Study on phytoconstituents and bioactive properties of stem lettuce *(lactuca sativa)*

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Abstract

In this present research, the stem of lettuce, family -Asteraceae was chosen to be studied. The nutritional values were determined by AOAC method, such as moisture, ash, protein crude fiber, fats and carbohydrate in sample. The values of the content are 9.01%, 5.80%, 26.55%, 6.50%, 1.53% and 50.61% respectively. The antioxidant properties of ethanol and watery extracts of leaves were investigated by DPPH free radical scavenging assay. The IC50 value of these extracts were observed to be 7.24 g/mL and 21.77 g/mL respectively.

Keywords: Stem lettuce, nutritional values, antioxidant activity.

Introduction

Celture, also called stem lettuce, celery lettuce, as paragus lettuce, or Chinese lettuce is a cultivar of lettuce grown primarily for its thick stem or its leaves. It is used as a vegetable and is especially popular in both main land and china and Taiwan, where the stem is interchangeably called quigsun or wosun. Lettuce belongs to the family Asteraceae and is known as slad - yo , in Myanmar. The stem is usually harvested at a length of around 15-20 cm and a diameter of around 3-4 cm. It is crisp, moist, and mildly flavoured , and typically prepared by slicing and then stir-frying with more strongly flavoured ingredients. It indicated in the treatment of inflammation and osteodynia (pain in the bones) , Alzheimers , lower blood pressure and prevent heart diseases, stomach cancer , lung cancer , diabetes , moderating sleep and provided a constant supply of antioxidants.

The present research work was aimed to study the chemical investigation such as phytochemical tests, nutritional values and elemental analysis. Moreover, the biological investigation of antioxidant activity of stem lettuce was studied.

Botanical description of Stem Lettuce *Lactuca sativa.* is as follows:

Species	-	L.sativa *(Lactuca sativa)*
Family	-	Asteraceae
Myanmar name	-	Slad – Yo
English name -	-	Stem Lettuce
Parts used	-	Stem

Figure 1. Photographs of Stem Lettuce *(Lactuca sativa)*

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Research Objectives

To investigate the nutrient values

To investigate the elemental analysis

To access the antioxidant activity of the sample

Materials and Methods

Plant Material

The stems of Lettuce were collected from the Taunggyi Township. The collected plant was identified as Lettuce *(Lactuca sativa)* by the authorized botanist at Department of Botany, Yangon University. The dried samples were cut into pieces and pulverized into powder using a grinding mill. The dried powdered samples were then stored in the air tight container.

Preparation of Plant Extract

The dried powder(100g) of as Lettuce *(Lactuca sativa)* was extracted with 70% EtOH at R.T(3 x 300 ml) for one week and filtered. The filtrate was evaporated by rotatory evaporator to get 70% EtOH extract. The dried powder of the plants was also boiled with water to get watery extract. The dried powdered samples (300g) were extracted with methanol (500 ml) for one week by maceration followed by filtration. This procedure was repeated three times. The total combined filtrate was concentrated by rotatory evaporator one-third volume and further partitioned between pet-ether and water to get the particular soluble extract. The aqueous layer was further dry over water bath to get dry methanol extract. The resulting extract was partitioned with ethyl acetate and ethyl-acetate soluble portion was concentrated by rotatory evaporator and dried over water-bath to get ethylacetate extract. The dried extracts obtained were then stored and kept in the refrigerator for the isolation of phytochemical organic constituents and for screening of bioactivity.

Determination of Nutritional Values, Phytoconstituents and Elementary Analysis

The amount of nutrients such as moisture, ash, fat, carbohydrate, fiber, protein, and calories in the samples were determined by recommended analytical methods. Preliminary phytochemical investigation was carried out according to the standard procedures (AOAC). For the standardization and quality control in dry powder samples were also determined by EDXRF spectrometer, at Taunggyi University.

(a) Determination of Moisture Content

The moisture content of the dried powder was determined by using Air Oven Method.

