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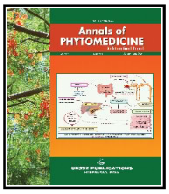

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Exploring the diverse bioactive compounds from chekkurmanis (*Sauropus androgynus* L.) leaves using HP-LC and GC-MS

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Abstract

Sauropus androgynus L. leaves are commonly called "protective food" due to their chief source of minerals and vitamins. The results of the study concluded that significantly highest carbohydrate (11.08 g/100 g), fiber (34.70 g/100 g), and iron (219.11 mg/100 g) contents in a combination of basal leaves on 180 days after planting, potassium (3392.65 mg/100 g), copper (9.81 mg/100 g) and zinc (14.46 mg/100 g) were in basal leaves on 120 days after planting, magnesium (269.50 mg/100 g) in basal leaves on 60 days after planting and vitamin B2 (riboflavin) (1.39 mg/100 g) and calorie (369.50 kcal) in terminal leaves on 60 and 120 days after planting. From the study, it could be revealed that basal leaves contained significantly higher nutritive value than the terminal whorls of *S. androgynus* leaves. According to days after planting, a slight increase in carbohydrate, fiber, and iron contents was recorded as the plant approached maturation from 60 to 180 days after planting whereas magnesium, and vitamin B2 values are high at 60 days after planting. This study exhibits that the nutritive composition of *S. androgynus* leaves is typically influenced by days after planting and the position of the leaves of plants. From GC-MS analysis, ethanol and hexane extract of *S. androgynus* leaf sample contains various phytochemical compounds; namely, 1H-idene, 1-methylene, 4,6,8 tetra decatriene, vitamin E, germacrene, cyclooctyne, tropone and bicyclogermacrene.

1. Introduction

S. androgynus the local names of the crop are; Katuk, Star gooseberry, and Sweet leaf bush, comes under the family 'Euphorbiaceae', is a glabrous shrubby perennial green vegetable, considerably grown in warm humid tropics with copious rainfall. It is congenital in the South and South East Asian region due to its therapeutic properties as stated by Padmavathi and Rao (1990). Intriguingly, in India, this is commonly known as "Multivitamin" or "Multigreen plant", because of its high nutritive value. *S. androgynus* is a prevalent green leaf vegetable with notable levels of carbohydrate, thiamine, vitamin A, vitamin B, vitamin C, calcium, iron, potassium, phosphorous, and protein. Owing to an aspect of antioxidant properties in leaves, this is extensively employed for healing many diseases, *viz.*, diabetics, cancer, allergy, microbial infection, cholesterol, and swelling (Paul and Anto, 2011).

Micronutrient deficiency has increased all over the world and it is called "Hidden Hunger". In most countries, predominantly women and children are malnourished (*i.e.*) strained by micronutrient deficiencies, which lead the way to high death rates worldwide, which is due to a lack of consumption of green leafy vegetables. In this regard, *S. androgynus* is the chief source of many minerals and nutrients as compared to any other green leafy vegetables. A required quantity of vitamins, minerals, and phytochemicals is essential for the typical performance of human metabolic activities. So, efforts are taken to analyze the characteristics of the nutrients that are available in *S. androgynus*. To observe the bioactive compounds of *S. androgynus* leaves at various growth stages and positions of leaves.

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2. Materials and Methods

The research trial was conducted at field number 26 Western Block Farm, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam. The soil nature of the experimental plot was sandy loam. Leaf samples were dried in a hot air oven at 60°C for 6 h until the leaves became crisp and brittle for estimating calorie, fiber, potassium, magnesium, iron, zinc, copper, and vitamin B_2 parameters. The identification of bioactive compounds by using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HP-LC) analyses have been done at the Department of Agricultural Biotechnology, Agricultural College and Research Institute, Madurai.

2.1 Treatment details for biochemical parameters

The basal (matured) and terminal (tender) whorls of *S. androgynus* leaf samples were collected at different growth stages, *viz.*, 60, 120 and 180 days after planting (DAP) for qualitative analysis. The statistical design adopted for this experiment was factorial randomized block design. All the analyses were carried out in three replications.

The details of the treatments are given below.

Factor: I	**Factor: II**
Position of a leaf **(P)**	Interval of days after planting **(D)**
P_1 = Terminal whorl (tender)	D_1 = 60 days after planting
P_2 = Basal whorl (matured)	D_2 = 120 days after planting
D_3 = 180 days after planting	

Correspondingly, there are six treatment combinations among leaf position and interval of days after planting are presented in the table.

