

Chemical compositions, nutritional values, total phenolic content, and antioxidant activity from *Eleocharis dulcis*

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Abstract: Several wild crops remain to be explored worldwide; among them, some have excellent medicinal and nutritional properties. In this study, *Eleocharis dulcis* has been selected for investigation of its chemical composition, nutritional values, total phenolic content, and antioxidant activity. The phytochemical constituents were screened by standard methods, indicating the presence of alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, polyphenol, reducing sugars, saponins, starch, and tannins. In a preliminary investigation by EDXRF, potassium, iron, chlorine, silicon, calcium, phosphorus, sulfur, manganese, bromine, copper, titanium, zinc, and rubidium elements were found in the EDXRF spectrum. It was found that potassium content is the highest among other elements. The nutritional values of the selected samples were determined by AOAC methods. The total phenolic content of aqueous extract ($5.115 \pm 0.05 \mu\text{g GAE/mL}$) was determined by Folin-Ciocalteu assay. The antioxidant activity of aqueous extract was evaluated by the 2, 2-diphenyl-1-picryl-hydrazyl free radical scavenging assay. It possesses nearly the antioxidant activity of standard ascorbic acid. It is suggested that *Eleocharis dulcis* exhibits great potential for antioxidant activity, and it is useful for nutritional and pharmacological functions.

Introduction

In recent years, there has been interest in phenolic compounds derived from fruits and vegetables for their possible health benefits [1, 2]. Fruits are a valuable nutritional component and they have essential nutrients such as minerals, vitamins, phytochemical constituents and bioactive compounds [3]. Numerous wild crops remain to be explored, among them, some have excellent medicinal and nutritional properties. Non-conventional foods, likewise conventional foods such as wild fruits, are consumed on special occasions [4]. Extensive research for the phytochemical and nutritional compositions of the available food sources continues to be conducted [5-7]. Although non-conventional foods are consumed widely and contribute to several health benefits, there is a need to evaluate the efficiency of wild and non-conventional fruit plants for the inclusion of the information regarding their health benefits [8].

Eleocharis dulcis, also called water chestnut in Myanmar, is a grass-like sedge native to Asia, tropical Africa, and Oceania. It is an aquatic plant that grows in marshes, underwater, or in mud [9, 10]. *Eleocharis dulcis* is one of

the most popular hydrophytic vegetables in Asia because of its unique taste [11]. It is grown in many countries for its edible corms. Nutritional and medicinal qualities have been recognized in India, China, Hong Kong, Malaysia, Thailand, and Russia. They also play a major role in the aging process [1, 12]. *Eleocharis dulcis* has also been used as a folk medicine to treat chronic diseases, hypertension, sore throat, laryngitis and enteritis [13]. According to the research information, the phenolic compound constituents present in *Eleocharis dulcis* and their antioxidant activity. The effects of phenolic compounds are reported to be generally associated with their antioxidant properties of eliminating free radicals [2, 14]. Production of excess number of free radicals and lipid peroxidation is involved in some chronic diseases such as cardiac, neurodegenerative disorders, carcinogenesis, diabetes, and rheumatic disorders [9, 15-17]. Traditional medicinal wild fruits also provide therapeutic benefits, including potent antioxidant actions [18-20]. Thus, the wild fruits have been investigated as possible food supplements and cost-effective alternatives to commercially available fruits. This study was to evaluate nutritional values, the total phenolic content and antioxidant properties from *Eleocharis dulcis*. According to the observations, the nutritional, and medicinal fruit, *Eleocharis dulcis*, may be useful to prevent the free radical reactions associated with diseases like tumors or cancer and age-related problems.

Materials and methods

Sample collection: Mature samples of *Eleocharis dulcis* were collected from Bilumyo village, Mohnyin Township, Kachin State, Myanmar and identified by the Department of Botany, Mohnyin University, Myanmar. The sample was sliced into small pieces and allowed to be air-dry at room temperature, and made into powder by using the grinding mill. The dried powdered was stored in an air-tight glass bottle for the whole investigation.

Preliminary phytochemical screening: The preliminary qualitative phytochemical screening for the detection of different phytoconstituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, polyphenol, reducing sugars, saponins, starch, and tannins was tested by using standard methods. Each of the tests was qualitatively expressed as negative (-) or positive (+) [21, 22].

