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ORIGINAL RESEARCH ARTICLE

Optimizing harvest time of *Cassia angustifolia* Vahl Pennel. for maximum total sennoside content: A fast UV spectrometric analysis approach

SNEHA A[2#] • NIRANJANA KUMAR A[1#] • KOTESH KUMAR J[1*] • SATYA SRINIVAS KVN[1*] KIRAN BABU GD[1] • VENKATESH B[1] • JNANESHA AC[1] • VINUTHA K[2]

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ABSTRACT

Cassia angustifolia, commonly known as Senna, is a well-known medicinal plant used in Ayurvedic formulations as a natural laxative. The medicinal property of Senna is primarily attributed to dimeric anthraquinone glycosides, known as sennosides. The qualitative and quantitative expression of secondary metabolites in medicinal and aromatic plants is influenced by various factors, including harvesting time, geographic location, seasonal changes, and cultivation practices. In this study, we investigated the impact of different harvesting times on the Total Sennosides Content (TSC) in different parts of Senna. The plant was harvested at five different time intervals during a three-and-a-half-month period from December 2019 to March 2020. The TSC was determined by using UV-Vis Spectrophotometer. Our results showed a significant increase in TSC in all parts of the plant with an increasing harvesting time of up to 105 days. This study provides new insights into the effect of harvesting time on the quality and quantity of sennosides in Senna, which could help optimize the harvesting practices and improve the therapeutic potential of this valuable medicinal plant.

INTRODUCTION

Cassia is a genus of the Leguminosae family, sub-family Caesalpiniaceae, consisting of approximately 580 species of herbs, shrubs, and trees. These plants are known for their diverse uses, including medicinal, ornamental, and economic purposes, as a source of tanning material. *Cassia senna* Vahl, also known as Alexandrian Senna, is native to Yemen, Somalia, Southern Arabia, and the Nile River region (Abulafatih 1987, Ghazanfar and Al-Sabahi, 1993) and is now cultivated worldwide for its medicinal properties. Another well-known species, *C. angustifolia*, commonly called Tinnevelly senna, is grown in Tamil Nadu (Pandse *et al*., 1974) and is found in the wild state in the Mundra coastal tract and Kutch region of India, where the soil is loamy with high pH and salinity (Gupta, 1974). It is a small, erect, perennial shrub that can reach a height of 100-120 cm. The plant's leaves are pinnate, with narrow, acute, lanceolate, and glabrous leaflets of pale green color. The pods are 4-7 cm long, containing 5-7 obovate, dark brown, and nearly smooth seeds.

*Corresponding Authors, E-mail: koteshkumarj@cimap.res.in; kvn.satyasrinivas@cimap.res.in
Institutional Communication No.: CIMAP/PUB/2023/47
[1]Phytochemistry Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092, India.
[2]Sri Venkateshwara College of Pharmacy, Hyderabad, India.
#A. Sneha and A. Niranjana Kumar have equal contributions and considered as first authors
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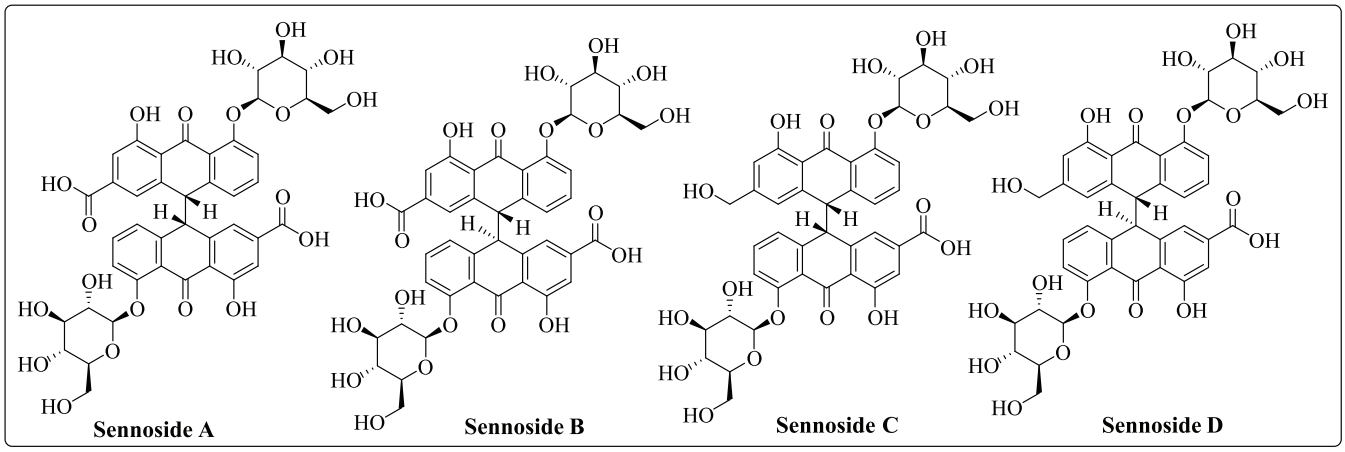