S.No.	**Treatment combination**	**Treatment details Position of leaf sample + Days after planting**
1.	P_1D_1	Terminal whorl (P_1) + 60 days after planting (D_1)
2.	P_2D_1	Basal whorl (P_2) + 60 days after planting (D_1)
3.	P_1D_2	Terminal whorl (P_1) + 90 days after planting (D_2)
4.	P_2D_2	Basal whorl (P_2) + 90 days after planting (D_2)
5.	P_1D_3	Terminal whorl (P_1) + 120 days after planting (D_3)
6.	P_2D_3	Basal whorl (P_2) + 120 days after planting (D_3)

The total calorie (kcal) value of *S. androgynus* leaves was estimated as per the method suggested by Onyeike *et al.* (1995). The carbohydrate (g/100 g) content of fresh leaves was analyzed by the anthrone method as suggested by Hodge and Hofreiter (1962). The fiber content was determined as per the method described by Watson (1994) and expressed in g/100 g. The concentration of potassium in the sample was deduced from the standard curve as described by Jackson (1973) and expressed in mg/100 g. Magnesium content suggested by Jackson (1973) and the values are expressed in mg/100 g. The Iron, Zinc, and Copper mg/100 g content of dried powdered leaf samples were analyzed as per the method illustrated by Lindsay and Norvell (1978). The amount of vitamin B2 content in the leaves was determined as per the method delineated by Seal *et al.* (2016).

2.2 Statistical analysis

All the estimations were carried out in three replicates and analysis of variance (ANOVA) was performed by utilizing the statistical package Windowstat to estimate the difference between two positions of leaves and three different stages of grow

3. Results

3.1 Calories

The calories of *S. androgynus* leaves varied from 336.30 kcal to 369.50 kcal. A maximum calorie value of 357.50 kcal was obtained in terminal leaves (P_1), followed by 347.06 kcal in basal leaves (P_2). The highest calorie value was registered in 120 DAP (D_2) with a value of 360.30 kcal, followed by 180 DAP (D_3) which recorded 359.05 kcal. The lowest calorie was obtained on 60 DAP (D_1) with a value of 337.50 kcal (Table 1). The interaction effect of the position of leaves and days after planting on calorie value was found to be non-significant.

3.2 Carbohydrate

Regarding the position of leaves, the highest carbohydrate content of 9.03 g/100 g was recorded in basal leaves (P_2) and the lowest 7.85 g/100 g in terminal leaves (P_1). Considering the days after planting, the highest carbohydrate value of 10.06 g/100 g was registered at 180 DAP (D_3), followed by 120 DAP (D_2) and 60 DAP (D_1) with a value of 9.32 and 5.95 g/100 g, respectively. Among the combination effect, a higher combination effect was identified in basal leaves on 180 DAP (P_2D_3) which exhibited 11.08 g/100 g and it was followed by basal leaves on 120 DAP (P_2D_2) which recorded 9.83 g/100 g (Table 1).

3.3 Fiber

The fiber content of leaves ranged from 27.30 g/100 g to 34.70 g/100 g. The maximum concentration of fiber 32.13 g/100 g was observed in basal leaves (P_2), followed by terminal leaves (P_1) with 28.16 g/100 g. Fiber content revealed a continuously increasing trend from 60 DAP to 180 DAP and increased with the age of the crop. Fiber content was the highest (31.34 g/100 g) in 180 DAP (D_3) and was on par (31.20 g/100 g) with 120 DAP (D_2). This was followed by 60 DAP (D_1) with a fiber content of 27.90 g/100 g (Table 1). The interaction effect of basal leaves on 180 DAP (P_2D_3) exhibited a higher fiber content of 34.70 g/100 g, followed by basal leaves (33.20 g/100 g) on 120 DAP (P_2D_2).

3.4 Potassium

The basal leaves (P_2) registered the highest potassium content of 1852.55 mg/100 g and the lowest value of 1735.96 mg/100 g was observed in terminal leaves (P_1). The highest value of potassium (3035.27 mg/100 g) was obtained at 120 DAP (D_2). The position of leaves and days after planting was found significant for potassium content. Basal leaves on 120 DAP (P_2D_2) recorded higher potassium content (3392.65 mg/100 g) and terminal leaves on 180 DAP (P_1D_3) recorded lesser potassium content of 1040.00 mg/100 g (Table 1).

3.5 Magnesium

Based on the position of leaves, magnesium content in basal leaves (P2) registered non-significantly highest value of 264.16 mg/100 g, but magnesium content was influenced by days after planting. Significant variation in magnesium content between the days after planting. Among the days after planting, the maximum magnesium content of 265.35 mg/100 g was recorded at 180 DAP (D3), and a minimum value of 252.60 mg/100 g (Table 1) was observed at 120 DAP (D2). The interaction effect of the position of leaves and days after planting was found to be non-significant on the magnesium content of leaves.