Determination of elemental analysis: The elemental analysis of *Eleocharis dulcis* was determined by Energy-dispersive X-ray fluorescence spectrometry (NEX CGII EDXRF spectrometer) at the Department of Chemistry, University of Mandalay.

Analysis of nutritional values: Analysis of the nutritional values of the sample was performed according to the procedures described by the AOAC methods. The moisture content was determined by the oven drying method, fat content by the Soxhlet extraction method, fiber content by the fiber cap method, ash content by ashing in furnace method, carbohydrate content by the phenol-sulphuric acid method, and protein content by the trichloroacetic acid method [23].

Quantitative determination of total phenolic content: Total phenolic content was determined quantitatively by using sophisticated methods, using Folin-Ciocalteu reagent by colorimetric method [24]. Apparatus used for the determination of total phenolic content were micropipettes, test tubes, quartz cells and water bath and the chemicals: Sodium carbonate, Folin-Ciocalteu reagent, gallic acid, and distilled water, respectively.

Preparation of standard gallic acid: 10.0 mg of the standard gallic acid was added to 10.0 mL of distilled water. 1.0 mL of this solution was taken in another test tube. The volume of the solution was made up to 10 mL with distilled water. The solution was taken into a series of 20 μ L, 40 μ L, 60 μ L, 80 μ L, and 100 μ L, respectively. Volume was made up to 1.6 mL, and then 100 μ L of Folin-Ciocalteu reagent and 300 μ L of saturated Na_2CO_3 (20.0%) solution were added. After each standard solution was heated at 40°C for 30 min, the absorbance of the various concentration solutions was measured by a UV-2550 spectrophotometer at 765 nm.

Determination of total phenolic content in *Eleocharis dulcis*: Total phenolic content in *Eleocharis dulcis* was measured with the Folin-Ciocalteu reagent. 10.0 µL of the sample was made up to 1.6 mL with distilled water and mixed with 100 µL of Folin-Ciocalteu reagent. Then, 300 µL of saturated sodium carbonate (20.0%) solution was added. Mixture was heated at 40°C for 30 min and then it was allowed to stand to cool. Absorbance of this prepared sample solution was measured at 765 nm using UV-2550 spectrophotometer. Then the total phenolic content in the sample was calculated from the standard curve of gallic acid.

Determination of antioxidant activity: Antioxidant activities of the sample were determined by DPPH free radical scavenging assay [25-27]. The control solution was prepared by mixing 2.0 mL of 60.0 µM DPPH and 2.0 mL of methanol. The test solutions were prepared by mixing 2.0 mL of the test sample solution and 2.0 mL of 60.0 µM DPPH solutions with various concentrations. The test sample solutions were allowed to stand at room temperature for 30 min. Then the absorbance of each solution was measured at 517 nm by using UV-2550 spectrophotometer.

Results and discussion

Preliminary phytochemical screening: Phytochemical screening of *Eleocharis dulcis* was screened by standard method, indicating the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, polyphenol, reducing sugars, saponins, starch, and tannins (**Table 1**). Therefore, this study may provide a valuable scientific base for nutritional values and their uses in the remedy.

Table 1: Phytochemical screening of *Eleocharis dulcis*

Compounds	Extract	Reagents	Observations	Remarks
Alkaloids	1.0% HCl	Dragendorff's reagent	Deep blue ppt.	+
		Wagner's reagent	Brown colour solution	+
		Mayer's reagent	No ppt.	-
Carbohydrates	H ₂ O	1.0% α-naphthol, conc. H ₂ SO ₄	Red ring colour	+
Flavonoids	Ethanol	Dil. HCl, Mg turning	Brown ppt.	+
Glycosides	H ₂ O	10.0% lead acetate	White ppt.	+
Phenolic compounds	Ethanol	1.0% FeCl ₃ , 1.0% K ₃ Fe (CN) ₆	Greenish blue colour solution	+
Polyphenols	Ethanol	1.0 % FeCl ₃	Bluish Black ppt.	+
α amino acid	Ethanol	Ninhydrin reagent	Purple colour on the filter paper	+
Reducing sugars	H ₂ O	Benedict's solution	Reddish Brown ppt.	+
Saponins	H ₂ O	Distilled water, shake	Froth	+
Starch	H ₂ O	1.0% Iodine solution	Blue ppt.	+
Tannins	H ₂ O	1.0% FeCl ₃ , 1.0% NaOH	Brown colour solution	+

-: negative and +: positive

Elemental analysis: The observed elements in *Eleocharis dulcis* by measuring EDXRF were organized in **Table 2**. From the results, many elements were present in the samples. It can be remarked that potassium is the highest value and followed by iron, chlorine, silicon, calcium, phosphorus, sulfur, manganese, bromine, copper, titanium, zinc, and rubidium. The trace amounts of other elements were also present in the samples.

