk-pan-ni* and *Pauk-pan-byu*) were collected near Professor Housing, West Yangon University, Yangon, Myanmar in the month of February 2019. The flowers and plant sample were authenticated by the botanists of Botany Department, West Yangon University.

The red and white flowers of *S. grandiflora* were harvested and carefully separated the flower petals from the other parts of flowers. The flower petals were dried under shade for one week and then dried in an air oven at 50 °C. The dried flower petals were ground by using a mechanical grinder. The dried powdered samples were stored in an air tight bottle and placed in cool and dry place.

Determination of Nutritional Values

The nutritional values such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrate contents and energy value of *S. grandiflora* flower petals were determined at Research Laboratory of Chemistry, West Yangon University. The analyses were carried out according to A.O.A.C method (A.O.A.C, 2000).

Phytochemical Screening in *S.grandiflora* Flower Petals

Preliminary qualitative phytochemical screening were carried out for alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, glycosides, α-amino acids, cyanogenic glycosides, starch, reducing sugars, carbohydrates, saponins and tannins following the standard procedures (Harborne, 1998; Kokate *et al*., 2009).

Preparation of Plant Extracts

Ethanolic extract: Each of the dried powder samples (50 g) was immersed in ethanol (500 mL) for 7 days at room temperature with frequent agitation. The ethanolic extract was filtered and filtrate was concentrated to dryness using rotatory evaporator under reduced pressure. The ethanolic extracts of each sample were kept in the desiccator for 3 days and then stored in airtight bottle at 4 °C.

Aqueous extract: Each of the dried powder samples (50 g) was boiled with distilled water (500 mL) on water bath for 30 min. The extract was filtered and the filtrate was evaporated on water bath. The resultant aqueous extracts were kept in the desiccator and then dry paste extracts were stored in airtight bottle at 4 °C until further use.

Determination of Phenolic Contents

The total phenolic content was determined for ethanolic and aqueous extracts of flower petals of *S. grandiflora* using the Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). 0.5 mL of sample extract solution (250 µg/mL in methanol) was mixed with 0.5 mL methanol and 5 mL Folin-Ciocalteu reagent (1:10 v/v). The mixture was incubated at 37 °C for 30 min. After 30 min, 4 mL of 1 M sodium carbonate was added to the above mixture and kept at room temperature for 15 min. The absorbance was measured utilizing a UV spectrophotometer (Shimadzu, UV-1800) at 760 nm against a blank without sample extract. The same procedure was repeated for the standard solution of gallic acid and the standard calibration line was constructed as shown in Figure 3. Then the content of phenolics in each extract was expressed in terms of gallic acid equivalent (µg GAE/mg of extract).

Determination of Flavonoid Contents

The content of flavonoids in the examined plant extracts (ethanolic and aqueous) was determined by using aluminium chloride colourimetric method (Afify *et al.,* 2012). An aliquot of 0.5 mL of each extract solution (250 µg/mL in methanol) was mixed with 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. The mixture was incubated for 40 min at room temperature followed with the measurement of absorbance at 415 nm using Shimadzu, UV-1800 UV-Visible spectrophotometer. The calibration curve was plotted using standard quercetin as shown in Figure 5. The total flavonoid contents of each sample extract were expressed as quercetin equivalent in terms of (µg QE/mg of extract).

DPPH Radical Scavenging Activity Assay

The radical scavenging activity (RSA) of the crude extracts was adopted the measure antioxidant activity using the DPPH method (Yan-Hwa *et al*., 2000; Aryal *et al*., 2019). Briefly, 1.5 mL of extract solution (25-800 µg/mL in ethanol) was added to 1.5 mL of DPPH (0.002 %) solution. The mixture was kept aside in a dark area for 30 min and absorbance was measured at λ_{max} 517 nm against equal amount of DPPH and ethanol as a control. The absorbance of sample in ethanol without DPPH was determined as sample blank. Ascorbic acid was used as standard antioxidant compound in the experiment. The percentage of DPPH radical scavenging (% RSA) was estimated using the equation:

$$\% \text{ RSA} = \frac{A_{control} - [A_{sample} - A_{blank}]}{A_{control}} \times 100$$

