

Proximate Composition, Nutritional Evaluation and Mineral Analysis in the Leaves of an Indigenous Medicinal Plant, *Alternanthera Sessilis*

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ABSTRACT

Plants are the good source of essential nutrients and minerals that contribute to the well being of an individual. On proper supplementation through diet, they can acquire, convert, allocate, distribute and pave way for proper utilization of all the essential components in the biological system. The present investigation is focused on the proximate analysis and nutritional evaluation of minerals in the leaves of the indigenous medicinal plant, *Alternanthera sessilis*. Percentage of moisture content, total ash, carbohydrate, protein, crude fat and crude fiber were analysed. It was revealed from the study that the edible portion, the leaves of the plant was found to contain high carbohydrate, protein and fiber. Poorer levels of fat were reported. Moisture content was also found to be higher. About 10 macro and microelements were evaluated by atomic absorption spectroscopy (Na, K, Ca, P, Mg, Mn, Cu, Zn, Fe and Cr). All the mineral contents correlated with RDA values. Among them, Iron and Manganese were present in higher proportions. The plant was found to be a rich source of macro and micro elements in sufficient quantities. The results of the present study indicated that the regular consumption of this plant would be a boon to combat malnutrition and other health disorders.

Keywords: *Alternanthera sessilis*, Proximate analysis, Mineral analysis, RDA, Macro and microelements, malnutrition.

INTRODUCTION

Plants are the ebullient gift of Mother Nature which houses an enormous amount of compounds that possess therapeutic properties. Naturally, plants possess primary and secondary metabolites which play a significant role in regulating some of the vital functions necessary for plant growth and development. Among them, the green leafy plants, consumed as food harbours essential minerals like Ca, Fe, Zn, Mg, Cu, Mn etc., and nutrients such as carbohydrates, proteins, fats and crude fiber. A huge amount of vitamins and hormone precursors are also present in the plants. [1]

The significant usage of medicinal plants from the ancient period to present date reveals the importance in the treatment of various diseases. The physicochemical studies deal primarily with the adulterants detection and also to ensure the quality and purity of the drug. [2]

Alternanthera sessilis belongs to the family Amaranthaceae. Traditionally, the leaves of the plant have been used in the treatment of skin and eye ailments, cuts and wounds and as an antidote for snake bite. [3] In the recent years, malnutrition is an overwhelming problem in the developing countries. Poor nutrition and inadequate

supplements in the food thrive to be a reason for many diseases. This could be surpassed by the intake of foods rich in all the essential minerals, trace elements and vitamins. [4] Therefore, the present study evaluates the physicochemical, nutrients and mineral composition of the indigenous medicinal plant *Alternanthera sessilis*.

MATERIALS AND METHODS

Collection of Plant material

The dried leaves of *Alternanthera sessilis* were used as a source of plant material for the present investigation. This material was purchased from the local market of Koyambedu, Chennai. The plant materials were taxonomically identified and authenticated by Dr. P.T. Devarajan, Associate Professor, Department of Plant Biology & Biotechnology, Presidency College, Chennai.

Fresh leaves of *Alternanthera sessilis* were separated, washed and shade dried for about 10 days. These dried leaves were ground to the coarse powder using a mechanical grinder. The powdered sample was used to determine the proximate analysis, minerals and nutrient composition.

PROXIMATE ANALYSIS

Moisture, ash, carbohydrate, protein, fat and crude fiber were estimated on the basis of the guidelines of AOAC, 1995. [5]

Estimation of Moisture content

Fresh sample materials were taken in a flat bottomed dish and kept overnight in a hot air oven at 100 - 110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of total ash

An ignited and weighed silica crucible which contains 2gm of the powdered sample was incinerated slowly by raising the temperature in a muffle furnace at 450°C for 4 – 5 hours. The sample was made carbon free. It was cooled and weighed. The crucible was cooled in a desiccator. The procedure was repeated until

a constant weight was obtained. The percentage of total ash was calculated.