Nutritional values: Nutritional values determined by standard AOAC methods were found to be ash (8.2%), carbohydrate (34.65%), fat (0.22%), fiber (7.6%), moisture (43.9%), and protein (1.11%), **Table 3**. Thus, carbohydrate content has the highest composition among other nutrients. Not only the carbohydrate but also the fat is the body's main fuel source and, important structural and metabolic functions. Moisture and ash contents are most important for measuring in the testing of food products and the quality of food.

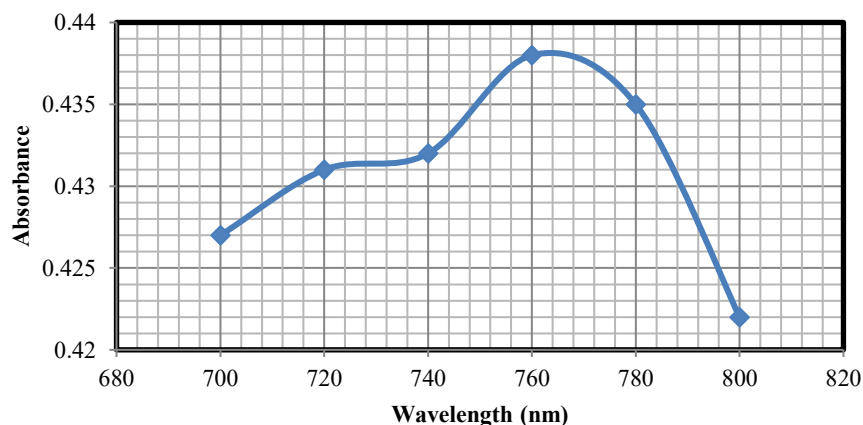
Table 2: Relative abundance of elements in *Eleocharis dulcis*

Components	Analyzed value (%)	Components	Analyzed value (%)
K	66.9000	Mn	0.2050
Fe	9.6100	Cr	0.1100
Cl	6.5600	Sn	0.1010
Si	4.0300	Rb	0.0992
Ca	3.9100	Br	0.0631
P	2.6400	Te	0.0516
Si	2.4300	Co	0.0458
Al	1.3100	Sr	0.0246
Ti	0.8300	Sb	0.0171
Ni	0.4830	W	0.0139
Cu	0.2790	Ga	0.0121
Zn	0.2540	V	0.0114

Table 3: Nutritional values in *Eleocharis dulcis*

Nutrients	(%)
Ash	8.2
Fat	0.22
Fiber	7.6
Carbohydrate	34.65
Moisture	43.9
Protein	1.11

Determination of total phenolic content: Total phenol content was measured with the Folin-Ciocalteu colorimetric method using gallic acid as a standard. The absorbance of *Eleocharis dulcis* (10.0 μ L) was measured with a UV-visible spectrophotometer at 765 nm with respect to the blank solution. The maximum wavelength and wavelength with corresponding absorbance of the standard gallic acid solution are shown in **Figure 1**. The results of the absorbance of standard gallic acid and the calibration curve are shown in **Figure 2**. Results of absorbance of *Eleocharis dulcis* were shown in **Table 4** and the total phenolic content in *Eleocharis dulcis* was shown in **Table 5**. Free radical scavenging of phenolic compounds is a primary important property of the various pharmacological and biological activities.

