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Estimation of nutritive composition of *Sauropus androgynus* (Multivitamin plant) at different growth stages and position of leaves

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Abstract

A study was designed to estimate the nutritive composition of Chekkurmanis (Katuk) leaves on two different growth stages viz., 60 & 120 days after planting and two different positions viz., terminal and basal whorls in plants. Utilization of green leafy vegetables differs from leaves of annuals and shrubs to leaves of trees. Katuk leaves rich in vitamins and minerals are known as protective food. The results of the study showed that Vitamin C, Protein, Calcium, Carbohydrate contents increased with the growth stages from 60 to 120 days. In contrast, Vitamin A content decreased as the plant approached 120 days. There is a significant difference in nutritive value from 60 to 120 days. Vitamin A was observed to be significantly high (4.11 mg/100g) in terminal whorl at 60 days after planting compared to 3.01 mg/100g in the terminal whorl at 120 days. Higher Vitamin A (5.12 mg/100g) was observed in basal whorl leaves at 60 days after planting, while Vitamin C (220.41 mg/100g), Protein (4.99 g/100g), Calcium (4.16%), Carbohydrate (9.83 g/100g) were observed in basal whorls on 120 days after planting. In conclusion, the basal whorls had more embedded nutritive value than the terminal whorl leaves. This study reveals that nutritive composition of *Sauropus androgynus* mainly depends on the growth stages and leaf position in the plant.

Keywords: Chekkurmanis leaves, nutritive analysis, growth stages and position (terminal or basal whorl) of leaves

Introduction

Chekkurmanis (*Sauropus androgynus* L.), the vernacular names of the crop are *Katuk* or *Sweet leaf bush*, belongs to the family Euphorbiaceae, is a shrubby glabrous perennial green leafy vegetable, extensively grown in warm humid tropics with ample rainfall. The crop is native of Indoburma region, later introduced to India from Malaya. It is widely distributed in Sikkim, Himalaya, Khasi, Abour and Arka hills, Western Ghats of Kerala. The plant is also cultivated in Australia, as it is nutritious, most prolific, high yielding with appetizing green leaves. Katuk is mainly propagated through semi hardwood cuttings of 20 - 30 cm length stem. The tender leaves and succulent shoots are used for cooking. In India, it is commonly known as Multivitamin or Multi green plant, because of its rich nutritive value, containing large amount of essential mineral elements, with high level of Vitamin A, Vitamin B, Thiamine (B1), Riboflavin (B2), Vitamin C, Carbohydrate, Calcium, Potassium, Phosphorous, Iron and Dietary Protein. Due to presence of antioxidants in the leaves, is utilized for treating many diseases viz., diabetics, cancer, allergy, microbial infection, cholesterol and swelling as reported by ^[29] Paul and Beena Anto (2011). In India, anciently Katuk leaves were used to improve the eyesight, to cure skin diseases, urinary problems, cardiovascular problems, relieves internal fever and several other illness as stated by ^[25] Ong (2003). Mineral deficiency and other related diseases can be overcome by intake of leafy vegetables in recommended quantities, as an appropriate amount of vitamins, minerals and phytochemicals are necessary for normal functioning of human metabolic processes. To understand the nutritive composition of Chekkurmanis at different growth stages and position of leaves, a study has been taken up at the Horticultural College and Research Institute, Periyakulam during 2019-2020.

Materials and methods

The healthy and disease-free rooted cuttings of *Sauropus androgynus* collected from Orchard, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU),

Madurai, Tamil Nadu, were used as planting materials for the present study. Field trial was taken up at Horticultural College and Research Institute, Periyakulam. The experiment was conducted at Field No. 26 of Western Block Farm, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam, which is located at 80° East longitude, 11° North latitude, at an altitude of 300 M above Mean Sea Level. The nature of soil in the experimental site was sandy loam. Chekkurmanis leaf samples were collected from terminal and basal whorls of the plants at 60 and 120 days after planting, to assess the nutritive composition of young (terminal whorls) and matured (basal whorls) leaves. The leaves were washed with tap water to remove adhering sand, dust and other contaminants on the surface. Fresh leaf samples were used for analyzing vitamins, carbohydrate and protein, while the leaf samples dried in hot air oven at 60° C for six hours were used for calcium analysis. All the analyses were carried out in three replicates.