Figure 1: Structures of Anthroquinine Glycosides from Senna (Sennosides A-D)

The plant is mainly valued for its cathartic properties and is especially useful in habitual constipation. The laxative principles sennoside A and sennoside B, isolated from leaves and pods of Senna, constitute important ingredients in purgative medicines. Senna export and processing industry needs to be rejuvenated, as the export of this medicinal plant is declining. Since it is one of largest foreign exchanges, earning medicinal crops, of the country's value addition by way of grading and extraction may result in an additional income of up to 25-30% more on net returns.

Previous studies on Senna mainly focused on developing strategies for quantifying and identifying sennosides and detecting adulterations in formulated herbal medicines (Sun and Su, 2002; Dhoble *et al.*, 2019; Demirezer *et al.*, 2011). However, accurately identifying the growth stage at which maximum secondary metabolite accumulation occurs can significantly reduce waiting time and expenses associated with maintaining the crop. In this study, we aimed to investigate the effect of Time of Harvest on the accumulation of Total Sennoside Content (TSC) (Sennosides A to D) in Senna. Short harvesting intervals, once every 15 days, were implemented during a three-and-a-half-month period from December 2019 to March 2020 to estimate the total sennoside content accumulated in different parts of the plant (Fig. 1).

MATERIALS AND METHODS

Collection of plant samples

The Senna sown at CSIR-CIMAP, Research Centre, Hyderabad, India, was collected every 15-day interval from December 2019 to March 2020. The plant was taxonomically identified and authenticated by Dr. Venkat Ramana, Assistant Professor, Department of Botany, Nizam College, Osmania University. Hyderabad, India (voucher specimen (CIMAP-2019/CA), stored at CIMAP Research Centre, Hyderabad, India).

Instrumentation

The TSC analysis was performed using the UV-Vis Spectrophotometer (Make: LABINDIA Pvt Ltd, Model: UV 3000$^+$), and UVWin5 Software version 5.2.0 was used.

Estimation of TSC

The following procedure estimated the total Sennoside content in different plant parts. 0.5 grams of senna plant powder (Leaf, Stem, Root, Flower and Pod) was taken into a conical flask, and 20 mL of distilled water was added and extracted in hot condition using sonication apparatus and filtered. The residue was twice re-extracted with water. All the filtrates were taken into a 100 mL volumetric flask and made up to the mark with distilled water. 20 mL of water extract was taken, acidified with 0.1 mL of 2N HCl, and extracted with chloroform (2 X 15 mL). The aqueous portion was neutralized (pH; 7-8) with a 5% Sodium carbonate solution. 6.3% of the Ferric chloride solution was added and heated in a water bath for 20 min without cooling then 1 mL of the con. HCl was added to the above mixture and heated for another 20 min. The solution was cooled to RT and extracted with ether (3 X 25 mL), and the organic layer was washed with distilled water (2 X 10 mL). The ether extract was taken in a 100

mL flask and made up to 100 mL with ether. 10 mL of this ether extract was slighlty concentrated, and 10 mL of 1N KOH solution was added. The colour intensity of the resulting solution was read at 500 nm using UV-Vis Spectrophotometer. Calculated the total sennoside content by the following formula:

$$\text{Formula: } \frac{\text{Optical density at 500 nm X 100 X 50}}{\text{130 X 4 X Weight of the sample}}$$

RESULTS AND DISCUSSION

Variation of total Sennosides

The total sennoside content in different parts of *C. angustifolia*, including root, leaf, flower, stem, and pods, was determined using UV-Vis spectrophotometry at 500 nm. The results showed that in the leaf part, the highest percentage of TSC was observed at 105 days (1.653%), with an increasing pattern noticed from 45 to 105 days. In contrast, samples from 15 and 30 days showed no sennoside content. The stems exhibited a lower percentage of TSC than leaf samples, with the total sennosides being highest at 105 days (0.961%) and a gradually increasing pattern from 45 to 105 days. Roots exhibited the most negligible percentage of TSC, compared to leaf and stem, with a high accumulation of sennosides at 105 days (0.596%) (Table 1) and a gradual increasing pattern from 45 to 105 days, similar to leaf and stem. The TSC was highest in the whole plant compared to stem and root but less when compared to leaf samples, with more accumulation of TSC, observed at 105 days (1.0%). An increasing pattern was noticed from 45 to 105 days. However, the flowers develop between 75 to 90 days, and their TSC was observed to be very low in percentage compared to other parts, with the TSC being constant at 0.020% at 90 and 105 days. Similarly, the pods developed at 90 days had high TSC next to the leaf, with 1.538% at 105 days. (See Figures 2 &3)