**Figure 1:** Maximum wavelength of standard gallic acid

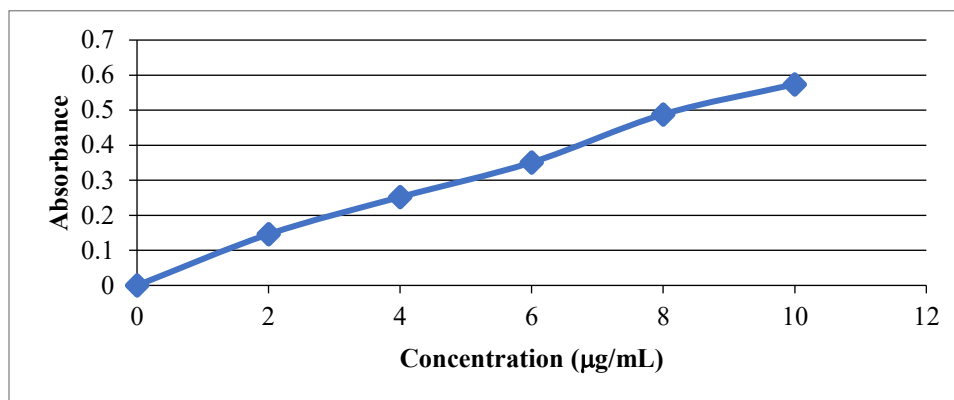


Figure 2: Calibration curve for standard gallic acid solution

Table 4: Findings of the absorbance of *Eleocharis dulcis*

Sample	Absorbance	Concentration (mg/mL)
Test 1	0.302	5.093
Test 2	0.301	5.076
Test 3	0.307	5.177

Table 5: Total phenolic content in *Eleocharis dulcis*

Sample	Phenol (mg/mL)	Phenol (mg/mL) Mean±standard deviation
	5.093	
<i>Eleocharis dulcis</i>	5.076	5.115±0.05
	5.177	

From these results, the amount of total phenolic content of the sample was obtained by using the standard graph. The total phenolic contents present in the sample were expressed as gallic acid equivalent, which was shown in **Table 5**, and there was found to be 5.115 mg of gallic acid equivalent per milliliter.

Antioxidant activity: The IC_{50} value is a widely used parameter. Radical scavenging activity was expressed in terms of IC_{50} . It means that the lower IC_{50} value, the higher antioxidant activity. The concentration, percentage of inhibition and IC_{50} values of standard ascorbic acid and aqueous extract of *Eleocharis dulcis* are shown in **Table 6**. According to the result, the aqueous extract exhibited significant IC_{50} values of antioxidant properties, which were compared with standard ascorbic acid. From the DPPH scavenging assay, the IC_{50} value of ascorbic acid was found to be 2.59 µg/mL and 0.486 mg/mL (486 µg/mL) for aqueous extract. The calibration curve for ascorbic acid and aqueous extract of *Eleocharis dulcis* in µg/mL (**Figures 3 and 4**). The comparison of the percentage of inhibition of standard ascorbic acid and aqueous extract of *Eleocharis dulcis* in µg/mL (**Figure 5**). Antioxidant potency of water extract was concluded to be medium compared with the potency of standard ascorbic acid. Therefore, the strong antioxidant activity of *Eleocharis dulcis* phenolics may be useful for their food nutrition and therapeutic functions. By preventing oxidative stress, antioxidants such as polyphenols become important for the maintenance of health and effective against cardiovascular diseases and cancer [19, 28, 29]. Present research was conducted with a view to recognizing the exact nutritive values and antioxidant potential of *Eleocharis dulcis* for human health, the possible role as a detoxifying agent clinically, and to augment awareness regarding *Eleocharis dulcis* consumption as a medicinal food among health-conscious people. Thus, a need to optimize extraction techniques for enhancing the yield and bioavailability of active compounds from *Eleocharis dulcis*. Clinical studies and *in vivo* experiments are vital to validate its antioxidant and therapeutic efficacy.

Table 8: Inhibition (%) and IC₅₀ of standard ascorbic acid and aqueous extract of *Eleocharis dulcis*

Sample	Parameters	Results					IC ₅₀ (µg/mL)
Ascorbic acid	Concentration	1	2	4	8	16	2.59
	% Inhibition	26.98	46.29	66.98	86.14	88.37	
Aqueous extract	Concentration	2	1	0.5	0.25	0.125	486
	% Inhibition	66.86	61.88	53.37	46.04	40.76	