Estimation of Vitamin A

The vitamin A was estimated by weighing 500 mg of fresh leaf sample from terminal and basal whorl of leaves separately and macerated with 10.0 ml of 80% acetone. The extract was centrifuged at 25000 rpm for 10 mins. Supernatant was collected and made up to a volume of 20.0 ml in a volumetric flask. Finally, the values of extract were read in spectrophotometer at 510 nm and 480 nm as described by [17] Jensen (1978) and the values are expressed in milligram per 100 gram.

Amount of Vit A in 100g leaf sample

$$= \frac{(7.6 \times \text{OD @ 480 nm}) - (1.49 \times \text{OD @ 510 nm}) \times V}{1000 \times W}$$

Where,

Weight (g) of sample taken = W

Volume (ml) of supernatant = V

Estimation of Vitamin C

The vitamin C content of the fresh leaves from the terminal and basal whorls were estimated separately by titrimetric method described by [13] Harris and Ray (1935) and the values recorded as milligram per 100 gram. Leaves (500 mg) from each sample were homogenized with 100 ml of 4% oxalic acid and centrifuged at 2000 rpm for 15 minutes. Then 5.0 ml of supernatant solution was pipetted out and 10.0 ml of 4% oxalic acid was added in porcelain basin. Finally titrated against dye solution.

Amount of ascorbic acid in 100 g leaf sample

$$= \frac{0.5 \text{ mg}}{V_1} \times \frac{V_2}{15 \text{ ml}} \times \frac{100 \text{ ml}}{w} \times 100$$

Where,

Weight (g) of sample taken = W

Volume (ml) of dye consumed by working standard = V₁

Volume (ml) of dye consumed by sample = V₂

Estimation of Carbohydrate

The carbohydrate content of fresh leaves from terminal and basal whorls were analyzed separately by anthrone method suggested by [15] Hodge and Hofreiter (1962) and the values are expressed in gram per 100 gram. 500mg of leaf sample was macerated with 80% hot ethanol, centrifuged at 2500 rpm for 20 minutes and the residue was collected. Again washing

was done with 80% hot ethanol till the anthrone reagent gives colourless solution. The residue was dried in water bath. Then 5.0 ml of distilled water and 6.5 ml of 52% per chloric acid were added. The extract was kept at 0°C for fifteen minutes. After centrifugation at 2500 rpm, the supernatant was collected. Extraction was repeated two to three times by using per chloric acid, centrifuged and the supernatants were pooled. The volume of each sample was made up to 100 ml and used as test solution. 0.1 ml of test solution was drawn and made up to 1.0 ml using distilled water. 4.0 ml of anthrone reagent was added and mixed thoroughly and kept in water bath for 8 minutes. Then the samples were cooled rapidly and read in spectrophotometer at 630 nm against the blank.

Amount of carbohydrate in 100 g leaf sample

$$= \frac{\text{Graph value}}{\text{volume of test soln.}} \times \frac{\text{total volume of extract}}{\text{weight of sample (g)}} \times 100$$

Estimation of Protein

The protein content of fresh leaves from terminal and basal whorls were analyzed separately by Biuret method as per [20] Layne (1957) and the results are expressed as gram per 100 gram. Leaves (1.0 g) from each sample was macerated with 10.0 ml phosphate buffer, centrifuged at 1500 rpm and then supernatant was collected. Extraction was repeated twice, supernatants were pooled and the volume was made up to 100 ml with phosphate buffer. Test solution (0.5 ml) was pipetted out and volume made up to 1.0 ml using distilled water. 4.0 ml biuret reagent was added, mixed thoroughly and incubated for 30 minutes at room temperature. The samples were read in spectrophotometer at 550 nm against the blank.