Procedure

Amount of 2-5 g well prepared test portion was placed in dish. The sample was dried in an air oven at 130°C for 1 hour. After drying was completed, lids were replaced on dishes and transferred to desiccators and then cooled for 30 minutes. It was weighed accurately until constant weight was achieved.

(b) Determination of Total Ash Content

The determination of percentage of the total ash and acid-insoluble ash values of powdered sample is carried out according to the Ash method (AOAC 2000, 930.05)

Procedure

The crucible appearance was checked. The empty crucible and cover were heated in Muffle furnace at 574 ± 25 °C for 15 minutes and placed in a desiccators and then cooled for 45 minutes when a porcelain or silica crucible was used or for 15

minutes in when using platinum. Nearest 0.1 mg of the prepared test sample was weighed. A suitable test specimen may be about 3 to 5 mg. At that time, test specimens were weighed for moisture determination. The test specimen was transferred to the prepared crucible and placed it, with the cover removed. The test specimen could also be carbonized on a gas burner over a low flame or other technique. The crucible with sample was placed in the Muffle furnace without cover. The temperature was raised gradually to 575 ± 25 °C. Heating should be done after placing the crucible with sample into the Muffle furnace. If Muffle furnace has been up before hand, sample is burned immediately and serenely. Muffle furnace should be cooled down before putting sample into it. The material was allowed to ignite at 575 ± 25 °C for a period of 4 hours or longer, if needed to turn away all the carbon. Complete ignition was indicated by the absence of black particles. After ignition was finished completely, the crucible was kept in the Muffle furnace for 2 hours for cooling down to 300 °C. The lids of Muffle furnace were not opened during the cooling down. After cooling down, the crucible was replaced into desiccators. The crucible was covered and cooled for 30 minutes. The calculation of ash results are shown in Table 1.

(c) Determination of Protein Content

Procedure

The protein content was determined by AOAC method (AOAC, 1990). The procedure was as follows: The representative sample (1 g), 50 mL of distilled water, 5 g of digestion mixture (4 g of potassium sulphate and 1 g of copper sulphate) and 15 ml of concentrated sulphuric acid were added into a preheated flask and gently shaken to 'wet' the sample with acid. The exhaust system was attached to the digestion tubes in the rack and wet the water aspirator to full effect. The loaded rack was exhausted into a preheated digestion block (420 °C). After about 5 minutes, turn down the water aspirator until the acid fumes were just contained within the exhaust head. Digestion continued until all samples were clear with a blue or green solution. The rack of tubes with exhaust still in place in the stand was removed to cool for 10-20 minutes. Using a commercial air blower can increase cooling. 80 mL of deionized water was added to the tubes. 25-30 mL of receiver was added to a conical flask and placed it into a distillation unit and then raised the platform so that the distillate outlet was submerged in the receiver solution. 50 mL of 40 % NaOH was dispersed into the tube. The steam valve on the Kjeldahl 1002 was opened and distilled for approximately 4 minutes. The end of the distillation cycle when using Kjeldahl 1002 was closed by the steam valve. The receiver solution in the distillate flask will now be green indicating the presence of an alkali-ammonia. The distillate was titrated with standardized HC 1 (usually 0.100 N or 0.200 N) until the blue or grey end point was achieved. The amount of the protein content was given in Table 1.

(d) Determination of Fiber Content

Procedure

The powdered samples (Ca. 2 g) were weighed and. extracted with petroleum ether (30 mL) by stirring, settling and decanting for three times. The extracted sample was air dried and transferred to a round-bottom flask. Sulphuric acid (0.1275 M) was added to disperse the sample followed by hot sulphuric acid (0.1275 M, 170 mL). The solution was then refluxed for about 30 minutes and filtered through a Buchner funnel. The insoluble matter was washed with boiling water until the final wash liquid was free from acid. The residue was rinsed back into the flask with sodium hydroxide (0.313 M, 30 mL) and 170 mL of boiling sodium hydroxide was added. The mixture was again refluxed for 30 minutes and filtered using a Gooch crucible with an

asbestos pad. The residue was washed with boiling water, 1 % hydrochloric acid and again with boiling water until wash liquor was free from acid. It was then washed twice with ethanol and three times with ether. The crucible was dried at 100 °C and weighed. The fiber content was determined by the loss in weight on ignition at 100 °C.

