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Original Research Article

Evaluation of Vitamins and Antinutrients in the Leaves of Traditional Medicinal Plant *Alternanthera Sessilis (L.) R.Br.Ex DC*

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ABSTRACT

Green plants have a magical way of filling the world with wonders. One of the important features of the plants is its healing properties. Plants not only heal but they also repair, revitalize and are curative in nature. *Alternanthera sessilis* is being used from prehistoric times for food, fodder and medicine. The plant gives golden luster to the body when consumed regularly. The quantitative determination of vitamins and antinutrients were carried out in the present study. Vitamins such as A, E,B_1 ,B_2 ,B_3 and C and antinutrients like phytate, oxalate, nitrate, nitrite, alkaloids, saponins, tannins and cyanogenic glycosides were analysed in the leaves of *A. sessilis*. It was elucidated by the study that the plant was found to contain significant amounts of fat and water soluble vitamins. These vitamins would play a vital role in the growth and development. The presence of vitamin C and E in considerable amounts would enable the plant to act as a potent radical scavenger. Antinutrients in lower amounts might be a factor for safer consumption of the plant as food. Hence, this study would recommend the inclusion of this traditional plant *Alternanthera sessilis* to combat malnutrition and also to provide a healthier lifestyle.

Key words: *Alternanthera sessilis*, vitamins, antinutrients, malnutrition, antioxidant.

INTRODUCTION

The outstanding gift of Nature is wrapped with full of therapeutic products. These are the medicinal plants which are packed with enduring healing properties. They could repair and rejenuvate human body. The plant foods are easily digestable and absorbed by the body without causing adverse effects. Any part of the plant such as root, stem, leaves, flower and fruit may contribute to macro and micronutrients. These nutrients namely carbohydrates, proteins, fats, minerals and vitamins in herbal plants provide strength, longevity and immunity. [1] Further, Phytochemical constituents in the plants possess antioxidant, antinflammatory, antibacterial, anticarcinogenic, antimutagenic, antifungal and antiviral properties. [2-4] Among the nutrients, vitamins are essential compounds found in plant foods that perform specific and vital functions of the body. They may act as a cofactor necessary to catalyze a biological reaction, required for proper vision, involved in cellular metabolism, cell growth, differentiation and development. [5] Biologically, antinutrients are protective molecules while nutritionally they hinder normal growth and development. Although, plants contain a variety of antinutrients, they reduce the utilization of nutrients, uptake of food, absorption and availability of

nutrients. Examples of antinutrients in plant foods are phytates, oxalates, phenolic compounds, saponins and so on. [6] The major cause of Malnutrition is the insufficient supply of micronutrients. Low amount of vitamin supplements would result in deficiency disorders. Some of the vitamin deficiency diseases are xerophthalmia, nyctalopia, scurvy, rickets, osteoporosis, pellagra and beriberi. [7] To conflict the diseases, a vitamin rich diet should be provided. Moreover, the green leafy vegetables provide a wholesome bowl of all the essential vitamins such as ascorbic acid, riboflavin, thiamine and beta carotene enriched with therapeutic properties. They are easily available and readily usable. [8]

The plant *Alternanthera sessilis* of Amaranthaceae family has been used in ancient India as an abortifacient, galactagogue, cholagogue, febrifuge, as an antidote for snake bite and skin diseases. [9,10] It also possess wound healing property, [9,11] hepatoprotective property, [9,12] improving memory and intelligence, recuperating skin tone, [13-15] eye complaints, [16,17] bone fractures and malaria, [16,18] antipyretic [16,19] and anticarcinogenic. [16,20] On thorough investigation on the ethnomedicinal features of the plant, this study was aimed to evaluate the profiling of vitamin content and antinutrients in the leaves of traditional 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. Deverajan, 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 vitamins and antinutrient content.