3.6 Iron

Significantly highest iron content of 212.76 mg/100 g was observed in basal leaves (P_2), which was followed by terminal leaves (P_1) with 206.43 mg/100 g. Considering the days after planting, the maximum content of 215.56 mg/100 g was registered at 180 DAP (D_3), and it was followed by 120 DAP (D_2), which recorded 211.50 mg iron per 100 g of leaf (Table 1). The interaction between the position of leaves and days after planting showed non-significant variation.

3.7 Copper

A higher level of copper content (8.71 mg/100 g) was observed in basal leaves (P_2), followed by terminal leaves (P_1) with copper content of 7.55 mg/100 g. Regarding days after planting, 120 DAP (D_2), the highest copper content of 9.11 mg/100 g was registered. The highest interaction effect on copper was expressed in basal leaves on 120 DAP (P_2D_2) which had 9.81 mg/100 g, followed by basal leaves on 180 DAP (P_2D_3) which registered 8.68 mg/100 g (Table 1).

3.8 Zinc

The maximum level of zinc was recorded in basal leaves (P_2) which was 13.45 mg/100 g, followed by terminal leaves (P_1) which had 12.51 mg/100 g. Significantly highest level of zinc content was noted at 120 DAP (D_2) with a value of 14.09 mg/100 g, followed by 180 DAP (D_3) which had 13.05 mg/100 g. The maximum interaction was recorded in basal leaves on 120 DAP (P_2D_2) which had 14.46 mg/100 g and the least interaction effect was observed in terminal leaves on 60 DAP (P_1D_1) which was 11.59 mg (Table 1) zinc per 100 g of leaves.

3.9 Vitamin B_2 (Riboflavin)

Terminal leaves (P_1) registered the maximum level of riboflavin (0.50 mg/100 g), followed by basal leaves (P_2) which was 0.32 mg/100 g. Among the days after planting, 60 DAP (D_1), Figure 1 exhibited significantly higher riboflavin (1.14 mg/100 g) and it was followed by 180 DAP (D_3), Figure 2 and 120 DAP (D_2), Figure 3 both of which were recorded 0.04 mg/100 g.

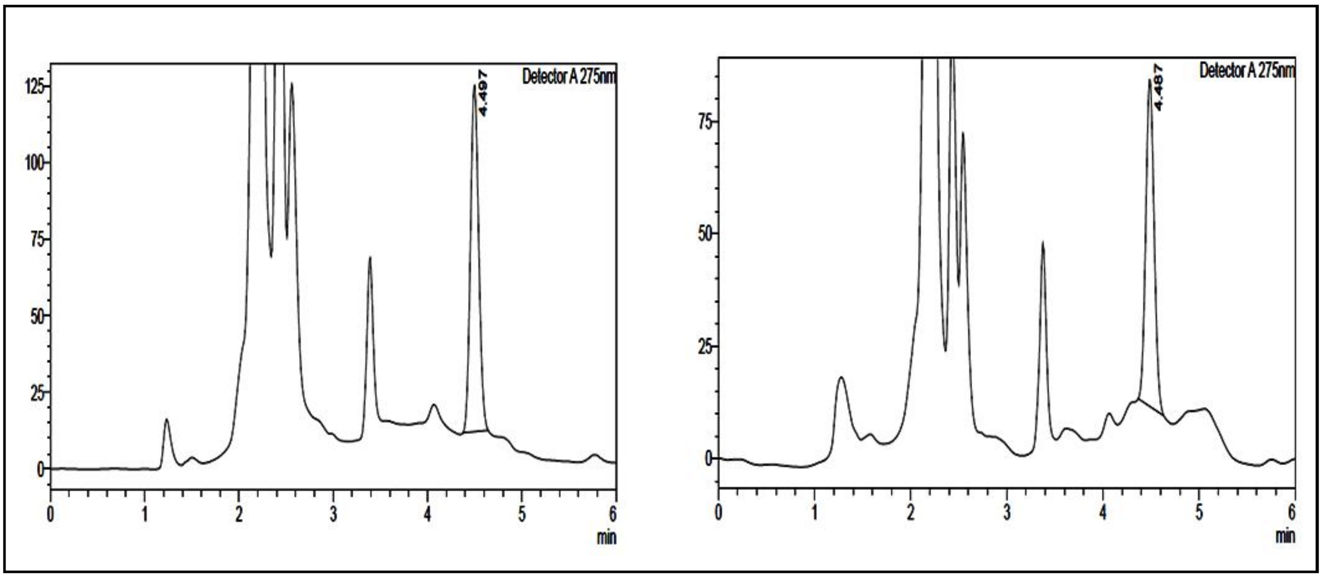


Figure 1: HP-LC analysis of terminal and basal *S. androgynus* leaves on 60 DAP.