(e) Determination of Fat Content

Accurately weighed 3 g of powdered sample (in a fine form) were weighed accurately and placed into a thimble lined with a circle of filter paper. Thimble and content were then placed in a Soxhlet extractor. Petroleum ether (60-80 °C) was poured into the extractor until some of it over-flowed into the flask. The petroleum ether was heated by means of a mantle. The extraction was assumed to be completed when a small amount of extract placed on a watch glass did not leave any residue on evaporation of solvent. A duration of about 6-8 hours was required for the complete extraction during which time the petroleum ether was recycled for about 27 times.

(f) Determination of Carbohydrate

Carbohydrate content of sample can be obtained as the difference between 100 and the sum of the percentage of moisture, ash, protein, fiber and fat contents (Vogel, 1956).

Screening The Antioxidatant Activity of Crude Extracts of as Lettuce *(Lactuca sativa)* **by DPPH Assay.**

Preparation of Solutions

(i) Preparation of 60 µM DPPH solution

2.364 mg of DPPH was thoroughly dissolved in 100 mL of 95% ethanol. This solution was freshly prepared in the brown coloured flask. Then, it must be stored in the fridge for no longer than 24 hours.

(ii) Preparation of Test Sample Solution

Accurately weighed 2 mg of each test sample and 10 mL of 95% EtOH were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. Desired concentrations 20µg/mL, l0µgmL4, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL and 0.625 µg/mL of each solution were prepared from this stock solution by dilution with appropriate amount of 95% ethanol.

(iii) Preparation of Blank Solution

Blank solution was prepared by mixing 1.5 mL of test sample solution with 1.5 mL of 95% ethanol.

Procedure

DPPH radical scavenging activity was determined by UV spectrophtometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol using shaker. The sample Solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solutions and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the equation and then IC_{50} (50 % inhibitory concentration) value was also calculated by linear regressive excel program. The percent inhibition of oxidation and 50 % inhibitory concentration of stem Lettuce *(Lactuca sativa)* were also presented in Table 3.

Results

Nutritional Values of as Lettuce *(Lactuca sativa)*

The quantitative analyses for the determination of total ash and moisture contents have been done according to methods described in the British Pharmacopoeia (1980), Myanmar medicine formulary (1989) and Ayuvedic formulation (1976). The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away.

The nutritional values such as protein, fiber, fat and carbohydrates were also determined by appropriate methods. The fat content determined by the soxelt extraction method was 1.53 %. In addition, the sample was also studied for fiber content by acid alkali treatment, protein content by AOAC method and ash content by using Muffle furnace. The protein, fiber, carbohydrates, moisture and ash contents for as Lettuce*(LACTUCA SATIVA)* were found to be 26.55 %, 6.50 %, 50.61 %, 9.01 % and 5.80 %, respectively. The results are shown in Table 1.

Table 1. Nutritional Values of the Stem Lettuce *(Lactuca sativa)*

No.	Test Parameter	Content (%)
1.	Moisture	9.01
2.	Ash	5.80
3.	Protein	26.55
4.	Fiber	6.50
5.	Fat	1.53
6.	Carbohydrate	50.61

EDXRF elemental analysis of stem Lettuce *(Lactuca sativa)* showed that Ca and K are predominantly present together with Fe, Mn and Zn. Ca is the key for the health of bones and teeth, but it also affects muscle, hormones and nerve function. The primary function of potassium in the body includes regulating fluid balance and controlling the electrical activity of heart and other muscles. Potassium supports blood pressure, cardiovascular health, bone strength and muscle strength (Andhiwal, C. K. *et al.*,1981). According to EDXRF, no toxic element was found in stem. The results are shown in Table 2.