$\% \text{ of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$

Determination of Water soluble ash

The ash was washed from the crucible into a 100ml beaker using 25ml of water and it was boiled for 5 minutes over a Bunsen burner and filtered through an ash less filter paper. The residue was washed with hot water twice, ignited to ash, cooled and weighed. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to air - dried drug.

Determination of acid insoluble ash

The ash was boiled with 25ml of concentrated hydrochloric acid for 5 minutes. The insoluble ash was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into a pre - weighed silica crucible. The percentage of the acid insoluble ash was calculated.

Calculation:

$\% \text{ of acid insoluble ash} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of the sample taken}} \times 100$

Determination of sulphated ash

A silica crucible was ignited at a temperature of 550° C to 650° C for 30 minutes, cooled and weighed in a desiccator. 1gm of powdered sample was taken in an ignited crucible and heated gently until the substance turns white in colour. A small amount of sulphuric acid was added, heated gently till the sample gets charred. After cooling, 1ml of sulphuric acid was added to moisten the sample and heated slowly so that white fumes were not formed. Complete incineration of the sample is done on igniting at 800° C + 25° C. The crucible was cooled in a desiccator and accurately

weighed. The percentage of the residue was calculated by repeating the procedure until a constant weight was obtained. (AOAC, 1990) [6]

Determination of Water soluble extractive value

In a stoppered conical flask, 5gm of powder was macerated with 5ml of chloroform and 95ml of water for 24 hours, shaking repeatedly for 6 hours. It was left undisturbed for 18 hours. The obtained filtrate (25ml) was evaporated on a flat - bottomed tarred dish and dried at 105° C and weighed. The procedure was repeated until a constant weight was obtained. The percentage of alcohol soluble extractive values with respect to air - dried material was calculated.

Calculation:

% of water soluble extract = $\frac{\text{Weight of extract obtained}}{\text{Weight of sample taken}} \times 4 \times 100$.

Determination of Alcohol soluble extractive value

5gm of the powdered sample was taken in a closed flask and macerated with 100ml of 90% ethanol for 24 hours. The solution was shaken repeatedly at frequent intervals for 6 hours and it was filtered. The obtained filtrate (25ml) was evaporated on a flat - bottomed tarred dish and dried at 105° C and weighed. The procedure was repeated until a constant weight was obtained. The percentage of alcohol soluble extractive values with respect to air - dried material was calculated.

Calculation:

% of alcohol soluble extract = $\frac{\text{Weight of extract obtained}}{\text{Weight of sample taken}} \times 5 \times 100$

NUTRITIVE ANALYSIS

Estimation of Crude Protein

Crude protein in the powdered plant material was estimated by Micro Kjeldahl Method. The first step is the conversion of organic nitrogen to ammonium sulphate which involves the digestion of the sample with concentrated Sulphuric acid and a catalyst. The ammonium sulphate was decomposed with Sodium hydroxide. The liberated ammonia was distilled with 2% boric acid. Titration was carried out with the nitrogen obtained using 0.05N HCl. Phenolphthalein was used as an indicator. The value of nitrogen obtained was multiplied by a factor 6.25 to give the percentage of crude protein.

Methodology

The process of digestion began with the addition of 10gms of Sodium sulphate, 0.5gm of Copper sulphate and 2g of the sample in a Kjeldahl flask. The contents of the flask were mixed well and heated for 15-20 minutes along with 25ml of Conc. H₂SO₄ in an inclined position. Glass beads were added to the flask to prevent spurting. The process was continued until a greenish colour was formed. The solution was allowed to cool. Then, 100ml of distilled water was added to the solution in the Kjeldahl flask. It was shaken well and transferred to a 250ml conical flask. The total volume was made up to 250ml with distilled water. 10-15ml of 2% Boric acid containing conical flask was placed below the distillation apparatus. In a Micro Kjeldahl steam distillation apparatus, 5ml of the aliquots was added followed by the addition of 1 drop of phenolphthalein indicator and 10-15ml of 40% Sodium hydroxide. The process of distillation was continued for 5-10 minutes until the solution was free from ammonia. The distilled product was titrated against N/10 H₂SO₄.