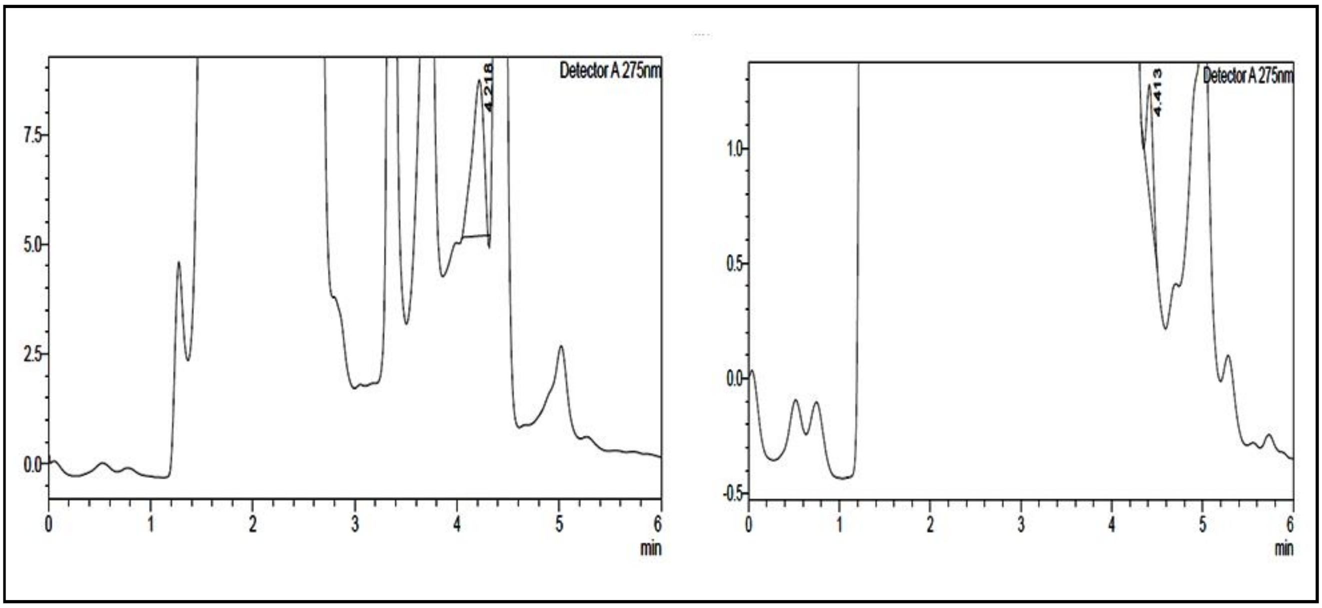


Figure 2: HP-LC analysis of terminal and basal *S. androgynus* leaves on 180 DAP.