Table 2 Relative Abundance of some Elements in stem Lettuce *(Lactuca sativa)*

No.	Elements	Relative Abundance (%)
1.	K	2.033
2.	Ca	0.321
3.	Fe	0.013
4.	Mn	0.073
5.	Zn	0.003
6.	S	0.317
7.	Rb	0.002
8.	Cu	0.002
9.	P	0.268

Antioxidant Activity of Crude Extracts from as stem Lettuce *(Lactuca sativa)*.

The present study was carried out to investigate the radical scavenging activity of the leaf samples using two crude extracts such as ethanol, ethyl acetate and water extracts, by using DPPH assay according to the spectrophotometric method. In this experiment, six different concentrations (0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wave length 517 nm using UV visible spectrophotometer. Each experiment was done triplicate. The results are shown in table 2 and figure 1 and 2.

The IC_{50} values were found to be 21.77 µg/mL for ethanol extract, 7.24 µg/mL for aqueous extract. Among these extracts, since the lower the $1C_{50}$ showed the higher and free radical scavenging activity ethanol extracts that were found to be more effective than aqueous extracts in free radical scavenging activity. However, it was observed that all of these extracts have the higher antioxidant activity than standard ascorbic acid ($1C_{50}$-1.92 µg/mL).

Table 3. Radical Scavenging Activity (%RSA) and IC_{50} values of Ethanol and Watery Extracts from Leaves and Standard Ascrobic Acid

Samples	% Inhibition (Mean ± SD) In Different Concentration (g/ml)						IC_{50} (g/ml)
	0.625	1.25	2.5	5	10	20	
Ethanol	21.52 ± 0.033	30.30 ± 0.002	35.33 ± 0.010	55.15 ± 0.003	70.30 ± 0.004	77.8 ± 0.001	21.77
Watery	34.55 ± 0.009	49.09 ± 0.001	54.85 ± 0.003	56.36 ± 0.012	59.70 ± 0.004	63.33 ± 0.003	7.24
Ascrobic acid	25.20 ± 0.00	45.58 ± 0.00	55.53 ± 0.00	64.82 ± 0.00	73.32 ± 0.00	75.23 ± 0.00	1.92

Conclusion

From the overall assessment of some chemical and biological investigation of banana flower, the following inferences could be deduced.

The nutritional values of as Lettuce *(Lactuca sativa)*. contain moisture (15.3%), ash (5.80%), fat (1.53%), fibre (6.50%), protein (26.55%), carbohydrate (50.61%) kilocalories in 100 g of sample. According to the results, the presence of the important nutrients like fat, fibre, protein, carbohydrate and the physical properties like moisture and ash mean the selected sample could be used as a nutritionally valuable and healthy ingredient to improve traditional medicinal formulation and to treat many diseases.

In the antioxidant activity, the IC_{50} values were found to be 21.77 µg/mL for 95 % ethanol extract and 7.24 µg/mL for watery extract and 1.92 µg/mL for standard ascrobic acid. Among these extracts, since the lower the IC_{50} showed the higher free

radical scavenging activity, watery extract was found to be slightly more effective than the 95 % ethanol extract.

From these observations, Lettuce *(Lactuca sativa)* has a lot of nutritional values and medicinal qualities.

Acknowledgements

I would like to express my profound gratitude to Rector, Kyaw Kyaw Gaung , and Pro-rector Dr. Nilar Aung , East Yangon University for their encouragements throughout the course of research work.

I would to express my sincere thanks to Dr. Tin May San (Professor & Ret) and Dr.Tin may San (Professor & Head) , Department of chemistry East Yangon University , for their kind heart with very effective suggestions , advices and encouragement and allowing me to use the facilities in the Department from the beginning of enrollment to the submission of this research paper.

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