$$\% \text{ of Nitrogen} = \frac{\text{ml of } \frac{N}{10} \text{ H}_2\text{SO}_4 \text{ used up} \times 250 \times 0.0014 \times 100}{\text{Volume of aliquot} \times \text{gm of the sample taken}}$$

% of Crude protein = % of Nitrogen x 6.25

Estimation of Crude Fat

Crude fat was estimated by Ether Extraction Method. An extraction thimble attached to the Soxhlet extractor was taken in which added 5gm of the powdered sample. A pre - weighed conical flask was attached to the extractor. Ether was poured into the extractor. The entire set of apparatus was placed in a water bath at 60 -

80°C. It was further connected to the condenser. The extractor was allowed to cool for about 8 hours with the help of a condenser which supplied cool water continuously. The thimble was removed from the extractor. Resetting the apparatus and heating in a water bath would result in ether recovery from the receiver flask. After disconnecting the receiver flask, it was dried in a hot air oven at 100°C for 1 hour. It was then cooled and weighed.

$$\% \text{ of crude fat} = \frac{(\text{Wt. of the crucible with dry residue} - \text{Wt. of the crucible with ash}) \times 100}{\text{gm of substance taken}}$$

Determination of Crude fiber

Under desired conditions, crude fiber was obtained from digestion of samples free from fat using 1.25% of Sulphuric acid and Sodium hydroxide solutions respectively.

$$\% \text{ of crude fiber} = \frac{\text{Loss of weight on ignition}}{\text{Weight of the sample}} \times 100$$

Determination of total carbohydrate

The percentage of carbohydrate was determined by subtracting the total ash content, crude protein, crude fiber and crude fat from the sample.

% of carbohydrate = 100 - (Crude protein % + Crude fat % + Crude fiber % + Total ash %).

Nutritive value

The Atwater method of energy calculation is used to determine the Nutritive value in the leaves of *A. sessilis*.^[7-8]

Nutritive value = 4 x % of protein + 9 x % of fat + 4 x % of carbohydrate.

MINERAL ANALYSIS

Estimation of Cu, Fe, Na, K, Zn, Mn, Mg, Cr, Ca and P

In a crucible, 0.5gm of powdered sample was placed in the muffle furnace and heated at 580°C for 3 hours. The obtained

ash was cooled in a desiccator, cooled and the sample was digested at high temperature with 10ml of concentrated Nitric acid, 4ml of Perchloric acid and 1ml of Sulphuric acid. The process was continued until it becomes the clear solution and cooled. The contents in the tube were transferred to 50 ml volumetric flask and the volume was made up to 50 ml with distilled water. The final solution was utilized for the estimation of minerals such as Cu, Fe, Na, K, Zn, Mn, Mg, Cr, P and Ca by Atomic Absorption Spectroscopy (Hitachi A - 1800 model) as per the methodology described by Ecrements and Burell., 1973.^[9] Standard solutions were prepared for each mineral and the concentration of various elements was determined.

STATISTICAL ANALYSIS

Statistical analysis of the results was carried out by using MS Excel 2007 statistical software and the mean values along with standard deviation were calculated.

RESULTS

Proximate analysis is an important criterion in the determination of contamination and the quality of the sample used for the experiment.^[10] The data of the various physicochemical parameters of *A. sessilis* were presented in Table 1. The leaves showed moisture content as 75.6 ± 2.56 which accounts for the succulent nature