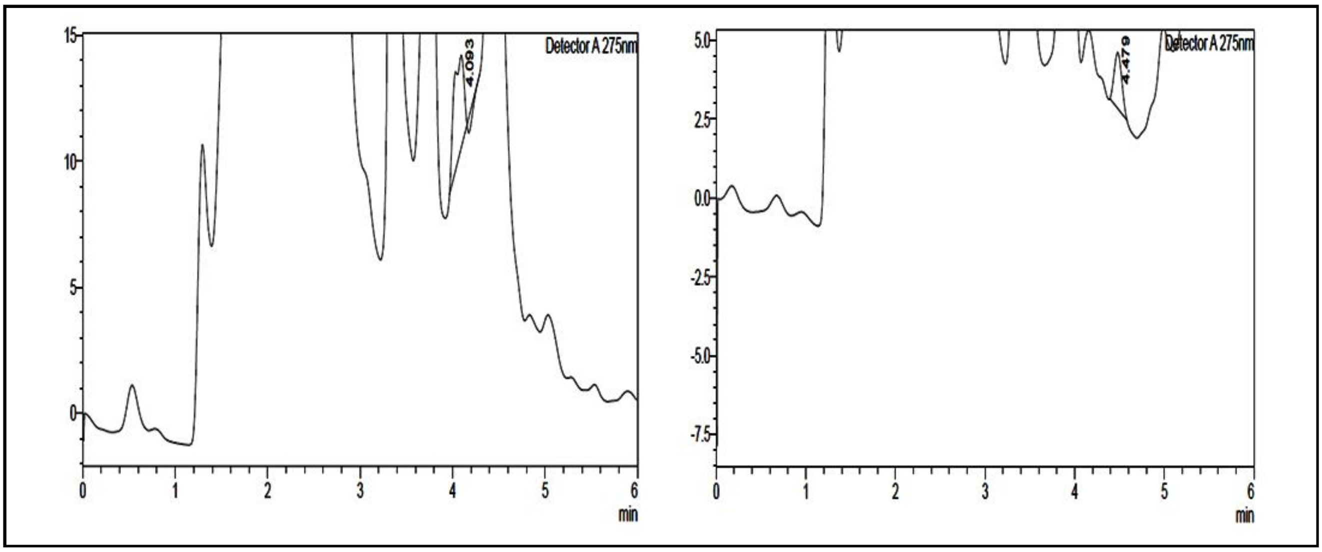


Figure 3: HP-LC analysis of terminal and basal *S. androgynus* leaves on 120 DAP.

With regard to riboflavin, terminal leaves on 60 DAP (P_1D_1) expressed the highest interaction effect of 1.39 mg/100 g, followed by basal leaves on 60 DAP (P_2D_1) which recorded 0.90 mg/100 g. The lowest interaction effect was observed in basal leaves on 120 DAP (P_2D_2) with a value of 0.02 mg/100 g (Table 1).

Table 1: Influence of Position of leaves and days after planting on biochemical parameters

Parameters	D1	D2	D3	Mean	Factors	SE (d)	CD (0.05)
Calorie (kcal)(P_1)	338.70	369.50	364.30	357.50	P	4.70	5.75*
(P_2)	336.30	351.10	353.80	347.06	D	10.47	12.82**
Mean	337.50	360.30	359.05	352.28	P x D	8.14	NS
Carbohydrate (g/100 g) (P_1)	5.71	8.81	9.03	7.85	P	0.10	0.23*
(P_2)	6.19	9.83	11.08	9.03	D	0.13	0.29**
Mean	5.95	9.32	10.06	8.44	P x D	0.18	0.14**
Fat (g/100 g) (P_1)	3.98	4.85	3.92	4.25	P	0.06	0.13**
(P_2)	4.71	4.86	5.88	5.15	D	0.07	0.16**
Mean	4.34	4.85	4.90	4.70	P x D	0.10	0.23**
Fiber (g/100 g) (P_1)	27.30	29.20	27.99	28.16	P	0.30	0.67**
(P_2)	28.50	33.20	34.70	32.13	D	0.37	0.82**
Mean	27.90	31.20	31.34	30.14	P x D	0.52	1.16**
Sodium (mg/100 g) (P_1)	145.39	129.50	147.60	140.83	P	1.99	4.44**
(P_2)	171.99	147.10	153.00	157.36	D	2.44	5.44**
Mean	158.69	138.30	150.30	149.09	P x D	3.45	7.70**
Potassium (mg/100 g) (P_1)	1489.99	2677.89	1040.00	1735.96	P	28.20	62.84**
(P_2)	1068.00	3392.65	1097.00	1852.55	D	34.54	76.97**
Mean	1278.99	3035.27	1068.50	1794.27	P x D	48.84	108.85**
Magnesium (mg/100 g) (P_1)	260.00	247.80	265.10	257.63	P	3.17	NS
(P_2)	269.50	257.40	265.60	264.16	D	3.88	8.65**
Mean	264.75	252.60	265.35	260.90	P x D	5.49	NS
Iron (mg/100 g) (P_1)	199.41	207.85	212.05	206.43	P	1.62	3.62**
(P_2)	204.03	215.16	219.11	212.76	D	1.99	4.43**

Mean	201.72	211.50	215.58	209.60	P x D	2.81	NS
Copper (mg/100 g) (P_1)	6.97	8.42	7.27	7.55	P	0.11	0.25**
(P_2)	7.64	9.81	8.68	8.71	D	0.13	0.30**
Mean	7.31	9.11	7.97	8.13	P x D	0.19	0.43*
Zinc (mg/100 g) (P_1)	11.59	13.73	12.21	12.51	P	0.11	0.25**
(P_2)	12.01	14.46	13.90	13.45	D	0.13	0.30**
Mean	11.80	14.09	13.05	12.98	P x D	0.19	0.43*
Vitamin (B_2) (mg/100 g) (P_1)	1.39	0.06	0.05	0.50	P	0.01	0.02**
(P_2)	0.90	0.02	0.04	0.32	D	0.01	0.03**
Mean	1.14	0.04	0.04	0.41	P x D	0.01	0.04**

4. Discussion

The results indicated that terminal leaves had higher calories as compared to basal leaves. Among the position of leaves, the maximum level of calories was registered in terminal leaves. A higher level of calories was observed at 120 DAP. Terminal leaves of plants are still growing and tender leaves with optical metabolic and physiological reaction triggering the calorie accumulation in terminal leaves then the basal leaves. An increase in calories with advances in stages of growth was observed by Adegbaju *et al.* (2019) in Celosia. The interaction effect of the position of leaves and days after planting on calorie value was found to be non-significant.