VITAMIN ANALYSIS

Vitamins such as Thiamine, Riboflavin, Niacin and Ascorbic acid were estimated by the method of Okwu et al., 2006. [21]

Determination of Thiamine (Vitamin B_1)

About 50 ml of ethanolic sodium hydroxide and 5 g of the sample were homogenized and filtered in a 100 ml flask. To the 10ml of the filtrate, 10 ml of potassium dichromate was added. A blank was prepared simultaneously and the colour developed was read at 360 nm.

Determination of Riboflavin (Vitamin B_2)

5 g of the sample and 100ml of 50% ethanol were extracted. This solution was shaken for about 1 hour and followed by filtration in a 100 ml flask. In a 50 ml of volumetric flask, 10ml of the extract was pipetted out. To this, 10 ml of 5% potassium permanganate and 10 ml of 30% hydrogen peroxide were added. It was allowed to stand on a hot water bath for 30 minutes. At this stage, 2 ml of 40% sodium sulphate was added and the solution was made up to 50 ml in a standard flask, aliquots were prepared. The absorbance was measured at 510 nm, spectrophotometrically.

Determination of Niacin (Vitamin B_3)

In a flask, 5 g of the sample was allowed to react with 50 ml of 1N Sulphuric acid. The contents in the flask were shaken for 30 minutes. To this solution, 3 drops of ammonia solution was added and filtered. A volumetric flask comprising of 10 ml of the filterate and 5 ml of potassium cyanide was taken and the solution was acidified with 5 ml of 0.02N Sulphuric acid. The absorbance was measured by using a spectrophotometer at 470 nm.

Estimation of Ascorbic acid (Vitamin C)

Ascorbic acid was estimated by the method of Omaye et al., 1971. [22]

A test tube consisting of 0.5 ml of the sample, 0.2 ml of distilled water and 0.5

ml of DTCS reagent were taken. The tubes were incubated at 37º C for 3 hours. Then, 1.5 ml of ice - cold 65% Sulphuric acid was added. The contents of the tube were mixed well and incubated at 37º C for 30 minutes. The colour developed was measured as absorbance at 520 nm.

Estimation of Vitamin A (Retinol)

The method adopted for the estimation of vitamin A was Nedd and Pearson., 1973. [23]

To 0.5ml of the sample, 0.5 ml of chloroform and 2 ml of Trifluoroacetate reagent (TFA)

were added in a test tube. The absorbance was recorded at 600 nm.

Estimation of β carotene

Nedd and Pearson., 1973 [23] was the method used to estimate β carotene. To 1 ml of the sample added 1ml of saponification mixture (2N Potassium hydroxide in 90% ethanol). It was mixed well and heated at 60º C for 20 minutes. The contents were allowed to cool and 25 ml of distilled water was added. This was transferred to a separating funnel. The solution was extracted with 25, 15 and 10 ml of petroleum ether at 40º C - 50º C respectively. This procedure was repeated for three times and the extracted contents were pooled and washed with 50 – 100 ml of distilled water. It was continued until the solution was made free of alkali. The extract was dried by the addition of anhydrous sodium sulphate. Now, by the careful separation of 3 ml of petroleum ether phase into a cuvette, the absorbance was measured at 420 nm against a reagent blank.

Estimation of Vitamin E (Tocopherol)

Tocopherol in the sample was measured by the method of Varley et al., 1976. [24]

Three stoppered centrifuge tubes labeled as Test (T), Standard (S) and Blank (B) was taken. 1.5 ml of the sample, 1.5 ml of standard solution and 1.5 ml of distilled water were taken in tubes T, S and B respectively. To the tubes T and B, 1.5 ml of ethanol was added and 1.5 ml of distilled water was added to the tube S. 1.5 ml of xylene was added to all the tubes. The contents of the tube were mixed well and centrifuged. 1 ml of xylene layer was separated from each tube and 1 ml of 2, 2' - dipyridyl reagent was added. All the tubes were mixed well. 1.5 ml of the mixture was pipetted out into the spectrophotometer cuvette and read at 460 nm against the reagent blank. Now, to these tubes, 0.33 ml of ferric chloride solution was added and mixed well. After 15 minutes, the colour intensity of the test and standard was measured against the blank at 520 nm. Vitamin E estimated was calculated as per the formula is given below.