of the plant. The percentage of total ash, acid insoluble, water soluble ash, sulphated ash value, water and alcohol soluble extractive value were given as 8.5 ± 1.67 , 3.2 ± 0.78 , 1.8 ± 1.25 , 1.8 ± 0.08 , 9.5 ± 0.05 , 18 ± 0.56 respectively. The composition of carbohydrate was found to be higher (74.56 ± 1.25) than protein (36.16 ± 1.90), crude fiber (25.54 ± 2.12) and Crude fat (3.87 ± 0.65). The energy value of total carbohydrates, fat and protein was found to be 760.0 ± 5.65 Kcal/day. The results on the mineral content of *A.sessilis* leaves expressed as mg/100g of dry weight. About 10 minerals were detected by Atomic absorption spectroscopy. The concentration of macro minerals such as Sodium, Potassium, Calcium, Phosphorus and magnesium were found to be 285.78 ± 5.95 , 4450 ± 2.50 , 510.35 ± 9.69 , 48.7 ± 2.45 and 80.0 ± 8.6 and the amount of trace elements like Zinc (8.9 ± 2.45), Iron (9.85 ± 1.98), Copper (1.6 ± 0.02), Manganese (6.97 ± 1.25), and Chromium (65 ± 0.05) present in the sample are presented in Table 2.

Table 1. Nutritional value of the leaves of *Alternanthera sessilis*

S.No.	Parameters	Values in g/100 gm on dry weight basis
1	Moisture content	75.6 ± 2.56
2	Total ash	8.5 ± 1.56
3	Water soluble ash	1.8 ± 1.25
4	Acid insoluble ash	3.2 ± 0.78
5	Sulphated ash value	1.8 ± 0.08
6	Water soluble extractive value	9.5 ± 0.05
7	Alcohol soluble extractive value	18 ± 0.56
8	Carbohydrate	74.56 ± 1.25
9	Protein	36.16 ± 1.90
10	Crude fat	3.87 ± 0.65
11	Crude fiber	8.80 ± 2.12
12	Calorific value Kcal/day	760.0 ± 5.65

Values are expressed as mean \pm SD

Table 2. Mineral composition of the leaves of *Alternanthera sessilis*

S.No.	Minerals	mg/100 gm dry weight
1	Sodium	285.78 ± 5.95
2	Potassium	4450 ± 2.50
3	Calcium	510.35 ± 9.69
4	Phosphorus	48.7 ± 2.45
5	Magnesium	80.0 ± 8.6
6	Zinc	8.9 ± 2.45
7	Iron	9.85 ± 1.98
8	Copper	1.6 ± 0.02
9	Manganese	6.97 ± 1.25
10	Chromium (μ g)	65 ± 0.05

Values are expressed as mean \pm SD

DISCUSSION

Plants contribute to satisfying human needs in terms of energy and nutrition. The nutrients present in plants are carbohydrates, proteins and fats. Minerals and Vitamins play a significant role in establishing a healthier management of human organ system. Water determines the energy value in terms of moisture. ^[11] The physicochemical parameters studied would be used to characterize and standardize the experimental plant. A Higher Moisture content of 75% in the leaves primarily explains the higher degree of food spoilage. The percentage of ash content defines the quality of a food material which gives an identity to a substance of its carbon free nature and also denotes the organic, inorganic matter and impurities present in the sample. The total ash content predicts the soluble and insoluble minerals in the sample. ^[12] The low values (acid insoluble ash and water soluble ash) in the leaves of *A.sessilis* may indicate that the sample would be free of foreign matter. Similarly, a lower sulphated ash value of the sample showed the meager amount of inorganic impurities. The presence of chemical components, its nature and the contaminants present in the sample would be analysed by extractive value. Water soluble extractive value gives an idea on the presence of sugars and inorganic compounds while alcohol soluble extractive value predicts the presence of ionic components in the sample. ^[13] The sample showed increased solubility in alcohol than water.

The basic nutrients present in the sample were found in the descending order as Carbohydrate > Protein > crude fiber > crude fat. These nutrients satisfy the RDA. The percentage of energy allowance reported in this study as Carbohydrate 74.5% (RDA value - 50 to 100g/day), Protein 72.3% (RDA value - 50g/day), Crude fiber 70.8% (RDA value - 12g/day) and crude fat 19% (RDA value - 20g/day). The leaves are rich in carbohydrates as they maintain the energy potential, proteins are considered to be the building block of cells, fats are the energy providers and aids in the

absorption of fat soluble vitamins and crude fiber is essential to enhance the digestion of food.^[14] The sample of the plant is found to be poor in lipids. It was also estimated that the plant provides sufficient amount of energy value as 760 Kcal/day. More or less the similar results were also expressed by Freedman., 2006^[15] and Pobi Gogoi et al., 2014.^[16]