Recovery of TSC

Significant variations in total sennoside content (TSC) were observed in different parts of Senna within days after sowing (DAS). In the leaf, TSC

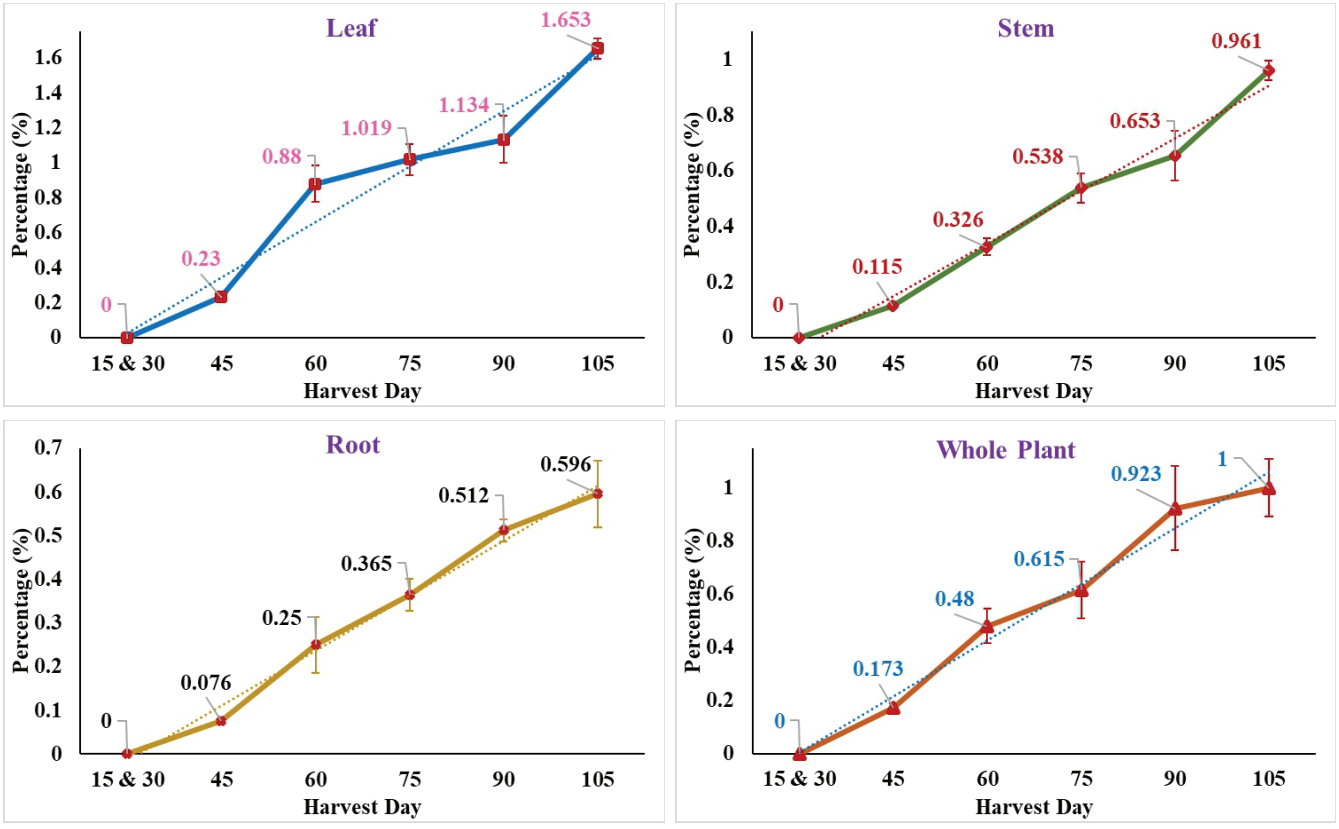

Figure 2: Total sennoside content of Senna plant part samples.