$$\text{Vitamin E } (\mu g/g) = \frac{(\Delta A520nm - \Delta A460\ nm) \text{ x conc. [S] x } 0.29 \text{ x Total volume}}{\Delta A520nm \text{ x Vol. of experiment x weight of the sample}}$$

ANTINUTRIENT ANALYSIS

The antinutrient content analysed in the present study are oxalate, phytate, nitrate, nitrite, saponins, tannins, alkaloids and cyanogenic glycosides..

Determination of oxalate

The method proposed by Day and Underwood (1986) [26] was adopted for the estimation of oxalate. [25] About 1 g of the sample was taken in a conical flask. To this, 75 ml of 3M Sulphuric acid was added. The contents of the tube were stirred for 1 hour using a magnetic stirrer. The solution was filtered using Whatmann No.1 filter paper. Then, 25 ml of the filtrate was taken and titrated against 0.05 M Potassium permanganate. The end point was the appearance of a faint pink colour which persists for about 30 seconds. The content of oxalate present in the sample was calculated by taking 1 ml of 0.05 M Potassium permanganate as equivalent to 2.2 mg oxalate. [27, 28]

Determination of phytate

Wheeler and Ferrel (1971) [30] method were used to estimate the phytate content. [29]

10 mg of the powdered sample was taken in a 125 ml Erlenmeyer flask. The contents of the flask were incubated for 30 minutes. 50

ml of 3% TCA was used for extraction. The flask was placed on a mechanical shaker for 45 minutes. The suspension was centrifuged and 10 ml of the supernatant was taken in a centrifuge tube. To this tube, added 4 ml of ferric chloride solution. The tubes were kept in boiling water bath for 45 minutes. Centrifuged for 15 minutes and the obtained precipitate were washed twice with 25 ml of 3% TCA. Heated the tubes for 5-10 minutes. Now, to the precipitate, a few ml of water and 3 ml of 1.5 N Sodium hydroxide were added. The volume in the tubes was adjusted to 30 ml and kept in a boiling water bath for 30 minutes. The hot solution was filtered using Whatman No. 2 filter paper. The precipitate was washed with 70 ml of hot water. The filtrate was discarded and the precipitate was dissolved with 40 ml hot 3.2 N Sulphuric acid in a 100 ml flask. The contents of the flask were cooled. In a 100 ml flask, 5 ml of the aliquot was taken and diluted to 70 ml. To this, add 20 ml of 1.5 N Potassium cyanide, diluted and the colour was read at 480 nm. The iron content was calculated from the ferric nitrate standard and the phytate phosphorus was calculated from the iron assuming 4: 6 (iron: phosphorus ratio).

Determination of nitrate and nitrite

The method based on Harper (1924) [32] and Bassir and Maduagwu (1978) [33] and Montgomery and Dymock (1961) [34] as described by Friday et al (2011) [31] was adopted for the estimation of nitrate and nitrite.

Processing of the sample

The leaves of the plant were separated and grounded in a mortar and pestle with 80 ml of distilled water. The contents were centrifuged and the supernatant was collected. To the supernatant, a spatula of mercuric chloride was added and filtered through a Whatman No.32 filter paper. A clear solution of the sample was obtained.

Estimation of nitrate

25 ml of silver sulphate (4g / l) was added to the obtained processed clear solution. This is done to remove chloride ions precipitated and removed by filtration. To the filtrate, added 0.2g of magnesium oxide. The yellow colour developed was read colorimetrically. A blank consisting of double distilled water was used. Potassium nitrate was used as a standard solution with the concentration of nitrate ranged from 0.0 to 20 µg N/ml. A calibration graph was used to estimate the amount of nitrate.