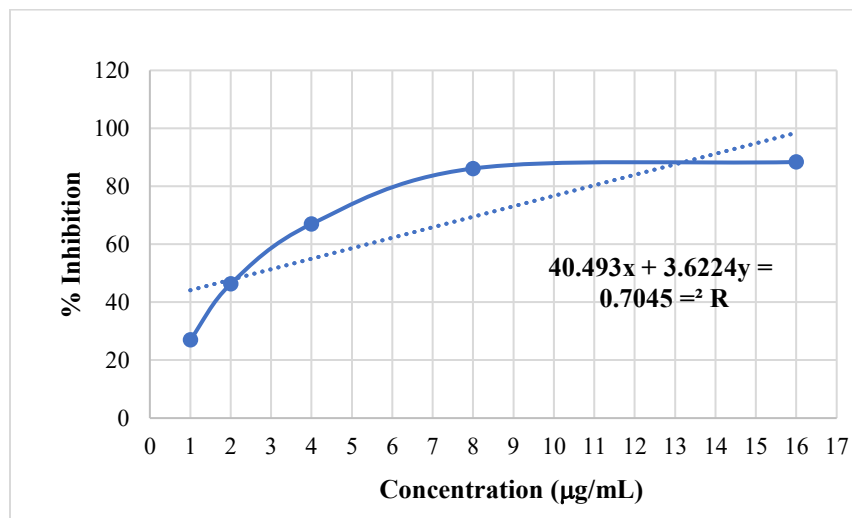


Figure 3: Calibration curve for ascorbic acid

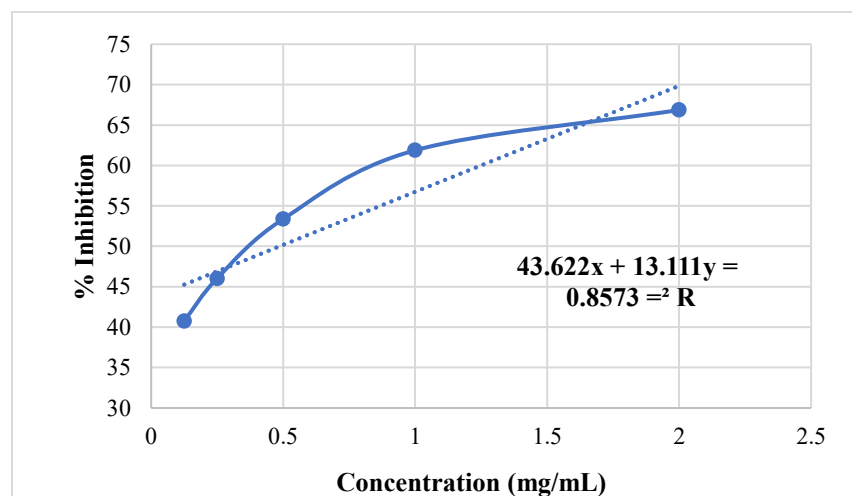


Figure 4: Calibration curve for aqueous extract of *Eleocharis dulcis*

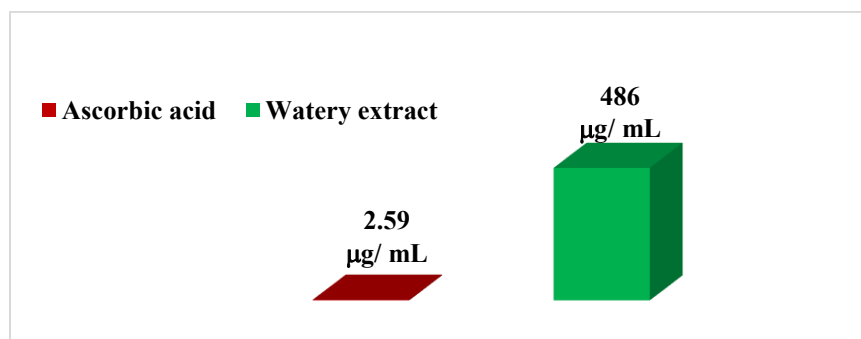


Figure 5: Inhibition of standard ascorbic acid and aqueous extract of *Eleocharis dulcis* (µg/mL)

Conclusion: Phytochemical screening confirmed several bioactive compounds, while elemental analysis showed high potassium and other essential minerals. Nutritional analysis revealed high carbohydrate and fiber content, supporting its dietary value. The total phenolic content and DPPH assay indicated moderate antioxidant activity compared to ascorbic acid. Overall, *Eleocharis dulcis* demonstrates potential as a nutrient-rich functional food with antioxidant and therapeutic benefits, supporting its application in diets, nutraceuticals, and further research in food science and pharmacology.

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Author contribution: KSSH conceived, designed the study and collected data Both authors performed and interpreted the analysis. Both authors drafted the manuscript and agreed to be accountable for its contents.

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Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.

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