The curative property of the plant mainly depends on the macro and micronutrients present in them. These elements contribute to the structural and functional properties of the plant. Table 2 describes the mineral composition of the leaves of *A.sessilis*. The leaves found to contain all the essential macronutrients and trace elements. A total of 10 minerals were analysed. Among them, the plant contains more or less of the estimated minerals in concentrations as per Recommended Daily Allowance (RDA). The minerals studied in the plant meets the RDA daily requirements. (NRC., 1989)^[17]

According to RDA, the daily requirement of Sodium 300mg/day, Potassium 4700mg/day, Calcium and Phosphorus is 800mg /day, Magnesium 210 - 350mg/day, Zinc and Iron 10 - 15 mg/day, Copper 2mg/day, Manganese 2 – 5 mg/day, and Chromium 200mcg/day. *A.sessilis* showed similar amounts of macro and micronutrients in RDA percentage as Sodium 95%, Potassium 94.6%, Calcium 63.7%, Phosphorus 6 %, Magnesium 66%, Zinc 89%, Iron 98%, Copper 80%, Manganese 98% and Chromium 32.5% respectively. From these results, it could be assessed that the plant contains a higher concentration of Iron, Manganese, Sodium and Potassium, moderate levels of Copper and Zinc and lower levels of Phosphorus.

Sodium and potassium are necessary for maintaining ionic stability. This relation between Na and K in foods significantly prevents hypertension and atherosclerosis.^[18-19] Calcium is an important mineral required for strong bones and teeth, provide structural rigidity of the body and involved in blood clotting and cellular permeability.

Manganese is required for enzyme structure and also plays a vital role in haemoglobin formation.^[20] Phosphorus is comparatively low which is an enzymatic component and essential for proper immune function, energy metabolism and acid - base balance of the body.^[21] The structural component of many enzymes is the mineral copper. It makes up the vital protein involved in the oxidation of iron, Ceruloplasmin.^[22] Magnesium is responsible for the formation of bones, energy metabolism and catalyzing enzymatic activity.^[23] Chromium was found to be moderately low when compared with other minerals. It has a special place in performing some of the important functions like the formation of muscle mass and stabilizing glucose level in blood.^[24] Zinc acts as a stimulator in activating beta cells of the pancreas to release insulin and maintains normal glucose tolerance. It is also required for the growth and tissue repair.^[25] Iron is essential for the formation of haemoglobin, transport of oxygen and providing immunity to the body.^[26] As the minerals Mn, Zn, Mg, Cu, Fe, Na, K, Ca and traces of chromium present in recommended amounts, *A.sessilis* might be beneficial for diabetic patients.^[27] Further, the low quantity of lipids in the plant would be considered as a dietary supplement for obese individuals.^[28] Further, the total element concentration becomes an important parameter to assess the stress to plants, its nutritive value, its toxicity and also a trademark on species related information.^[29] A wider understanding on the significance of the minerals and valuable bio molecules in plants paves a way to use the medicinal plants as food and phyto - medicine.^[30-34] Moreover, minerals are being utilized by the plants as structural components for carbohydrate and proteins.^[30,35] Thus, the presence of high protein, carbohydrate, fiber and minerals in the leaves of *Alternanthera sessilis* would definitely increase the food value, including it in the daily food regimen to stay healthy.

CONCLUSION

Alternanthera sessilis, the perennial herb possess good nutritive value and may be used as a food and fodder. It also found to contain a good amount of various macro and micro elements in appreciable quantities. This plant would be a promising source of carbohydrate, protein fat and fiber and may be recommended as nourishment to people suffering from malnutrition. Moreover, the minerals present in the leaves found to satisfy the RDA requirements. The presence of an optimal level of nutrients in the plant at right proportions could be beneficial to human body.

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