Estimation of nitrite

Nitrite could be estimated from the processed clear extract solution. The sample was used as a blank and sulphanilic acid was replaced with double distilled water. The pink colour developed was measured colorimetrically at 550 nm. A standard solution with 1 ml aliquots of sodium nitrite containing 0.0 to 2 µg nitrite N/ml was prepared. The levels of nitrite obtained from standards and sample were extrapolated to quantify the amount of nitrite.

Estimation of Tannins

The amount of tannins present in the plant was estimated spectrophotometrically by Folin-Denis method. [35-37]

About 5g of the powdered leaves were taken in a 250 ml conical flask. To this added 75 ml water and boiled for 30 minutes. The contents were centrifuged at 2000rpm for 20 minutes. The supernatant was made up to 100 ml with distilled water. 1 ml of the sample and 1 to 10 ml aliquots of standard tannic acid (50 µg/ml) was taken and to this 0.5 ml of Folin Denis reagent and 1 ml of sodium carbonate were added and made up to 10 ml with distilled water. The absorbance was measured at 700 nm. The tannic acid content was expressed as mg of tannic acid equivalent to per gram of the extract.

Estimation of Saponins

Total Saponins were determined by the method of Obdoni et al., 2001. [38] The plant samples were ground and 20 g of each plant sample is put into a conical flask and 100 ml of 20% ethanol is added to the plant sample. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture is then filtered and the residue re-extracted with another 200 ml

of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90ºC. The concentrate is then transferred into a 250 ml separating funnel and 20 ml of ethyl acetate is added to the extract and vigorously shaken. The aqueous layer is recovered while the ethyl acetate layer is discarded and the purification process is repeated. 60 ml of n-butanol is added and the combined n-butanol extracts is washed twice with 10 ml of 5% NaCl. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

Estimation of Alkaloids

Alkaloids were determined using Harborne, 1973 method. [39] 5 gms of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one - quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to collect and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid which was dried and weighed.

Determination of Cyanogenic glycosides

Alkaline picrate method of Sarkiyaki (2010) [40] was adopted from Charles et al., 2016 [41] to estimate cyanogenic glycosides content in an edible plant. 500 mg of the sample was boiled for 4 hours with 20 ml of 1N HCl. The solution was filtered and 50 ml of petroleum ether was added. The filtrate was evaporated at 60º C. To the residue added, 5 ml of acetone - ethanol mixture. 0.4 ml of the sample was taken in labelled test tubes. To this, 6 ml of ferrous sulphate reagent and 2 ml of concentrated sulphuric acid was added. The mixture was incubated for 10 minutes and the absorbance was measured at 490 nm.

RESULTS

Vitamin deficiency in food would cause adverse effects on the human body. They are necessary even in trace amounts for the proper functioning of the body. Table 1 shows the different concentration of vitamins in *A. sessilis* leaves. It was observed that the B complex family of vitamins such as B_1, B_2 and B_3 were found to be high. Among them, B_3 having the highest concentration of 14.56 ± 2.96, followed by vitamin B_1 and B_2 showing 1.45 ± 2.34, 1.86 ± 0.85 respectively. The plant was also found to contain significant amounts of vitamin C and vitamin A 52.25 ± 4.65 and 956.02 ± 3.92. The higher content of β – carotene was also reported in A. sessilis. A meager amount of vitamin E was also present in the plant, 1.54 ± 0.05.

Table 2 represents the amount of antinutrients in the leaves of A. sessilis. The amount of antinutrients such as alkaloids (28.32 ± 1.22) and saponins (22.08 ± 1.22) were higher followed by tannins (8.56 ± 2.65). Smaller amounts of phytate (3.25 ± 1.45), oxalate (0.2156 ± 1.25) and least value of nitrate (0.06 ± 0.05) and nitrite (0.03 ± 0.07) and cyanogen glycoside (0.01 ±0.05) were reported.