Where; % RSA= radical scavenging activity

$A_{control}$ = absorbance of DPPH in ethanol

A_{sample} = absorbance of DPPH with sample solution

A_{blank} = absorbance of sample in ethanol without DPPH

Results and Discussion

Nutritional Values

Proteins, carbohydrates, fats, vitamins, minerals and water are the nutrients that are essential for life and contribution to the caloric value of the body. In the present study, proximate analyses were carried out for the red and white flower petals of *S. grandiflora* to know the nutritional significance of the frequently consumed species in the traditional medicines. Proximate compositions *viz*. moisture, ash, crude protein, crude fat, crude fiber, carbohydrates and energy values were carried out using standard methods for food analysis (A.O.A.C, 2000). The analyses of nutritional values were performed at Department of Chemistry, West Yangon University. These analyses revealed some interesting findings and the results obtained from proximate analysis are presented in Table 1. These flower samples are low in protein and fat, however rich in carbohydrates. Moreover, the flowers of *S. grandiflora* are energy's high source, as 100 g of plant materials can give approximately 357 kcal and 352 kcal energy for red and white flowers, respectively.

Table 1 Nutritional Values of Red and White Flower Petals of *S. grandiflora*

Parameter	Nutritional Value (%)*	
	Red Flower	White Flower
Moisture	6.13	5.28
Ash	4.37	5.10
Fiber	1.40	3.60
Protein	5.99	8.32
Fat	0.83	1.53
Carbohydrate	81.28	76.17
Energy Value (kcal/100 g)	357	352

* based on the weight of dried sample

Phytochemical Constituents

The qualitative tests for phytochemicals in red and white flower petals of *S.grandiflora* were carried out and a number of phytochemicals showed positive results in their specific tests. In the present study, the phytochemical screening of flowers of *S. grandiflora* revealed the presence of alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, glycosides, α-amino acids, starch, reducing sugars, carbohydrates, saponins and tannins. The results are indicated in Table 2.

Table 2 Phytochemicals of Red and White Flower Petals of *S. grandiflora*

No	Phytochemical Constituents	Red Flower	White Flower
1	Alkaloids	+	+
2	Phenolic compounds	+	+
3	Flavonoids	+	+
4	Steroids	+	+
5	Terpenoids	+	+
6	Glycosides	+	+
7	α-Amino acids	+	+
8	Cyanogenic glycosides	-	-
9	Starch	+	+
10	Reducing sugars	+	+
11	Carbohydrates	+	+
12	Saponin	+	+
13	Tannins	+	+

Note: (+) = presence (-) = absence

Total Phenolic Content

The total phenolic content of ethanolic and aqueous extracts of red and white flowers of *S.grandiflora* was estimated by Folin-Ciocalteu's method using gallic acid as standard. The reagent is formed from a mixture of phosphotungstic acid and phosphomolybdic acid which after oxidation of phenols is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue coloration produced has a maximum absorption in the region of 760 nm and proportional to the total quantity of phenolic compounds originally present. The gallic acid solution of concentration (3.125 -100 µg/mL) was used for the construction of calibration curve ($y=0.0052x + 0.1073$) with a regression coefficient ($R^2=0.9907$) as shown in Figure 3.

The total phenolic content of red and white flowers extracts were expressed as µg GAE/mg of plant extract and shown in Table 3 and histogram is shown in Figure 4. Among the extracts, the higher phenolic contents (185.15 and 134.38 µg GAE/mg) were found in ethanolic extracts of red and white flower petals, respectively. Significant amount of phenolic content was observed in aqueous extracts of red flower (82.08 µg GAE/mg) and white flower (55.92 µg GAE/mg).