Amount of protein in 100 g leaf sample

$$= \frac{\text{Graph value}}{\text{volume of test soln.}} \times \frac{\text{total volume of extract}}{\text{weight of sample (g)}} \times 100$$

Estimation of Calcium

The versenate method was used for estimation of calcium content in leaf samples. 0.5 g of dried leaf sample from terminal and basal whorl of leaves were taken separately in conical flask and 5.0 ml of triple acid extract pipetted out into conical flask. Then kept in sand bath for 5.0 hours. After that 10% of sodium hydroxide (NaOH) was added drop by drop to neutralize the acidity and another 5.0 ml was added to maintain the pH at 12. Then small quantity of murexide indicator was added and finally titrated against 0.02 N EDTA. The color change of violet from pinkish red was observed as suggested by [16] Jackson (1973) and the values are expressed in percentage.

Percentage of Calcium in leaf sample

$$= 0.0004 \times B \times \frac{V}{5} \times \frac{100}{W} \times \frac{100}{(100 - M)}$$

Where,

Weight (g) of sample taken = W

Volume (ml) of triple acid extract prepared = V

Volume (ml) of 0.02 N EDTA used for Calcium = B

Moisture content (%) of the sample = M

Statistical analysis

All the analyses were performed in triplicate and Analysis of variance (ANOVA) was carried out using statistical package

WINDOWSTAT to evaluate variation between two levels of growth stage and leaf position of the plant.

Result and discussion

Table 1 represents the nutritive composition of terminal and basal whorls of Chekkurmanis leaves at two different growth stages viz., 60 and 120 days after planting and two positions in plants.

Vitamin A

The fresh leaves from terminal and basal whorl of *Sauropus androgynus* contained 4.11mg/100g and 5.12mg/100g Vitamin A respectively on 60 days, while 3.01mg/100g and 4.26mg/100g on 120 days. There existed significant difference ($P < 0.05$) in Vitamin A content in terminal and basal whorl leaves on 60 & 120 days. High Vitamin A content was found in basal whorl leaves on 60 days compared to terminal leaves on 120 days. This trend of Vitamin A content

in Chekkurmanis leaves is analogous with results reported by [27] Padmavathi and Rao (1990) and less than the values reported by [10] Devi *et al.* (2007). This content considerably reduced with later growth stage of the plant. This ensures the fact that Vitamin A is redistributed to every organ of plant as reported by [14] Hocmuth *et al.* (2004). It is essential for effective functioning of visual system, reproduction, immune system, growth and development as stated by [33] Sommer and WHO (1995).

In the present study, Vitamin A content of Chekkurmanis decreased when the plant were in advanced growth stage. Similar aspect was identified in *C. argentea* by [3] Adegbaaju *et al.* (2019) and [7] Biesiada *et al.* (2007) for Leek, Zucchini and Kohlrabi. The values obtained from this study shows higher Vitamin A than *Amaranthus digitata*, *Hibiscus sabdariffa* and *Vigna unguiculata* as noticed by [28] Patricia *et al.* (2014) and moringa leaves by [1] Abbas *et al.* (2018).

Table 1: Nutritive composition of terminal and basal leaves of Chekkurmanis leaves

Days after planting (DAP)	Position of leaf	Vitamin A (mg/100g)	Vitamin C (mg/100g)	Carbohydrate (g/100g)	Protein (g/100g)	Calcium (%)
60 DAP	Terminal whorl	4.11 ^b	112.26 ^b	5.71 ^b	2.63 ^b	1.69 ^b
	Basal whorl	5.12 ^a	123.33 ^a	6.20 ^a	3.04 ^a	2.08 ^a
120 DAP	Terminal whorl	3.01 ^b	199.30 ^b	8.81 ^b	4.25 ^b	2.94 ^b
	Basal whorl	4.26 ^a	220.41 ^a	9.83 ^a	4.99 ^a	4.16 ^a
SEd		0.114	4.492	0.139	0.101	0.056
CD ($P < 0.05$)		0.280	10.992	0.342	0.248	0.137