Table 1: Vitamin analysis in the leaves of *A.sessilis*

S.No	Vitamin	mg/100g
1	Vitamin A (µg)	956.02 ± 3.92
2	Vitamin B_1	1.45 ± 2.34
3	Vitamin B_2	1.86 ± 0.65
4	Vitamin B_3	14.56 ± 2.96
5	Vitamin C	52.25 ± 4.65
6	Vitamin E (µg)	1.54 ± 0.05
7	β carotene	1090 ± 8.95

Values are expressed as mean ± SD

Table 2: Antinututrient Analysis in the leaves of *A.sessilis*

S.No	Antinutrient	mg/100g
1	Oxalate	0.2156 ± 1.25
2	Phytate	3.25 ± 1.45
3	Nitrate	0.06 ± 0.05
4	Nitrite	0.03 ± 0.07
5	Tannins	8.56 ± 2.65
6	Saponins	22.08 ± 1.22
7	Alkaloids	28.32 ± 1.22
8	Cyanogen glycosides	0.01 ± 0.05

Values are expressed as mean ± SD

DISCUSSION

The organic micronutrients vitamins, although required in minimal amounts are essential for normal functioning of the body.

[42] *Alternanthera sessilis* is being considered as the indigenous medicinal plant and has been used in traditionally for various ailments. The present study had shown that the plant was found to contain appreciable amounts of vitamins A, B_1, B_2, B_3, C and β – carotene. Moreover, these vitamins do not serve for providing energy but are essential for the proper functioning of the body. The amount of vitamins consumed by an individual to stay healthy is given by Recommended Daily Allowance (RDA) which determines the average requirement of a person to meet the daily needs. [43] It could be predicted from the study that the plant was found to satisfy the RDA daily requirements. (NRC, 1989) [44]

As suggested by NRC, the Recommended Daily Allowances (RDA) for vitamins such as vitamin A (750-1200 µg/day), vitamin E (11-15 mg/day), vitamin B_1 (1.1-1.5 mg/day), vitamin B_2 (1.2-1.7 mg/day), vitamin B_3 (12-16 mg/day) and vitamin C (40-90 mg/day) suggested that the plant *A. sessilis* is a rich source of all the essential micronutrients.

The fat soluble vitamin A is required for normal growth and development, vision, healthy skin and acts to combat infectious diseases. Pro - vitamin form of A namely carotenoids play a major role in maintaining the levels of vitamin A in blood. Vitamin A is supplied to the body in the form of β - carotene. B-Carotene is essential for vitamin A activity and primarily acts as an antioxidant. Green leafy vegetable like *A. sessilis* would serve the purpose of maintaining healthy bodily functions as well as the economy needs of an individual. [45] The presence of vitamin E would contribute to maintaining the antioxidant integrity of cell membranes and prevents autoxidation of lipids. Since it is lipophilic in nature, it gives protection to PUFA in the cell membrane. [46] On the other hand, water soluble vitamins including B complex family of vitamins and Vitamin C act primarily as coenzymes and cofactors in various biological reactions. [47] Vitamin C functions as a potent free radical scavenger, helps in the absorption of iron, possess wound healing property, collagen synthesis and exhibits anti inflammatory, anticarcinogenic action. [48] In the indigenous system of medicine, the plant has a significant role acting as an antidote to cough, healing of wounds and protection against skin infections. This could be attributed to the vitamin C content of the plant. Further, the oxidation of bioconstituents in the plant may be protected by the substantial amounts of vitamin C. [49,50]

Thiamine (Vitamin B_1) is essential for nervous system function, stimulating appetite and as a coenzyme in metabolism of carbohydrates. Vitamin B_2 (Riboflavin) catalyses the formation of niacin, energy production from the food stuffs and cofactors in oxidation and reduction reactions. [51] Niacin (VitaminB_3) is required for maintaining healthy skin, enhances digestion, activating appetite, stimulating nerves and aids in catalysing enzymatic reactions. [47, 51] Any deficiency of vitamin would result in disorders that disturb the general functioning capability of the various systems. As these micronutrients possess immunomodulating functions, their underutilization would result in malnutrition. [52] Since, the plant has the higher quantity of vitamins satisfying the dietary allowances, *A. sessilis* would be a good alternative source to combat malnutrition.