The fact that why carbohydrates are richer in basal than terminal leaves may presumably be due to more production intending to supply energy for metabolic activities and the development of plant parts. Similar trends of virtuous carbohydrate proportion in basal leaves compared to terminal leaves have been reported by Mataa *et al.* (2020) in *Hibiscus sabdariffa* and Kagale and Surekha (2023) in *Colocasia esculenta* leaves.

In the present study, maximum fiber content was recorded in basal leaves rather than terminal leaves which may be due to lignification of cell wall components leading to more fiber accumulation. A similar trend in the position of leaves was reported by Platel and Srinivasan (2017) in *S. androgynus*. As days after planting increased, fiber content also increased. The gradual increase in fiber content as the plant leaves approached maturation was well supported by Adegbaju *et al.* (2019) in Celosia and Kumar *et al.* (2023) in *Daucus carota*.

It is a mobile mineral element. The present study showed that basal leaves expressed higher potassium content compared to terminal leaves in *S. androgynus*. The observed pattern of variation in potassium content along with the age of the leaf has been previously noticed by several workers and in Amaranthus by Makobo *et al.* (2010).

Non-significantly basal leaves contained relatively higher magnesium content than terminal leaves. A higher amount of magnesium content was observed on 180 DAP. Data on magnesium content indicates that when a plant approaches maturation, the content also increases. The phenomenon of increased magnesium values is in harmony with earlier workers and in Hibiscus species by Khader and Rama (2003).

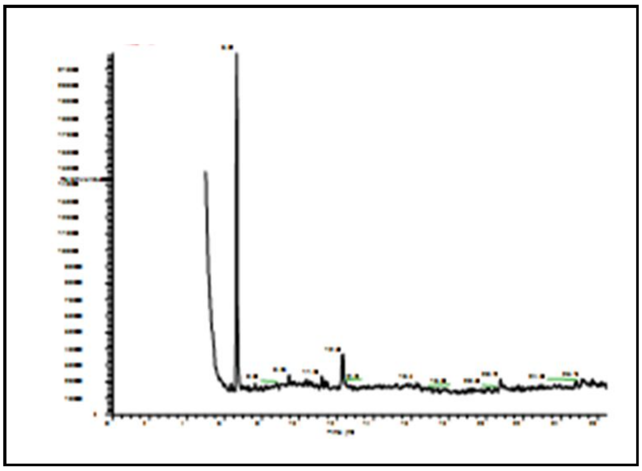
Basal whorls of leaves recorded significantly higher iron content than terminal whorls. This is in accordance with the reports of Srinivasan and Murali (2022) on *S. androgynus*. Among the three different stages of sample collection, iron content was found to be significantly higher at 180 DAP. Considering the days after planting, the iron content of leaves gradually increased from the initial to the later stage of plant growth. This is in accordance with Uddin *et al.* (2012) in purslane and Singirikonda *et al.* (2021) in *Cochlospermum religiosum*.

A higher level of copper content was observed in basal leaves, followed by terminal leaves (P1). This result is in agreement with work on *S. androgynus* by Platel and Srinivasan (2017). The observation recorded in terms of days after planting indicated that the copper content significantly increased from 60 DAP to 120 DAP and then gradually decreased towards 180 DAP. An identical variation trend for this micronutrient was previously reported by Acikgoz (2011) in *Brassica oleraceae var. acephala.*

The result perceived from the present investigation showed that the level of zinc in basal whorl leaves was significantly higher than in terminal whorl leaves. This is in harmony with the previous reports of Musa and Ogbadoyi (2012) in *Telfairia occidentalis* and Platel and Srinivasan (2017) in *S. androgynus.* Significantly highest level of zinc content was noted followed by 180 DAP (D3). This finding is in accordance with Adegbaju *et al.* (2019) in *Celosia argentea*.

Both position of leaves and days after planting had significant influence on riboflavin. In the present study, terminal leaves obtained the maximum riboflavin followed by basal leaves. Similar trend on higher vitamin B_2 in basal leaves also previously reported by Srinivasan and Murali (2022) in *Sida acuta*. With respect to days after planting, at 60 DAP the riboflavin content was maximum at 120 and 180 DAP and showed slight decrease at 60 DAP. As the plant grew older, it undergoes some metabolic changes and common physiological process.