Mean values carrying superscript letters represent significant difference at $P < 0.05$

Vitamin C

The Vitamin C content in terminal and basal whorl of fresh Chekkurmanis leaves were 112.26 mg/100g and 123.33 mg/100g respectively on 60 days, while on 120 days after planting the content were 199.30 mg/100g and 220.41 mg/100g respectively. The Vitamin C content raised considerably with age. There existed significant difference ($P < 0.05$) in Vitamin C content in terminal and basal whorl leaves on 60 & 120 days. High Vitamin C (220.41 mg/100g) content was noted in basal whorl leaves on 120 days and lesser value (92.26 mg/100g) in terminal leaves on 60 days. Similar trend was observed in *Telfairia occidentalis* by [18] Platel and Srinivasan (2017) and [5] Musa *et al.* (2012). The previous study in Chekkurmanis by [22] Padmavathi and Rao (1990) and [31] Singh *et al.* (2011) reported that vitamin C content was 244.00 mg/100g and 314.30 mg/100g respectively in terminal and basal whorls, which was higher than this study result. According to [21] Lee and Kadar (2000), the maturity of leaves is a major factor that determines the compositional value of vegetables. Further [19] Korus (2010) observed that Vitamin C content depends on degree of plant maturity and variety. In this study, Vitamin C attained the maximum value of 220.41 mg/100g in basal whorl leaves on 120 days. It is very high when compared with *Amaranthus digitata* (70.00 mg/100g) and *Hibiscus sabdariffa* (30.00 mg/100g) as reported by [28] Patricia *et al.* (2010) and *Moringa oleifera* (0.56 mg/100ml) as reported by [2] Achikanu *et al.* (2013).

Vitamin C supports synthesis of hormone, iron absorption and collagen, which is extensively used in cosmetics and pharmaceuticals. It acts as water soluble antioxidant, which plays a major role in free radical scavenging activity and activates immune system [22] (Locato *et al.*, 2013).

Carbohydrate

The carbohydrate value observed in terminal and basal whorl

of fresh leaves were 5.71g/100g and 6.20g/100g respectively on 60 days, while 8.81g/100g and 9.8 g/100g respectively on 120 days. Carbohydrate content was high (9.83g/100g) in basal leaves on 120 days and low (5.71g/100g) in terminal leaves on 60 days. The basal whorl leaves consisted of more carbohydrate (6.20g/100g & 9.8g/100g) when compared with terminal whorl leaves (5.71g/100g & 8.81g/100g) on 60 and 120 days. There existed significant difference ($P < 0.05$) in carbohydrate content on the terminal and basal whorl leaves on 60 & 120 days. The content also significantly increased which respect to the maturation of the plants and this finding is in line with the findings of [3] Adegbaaju *et al.* (2019). The amount of carbohydrate observed in this study was identical with earlier research reported in *Sauropus androgynus* by [9] Chakraborty *et al.* (2019), but lower than as reported by [10] Devi *et al.* (2007). Carbohydrate is a fundamental element for normal functioning of human body. Ingestion of carbohydrate enriched leaves adds energy level to diet by providing favourable nutrition. Amount of carbohydrate in basal leaves of katuk is higher than *T. occidentalis* but lower than *C. aconitifolius* as indicated by [26] Otitoju *et al.* (2014) and equal to the *Senna occidentalis*, *Solanum nodiflorum*, *Physalis viscose* by [24] Odhav *et al.* (2007), moringa leaves as observed by [1] Abbas *et al.* (2018).

Protein

Fresh leaves of terminal and basal whorl leaves of Chekkurmanis contained protein content in the range of 2.63 g/100g & 3.04 g/100g respectively at 60 days, while it was 4.25 g/100g & 4.99 g/100g respectively at 120 days. There existed significant difference ($P < 0.05$) in protein content on the terminal and basal whorl leaves on 60 & 120 days. Previously [32] Singh *et al.* (2011) published the literature and reported that protein content of Chekkurmanis leaves were 5.25 g/100g. From the result of this study, the protein content of *Sauropus androgynus* increased with age of the plant.