Antinutrients are the substances which are essential for metabolism in plants but interfere with the various biological processes like digestion, absorption and utilization producing undesirable effects. [53] The plant *A. sessilis* found to contain low amounts of antinutrients such as oxalate, nitrate, nitrite and moderate amounts of phytate, saponins, tannins and higher levels of alkaloids as given in Table 2. Antinutrients in plants provide self defense and also would optimally decrease the exploitation of nutrients such as proteins, vitamins and minerals.

Generally, Phytate found to decrease the absorption of minerals such as iron, zinc, magnesium and calcium from foods. [54] But the lower phytate content in the leaves of *A. sessilis* enumerates increased absorption and utilization of minerals in humans. [55,56] Oxalate could affect the absorption and utilization of calcium in the body and may result in irritation to the gut. Oxalate could bind with calcium and converted to an insoluble calcium oxalate. [57] The quality of leafy vegetables is measured by the amount of nitrate and nitrite present in it. Nitrate is non – toxic whereas nitrite may lead to drastic effects. The acceptable limits of nitrate and nitrite as given by WHO were 3.7 mg/kg and 0.06 mg/kg body weight respectively. [58,59] The values of nitrate and nitrite in the plant were found to be within the permissible limits. Tannins in foods would inhibit the activities of enzymes such as trypsin, chymotrypsin and lipase. It also decreases iron absorption and quality of foods. [60] Saponins found to exhibit hypocholesterolemic, immunostimulatory and anticancer properties. The food rich in saponins helps to control cholesterol, preventing peptic ulcer and osteoporosis and decreases the risk of heart diseases. [61] Alkaloids as antinutrient, acts on the nervous system by disrupting the transmission of impulses and cell membrane in the GI tract. [62]

Cyanogenic glycosides helps plants in case of sudden injury and serve as mobile nitrogen storage compounds during germination. Higher values results in toxicity. [63] The low value of cyanogenic glycosides in the plant A. sessilis may contribute to the growth and development.

The permissible or physiological tolerance levels of oxalate (2- 5 g), cyanogenic glycosides (20 mg/ HCN equivalent per kilogram sample recommended by Standard Organisation of Nigeria, SON. [64]

In general, processing would reduce the amount of antinutrients to their permissible levels. Reduction of antinutrients to a certain extent would be possible through the process of soaking, cooking and blanching. Soaking decreases phytate, tannins and oxalate contents in foods. [65] Soaking for 30 minutes in water and boiling effectively reduces the content of oxalate in the leaves. [66] Further, calcium oxalate may be reduced by 19-87% in boiled green leafy vegetables. [67]

Basically, a larger cooking time reduces the content of antinutrients. Combination of methods like soaking and boiling would drastically reduce the antinutrients. Moreover, blanching may be the most effective method in the reduction of cyanogenic glycosides. [63] The plant *Alternanthera sessilis* is included in the food regime of South India. As the plant was found to contain a majority of antinutrients in lower amounts, this plant is considered to be safe for consumption.

CONCLUSION

Traditional green leafy vegetables play a vital role in contributing vitamins for a healthier well being. They are the cheapest source of nutrition, readily available and impart medicinal properties. The presence of natural antioxidants such as vitamin C and E significantly promotes the beneficial application of the plant in medicine and pharmaceuticals as a potent drug used to cure various ailments. The presence of antinutrients in lower amounts would be helpful in categorising the plant *Alternanthera sessilis*, safer for consumption. Although, if present in higher amounts, the process of soaking, Cooking and blanching may reduce the level of antinutrients. All the vitamins studied, meets the RDA requirements. Hence, the plant *Alternanthera sessilis* may be consumed to balance malnutrition. Considering the rich resources of ingredients, the plant is rightly known as “King of Greens” which may be utilized to cure certain illness or to lessen the symptoms of an ailment. With their demonstrated nutritional qualities and low antinutritional factors, *Alternanthera sessilis*

may be included in the dietary regime to compensate for nutritional deficiency

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