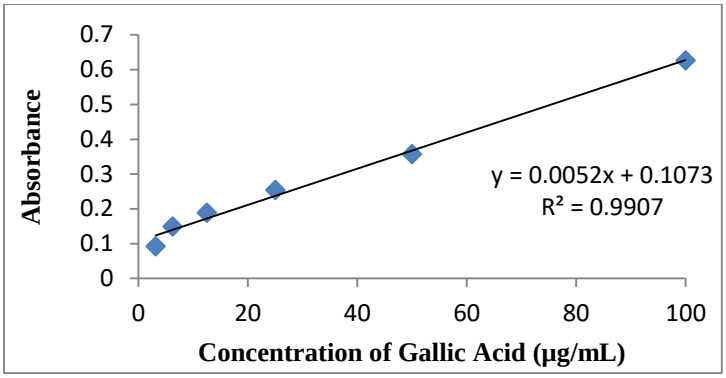

Figure 3 Standard curve of gallic acid

Table 3 Total Phenolic Contents in Red and White Flower Petals of *S. grandiflora*

Extract (250 µg/mL)	Total Phenolic Content (µg GAE/mg of extract)	
	Red Flower	White Flower
Ethanol	185.15	134.38
Aqueous	82.08	55.92

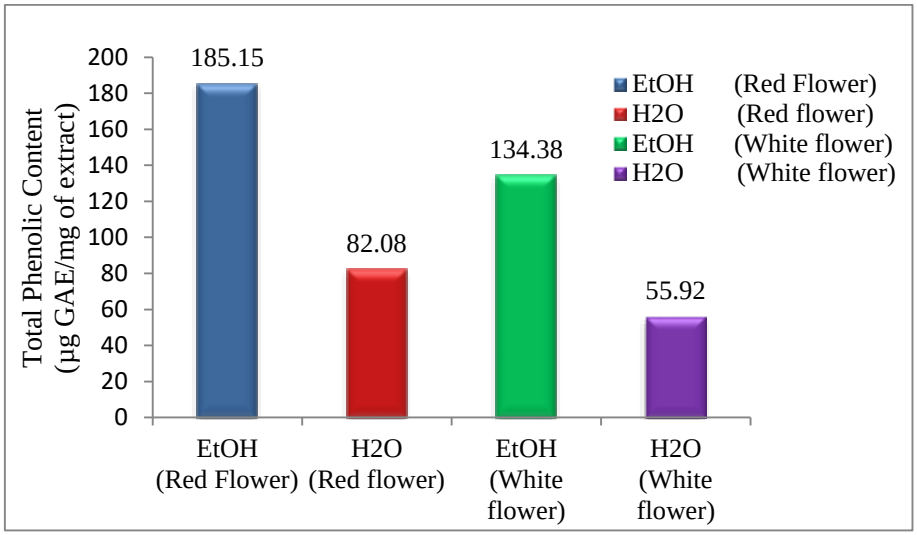

Figure 4 Histogram of total phenolic content of flower petals of *S.grandiflora*

Total Flavonoid Content

The total flavonoid content in ethanolic and aqueous extracts of the red and white flower petals of *S. grandiflora* were determined using spectrophotometric method with aluminium chloride. Quercetin was used as standard and total flavonoid content was expressed in µg quercetin equivalence (µg QE/mg of extract). The total content of flavonoids in extracts was determined from the regression of the calibration curve (y=0.0033x–0.0064, R^2 = 0.9934) expressed in Figure 5. The total flavonoid contents in ethanolic and aqueous extracts of red and white flowers are indicated in Table 4 and histogram shown in Figure 6. It was observed that red flower variety possessed the highest amount of flavonoids than those of white flower variety. Both ethanolic and aqueous extracts of red flower contained 356.85 µg QE/mg and 285.33 µg QE/mg of extract, respectively, however, 45.33 µg QE/mg and 22.30 µg QE/mg of total flavonoids were observed in

white flowers. Since the various colour of plant parts are caused by the flavonoid compounds, red flowers of *S. grandiflora* contain more content of flavonoids.