Table 1: Total sennoside content (%) in different plant parts

Plant Part	Days After Sowing (DAS)*					
	15 & 30	45	60	75	90	105
Leaf	NiL	0.23 (±0.027)	0.88 (±0.103)	1.019 (±0.089)	1.134 (±0.135)	1.653 (±0.059)
Stem	NiL	0.115 (±0.010)	0.326 (±0.031)	0.538 (±0.052)	0.653 (±0.089)	0.961 (±0.034)
Root	NiL	0.076 (±0.008)	0.25 (±0.063)	0.365 (±0.036)	0.512 (±0.025)	0.596 (±0.076)
Whole plant	NiL	0.173 (±0.014)	0.48 (±0.063)	0.615 (±0.105)	0.923 (±0.157)	1.000 (±0.107)
Flower	--	--	--	--	0.020 (±0.003)	0.020 (±0.004)
Pods	--	--	--	--	1.115 (±0.124)	1.538 (±0.127)

Note: The data mentioned in the parenthesis are standard deviation from the corresponding mean values of the three replicates.

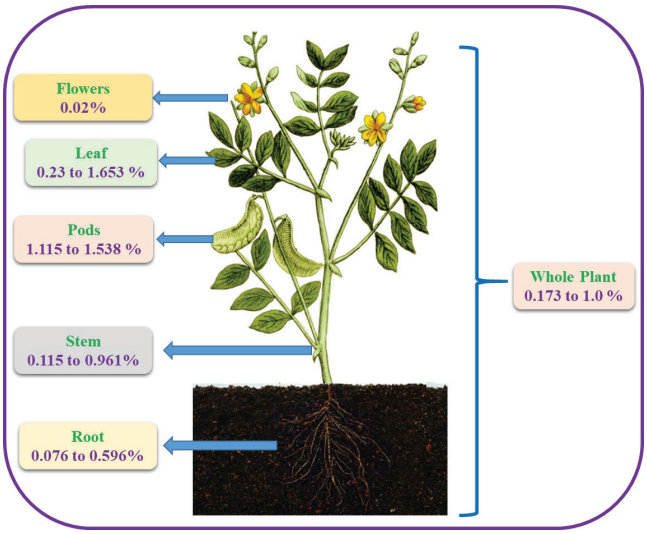

Figure 3: Total sennoside content in plant part wise

recovery ranged from 26% to 45%. The highest TSC content was recorded at 60 days (45%), after which it decreased due to the formation of flowers and pods (Fig. 4).

In the stems, TSC was recorded from 15% to 21%. TSC content recovery decreased up to 90 DAS and then slightly increased due to the hardening of the stem at 105 days. A decreasing pattern in TSC recovery was noticed in the whole plant from 45 to 105 days (29% to 17%). In the roots, minimal recovery was obtained (10% to 14%), and significant variations were not noticed. The flowers developed between 75 to 90 days, and their TSC recovery was meager compared to other parts (0.34% to 0.45%). The pods developed at 90 days and had a high TSC recovery next to the leaf part, except in the whole plant at 45 days.

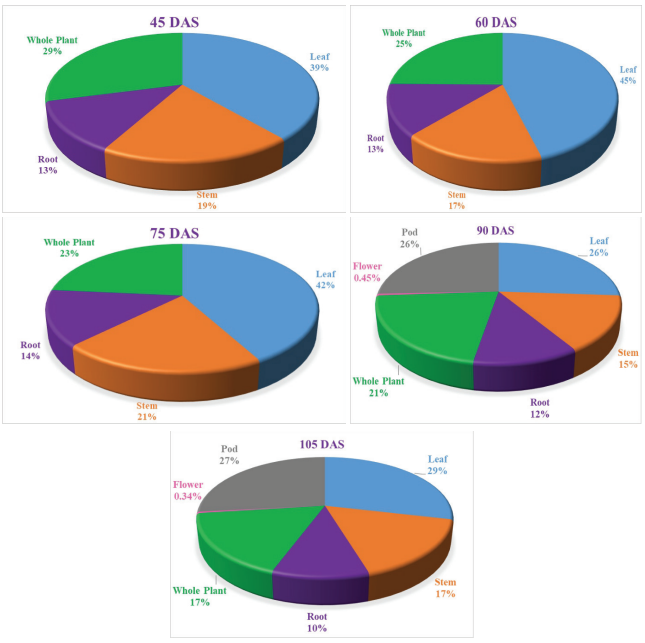

Figure 4: Recovery of TSC from parts

CONCLUSION

In conclusion, the study examined the changes in the TSC of different parts of Senna over three and a half months, with short harvest intervals. The leaf part had the highest TSC percentage (1.653%) at 105 days after sowing, while the least (0.020%) was observed at 90 and 105 days in floral parts. Overall, the total sennoside content in all plant parts increased up to 105 days after harvest. Farmers harvest the leaves and pods, typically discarding the stem and root. However, the experiments conducted in this study indicate a significant quantity of TSC present in these discarded parts, providing an opportunity for farmers to generate additional income.

Declaration of competing interest

The author declare that no conflict of interest exists in submitting this manuscript.

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