All the volatile compounds in ethanol extract from terminal leaves of *S. androgynus* were identified by GC-MS analysis and prevailed the occurrence of different components with various retention times. The mass spectrometer examined the components exhibited at various retention times to distinguish the structure and nature of the constituents. The major constituents are 1H-indene, 1-methylene, 4,6,8-tetradecatriene, and vitamin E compounds which play a role in the nutritive potential of the plant. The highest peak (6.67 RT) was due to the appearance of 1H-indene, 1-methylene (Figure 4).

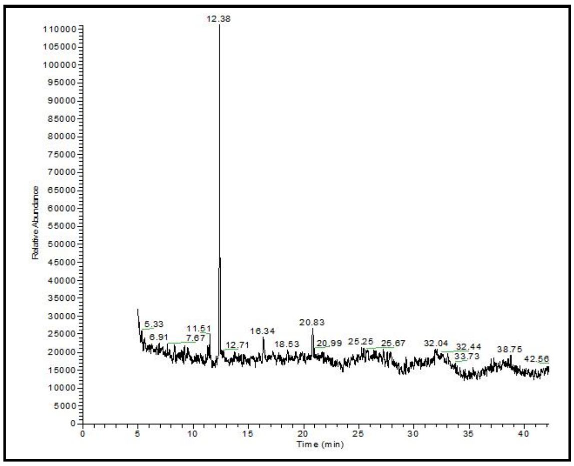


Compound name	Molecular formula	Molecular wt. (g/mol)	Retention time (min)	Area (%)	Height (%)
1H-indene, 1-methylene	$C_{10}H_8$	128.17	6.67	70.61	74.91
4,6,8-tetradecatriene	$C_{14}H_{16}O_2$	216.27	12.35	12.37	7.38
Vitamin E	$C_{29}H_{50}O_2$	430.71	20.80	2.43	2.21

Figure 4: GC-MS analysis of ethanol extract of terminal leaves of *S. androgynus*.

The other names of these compounds are: 1-methylene-1H-indene, benzofulvene and 1 methylideneindene. The high-ranking compound (peak height 74.91%) of this extract 1H-indene, 1-methylene- has been recognized as an aromatic hydrocarbon constituent utilized as a fragrant or odor agent and finds application in other industrial purposes. It comprises a benzene ring, which is linked with a cyclopentene ring. The next highest peak (12.35 RT) found was 4,6,8-tetradecatriene (peak height 7.38%). It is a polyethylene alkyne compound reported to have biological activities namely antibacterial, anti inflammatory, and antiarrhythmic activities. Furthermore, a well-known antioxidant, vitamin E is also available in this leaf extract at 20.80 RT and 2.21% height. It is a lipid-soluble compound in cell antioxidant safeguarding system. It prevents oxidative stress, conserves cell membrane, governs platelet aggregation, and activates protein kinase as well as serves as an antioxidant, acts against inflammation, cancer, heart disease, and cataracts, and stimulates the immune system (Rizvi *et al.,* 2014).

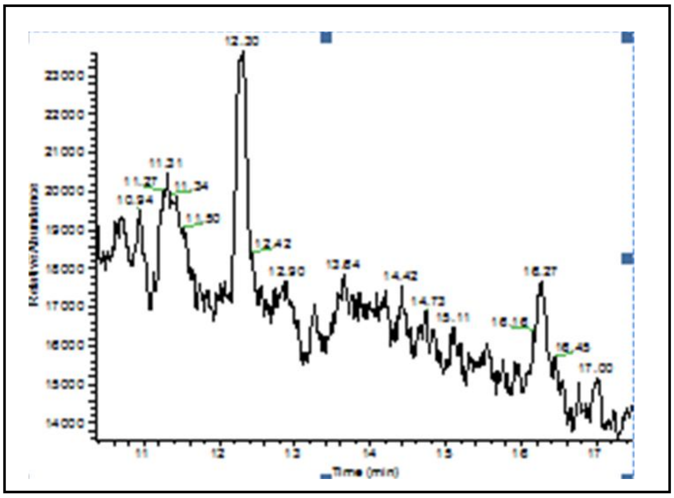
In the GC-MS study, ethanol extract of basal leaves showed the highest peak due to the presence of germacrene component identified on 12.38 retention time with 62.51% height (Figure 5). This compound comes under the class of natural volatile hydrocarbon, particularly sesquiterpene compound commonly generated in many plant species in order to its insecticidal and antimicrobial activities, however functions as insect pheromones (Adio, 2009), Devi *et al.* (2021) in *Curcuma angustifolia* and Kurkcuoglu *et al.* (2021) in *Stachys megalodonta.*



Compound name	Molecular formula	Molecular wt. (g/mol)	Retentiontime (mins)	Area (%)	Height (%)
Germacrene	$C_{15}H_{24}$	204.35	12.38	63.86	62.51

Figure 5: GC-MS analysis of ethanol extract of basal leaves of *S. androgynus*.