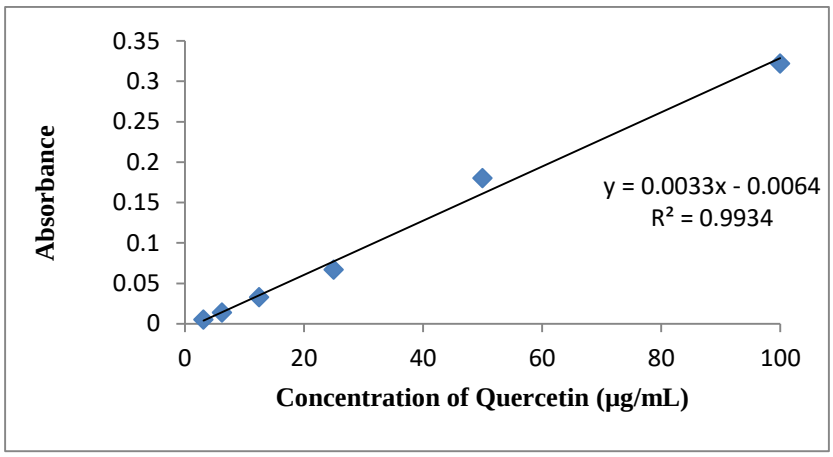

Figure 5 Standard curve of quercetin

Table 4 Total Flavonoid Contents in Red and White Flower Petals of *S. grandiflora*

Extract (250 µg/mL)	Total Flavonoid Content (µg QE/mg of extract)	
	Red Flower	White Flower
Ethanol	356.85	45.33
Aqueous	285.33	22.30

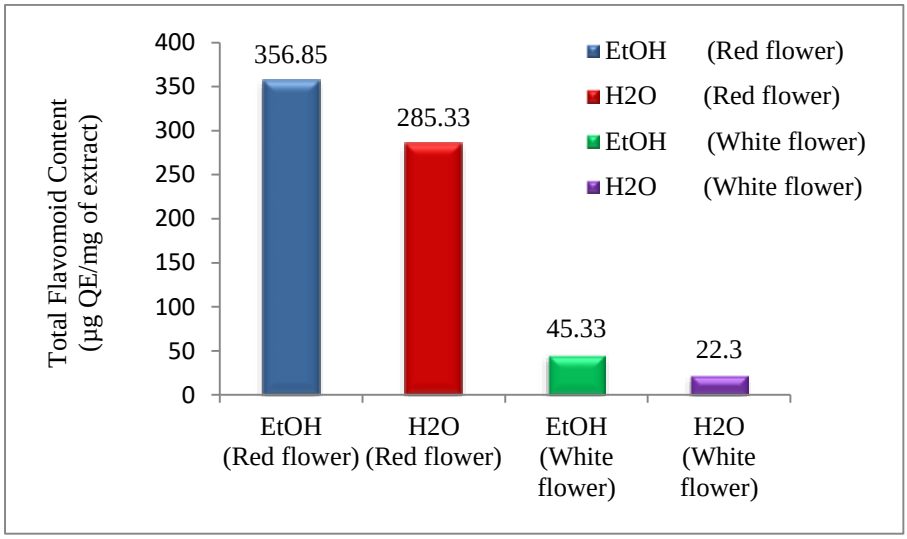

Figure 6 Histogram of total flavonoid content of flower petals of *S.grandiflora*

Evaluation of Antioxidant Activity

The antioxidant activity of ethanolic and aqueous extracts from flower petals of *S. grandiflora* was determined using DPPH reagent. DPPH is very stable free radical. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench DPPH free radicals and converted them into a colourless or yellow product, resulting in a decrease in absorbance at 517 nm (Amarowicz *et al*., 2003).