According to ^[6] Bamishayie (2011), the protein content of moringa leaves was moderately low at initial stage (27.61%) and finally reached higher values on later stage (28.08%). The highest protein content (4.99 g/100g) was recorded in basal whorl of the plant on 120 days after planting and the lowest content (2.63 g/100g) was recorded on terminal whorl of the plant 60 days after planting. The protein values found in basal leaves of Chekkurmanis on 120 days after planting were higher than *Petroselinum crispum* (2.97 g/100g), *Anethum graveolens* (3.46 g/100g), *Lactuca sativa* (1.62 g/100g), *Brassica oleraceae* (1.21 g/100g) as reported by ^[8] Caunii *et al.* (2010), but lower than *Moringa oleifera* leaves (6.7 g/100g) as reported by ^[11] Gopala Krishnan *et al.* (2016). Therefore, *Sauropus androgynus* has the potential to serve as a very good source of protein for the reduction of protein malnutrition. It also acts as neurotransmitter and carries the oxygen in blood. Deficiency of protein causes malnutrition diseases viz., kwashiorkor and marasmus and results in stunted growth. It is also useful for immune function, creation of enzymes and hormones and maintains fluid balance in the body.

Calcium

Calcium content of dried leaf samples collected from both terminal and basal whorl of leaves was in the range of 1.69% and 2.08% respectively on 60 days, whereas it was 2.94% and 4.16% respectively on 120 days. There existed significant difference ($P < 0.05$) in calcium content in the terminal and basal whorl leaves on 60 & 120 days after planting. Calcium percentage obtained in this study is more (4.16%) than that (0.7%) as stated by ^[27] Padmavathi and Rao (1990). In the present study, the highest calcium content observed in basal leaves at 120 days was 4.16%. This finding is in agreement with ^[23] Modi (2007), who observed more calcium content in long day interval than short day interval in amaranthus plant. In contrast, the lowest calcium content of 1.69% was recorded in the terminal leaves on 60 days. The highest calcium content in basal leaves might be due to immobility (non-translocation) of calcium in the plant. This concept is in agreement with the authors ^[35] Taiz & Zeiger (2002) and ^[14] Hochmuth *et al.* (2004).

When the plants approach maturity, the nutrient content (calcium) in the leaves gets increased. This concept is in agreement with the values reported by ^[18] Platel and Srinivasan (2017) for Chekkurmanis crop and similar trend with age of plant was observed in tender and mature stages of *Celosia argentea* plant by ^[3] Adediran *et al.* (2015). Calcium plays an important role in nervous system, cell signaling and muscle contraction. It also helps to maintain healthy teeth and bones. Calcium is also utilized by cells for transferring ions and triggers several enzymes secretion in human body by ^[31] Sadler (2011). The values found in this study are higher than some of underutilized green leafy vegetables such as *Digera arvensis* (0.50%), *Amaranthus tricolor* (0.23%) and *Cucurbita maxima* (0.30%) as reported by ^[12] Gupta *et al.* (2004), *Hibiscus sabdariffa* (0.4%) and *Ipomea aquatica* (0.09 %) by ^[32] Singh *et al.* (2011), *Alternanthera sessilis* (2.0 %) and *Celosia argentea* (2.03%) as reported by ^[30] Rekha Sinha (2018) and *Moringa oleifera* (1.14%) by ^[34] Srivastava *et al.* (2018).

Conclusion

The results of the study indicate that *Sauropus androgynus* is an excellent source of Vitamin A & C, Carbohydrate, Protein and Calcium than any other leafy vegetables. Further, the

green leafy vegetables are fine source of vitamins, minerals and other minor elements. The study reveals that basal whorl of leaves consist of more nutrient content than terminal whorl leaves and there existed significant difference between the nutritional constituents in leaves upon growth of the plant.

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