The GC-MS chromatogram of hexane extract of terminal leaves of *S. androgynus* indicated that the largest peak was due to the existence of cyclooctyne on 12.30 retention time with 21.11% height (Figure 6), otherwise known as 1-cyclooctyne. Cyclooctyne is the smallest ring structure under the class of cycloalkyne which can be isolated in unadulterated form because of its appreciable energy.

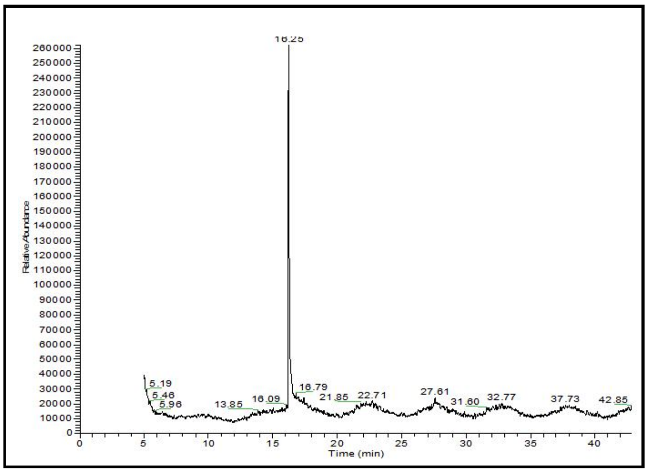


Compound name	Molecular formula	Molecular wt. (g/mol)	Retentiontime (mins)	Area (%)	Height (%)
Cyclooctyne	C_8H_{12}	108.8	12.30	21.13	21.11
Tropone	$C_8H_{15}NO$	141.212	16.27	7.05	8.06

Figure 6: GC-MS analysis of hexane extract of terminal leaves of *S. androgynus*.

It has a wide range of applications in chemical biology, drug discovery, nanotechnology, and surface functionalization and it is capable of forming complexes with copper, zinc, cobalt, molybdenum, manganese, and tungsten. In organic chemistry, a cyclooctyne is the cyclic analog of an octyne as stated by Baskin *et al.* (2007). In addition to this, the second-highest compound tropone was detected at 16.27 retention time with 8.06% height. Tropone is an organic compound that has several influences on organic chemistry due to its non-benzenoid aromatic compound and is also used as a building block in different cyclo addition reactions. It comprises seven carbon atoms conjoined with three ketone and alkene groups and perceives aromatic properties. Some proportion of this component was discovered in biomolecules including colchicine and stipitatic acid. It possesses pharmaceutical properties, *viz.*, antifungal, antibacterial, antiviral, and antitumor (Ononye *et al.,* 2014).

In the GC-MS analysis, phytochemical compounds were determined in the hexane extract of basal leaves. The largest peak compound bicyclo germacrene was noted on retention time 16.25 with 89.86% height (Figure 7).



Compound name	Molecular formula	Molecular wt (g/mol)	Retentiontime (mins)	Area(%)	Height(%)
Bicyclogermacrene	$C_{15}H_{24}$	204.35	16.25	84.01	89.86

Figure 7: GC-MS analysis of hexane extract of basal leaves of *S. androgynus*.

Bicyclogermacrene comes under the organic compound class, also called bicyclogermacrane and isolepidozanesesquiterpenoid, a compound that is obtained from germacrene through cyclization and dehydrogenation processes. This compound was found to have a reasonable impact on Gram-negative and Gram-positive bacteria. Despite that, it possesses considerable consequences for fungi (Arn and Acree, 1998).

5. Conclusion

From this study, it could be concluded that carbohydrate, and fiber iron contents increased with plant maturity which reinforces its anatomical characteristics whereas, magnesium, zinc, copper, and vitamin B_2 contents expressed gradually decreased progression on maturation of plants. The nutritive composition of leaves differs substantially towards the growth of plants. The plant maturation process is extensively synchronized with biochemical variation and the correct stage of harvest is more important in order to obtain quality produce. Furthermore, the basal leaves are more nutritious than the terminal leaves. Hence, it can be concluded that the position of leaves and different stages of growth influenced significant variations in the nutritional value of leaves.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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