The antioxidant activity of different extracts from flowers of *S.grandiflora* is expressed in terms of percentage of radical scavenging activity (% RSA) and IC_{50} values. The radical scavenging activities of flower extracts of *S. grandiflora* are shown in Table 5 and Figure 7. The obtained values of % RSA varied from 5.20 % to 82.13 % in various dosages of sample extracts. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50 % of DPPH radical. The lower the IC_{50} value, the higher the antioxidant activity of the samples (Li *et al*., 2009). The observed IC_{50} values of each extract and standard ascorbic acid are expressed in Table 6. The highest capacity to scavenge DPPH radicals was found in ethanolic extracts of both red and white flowers varieties with IC_{50} values of 213.67 µg/mL and 322.21 µg/mL, respectively. A moderate activity was observed in aqueous extracts of both red and white species of *S.grandiflora*.

Table 5 Radical Scavenging Activity of Red and White Flower Petals of *S.grandiflora*

Concentration (µg/mL)	% RSA			
	Red Flower		White Flower	
	EtOH extract	H_2O extract	EtOH extract	H_2O extract
25	14.61	5.33	10.50	5.20
50	18.54	8.00	14.67	7.82
100	33.03	12.00	20.43	10.34
200	48.31	23.33	36.85	20.57
400	73.03	49.33	58.37	40.24
800	82.13	64.00	70.32	50.09

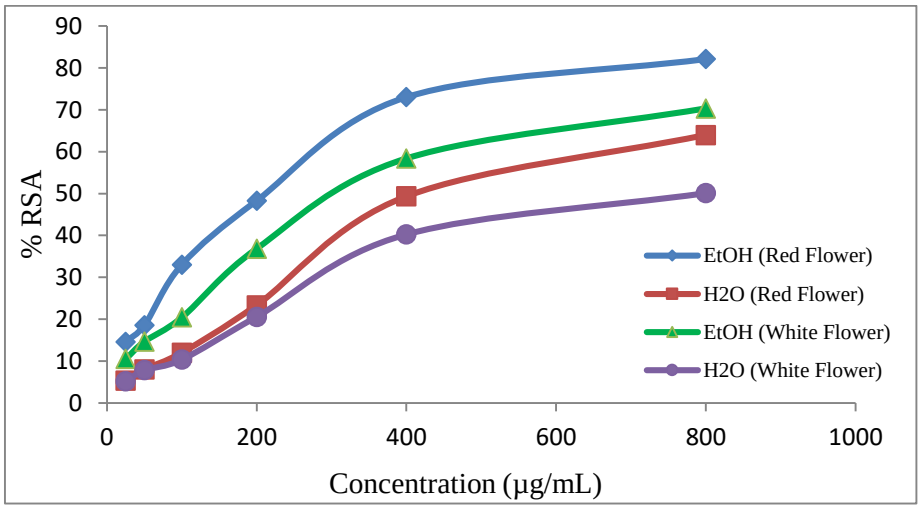

Figure 7 Radical scavenging activity of red and white flower petals of *S.grandiflora*

Table 6 IC_{50} Values of Ascorbic Acid and Extracts of *S. grandiflora*

Sample		IC_{50} Value (µg/mL)
Red Flower	EtOH extract	213.67
	H_2O extract	417.98
White Flower	EtOH extract	322.21
	H_2O extract	797.15
	Ascorbic Acid	6.25

Conclusion

The present study reported the nutritional values, phytochemicals, the total phenolic, total flavonoid contents and antioxidant activity of red and white flower varieties of *S. grandiflora*. Ethanolic extracts of both varieties were found to possess the higher phenolic and flavonoid contents and showed the higher antioxidant activity compared to aqueous extract. The results of this work suggested the importance of *S. grandiflora* as a source of active constituents known for their antioxidant properties.

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