

Evaluation of α -glucosidase inhibitory potential of different extracts of *Cassia angustifolia* extracts.

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Abstract: This study investigated the inhibitory effect of methanol-water extract and different fractions on soil properties, herbage yield, and quality of the medicinal herb Senna. The results showed that the methanol-water extract and fractions had no significant anti-tyrosinase activity, but some fractions were active toward α -glucosidase. The hexane fraction and remaining crude extract demonstrated the highest inhibition on α -glucosidase compared to acarbose. ENC and BFM were compared with conventional farmyard manure (FYM) and chemical fertilizers (CF) in a pot experiment. Both ENC and EBC improved soil quality and fertility by increasing soil organic carbon, available nutrients, microbial biomass, and enzyme activity. ENC and BFM increased total herbage yields by 21 and 16.3% compared to FYM. The ethyl acetate fraction contained high phenolic and hydroxy anthraquinone derivatives, but no correlation was observed between the phenolic and sennoside contents of different fractions and their α -glucosidase inhibitory effect. The hexane fraction of *C. angustifolia* could be valuable for in vitro and in vivo antidiabetic studies and further phytochemical studies.

Keywords: *Cassia angustifolia*, α -Glucosidase inhibition, Methanol extract, Phytochemicals Activity, Antidiabetic activity.

INTRODUCTION

Cassia angustifolia Vahl. is a significant medicinal plant that is a member of the family fabaceae, which is also known as Indian senna. This plant is very popular as an agent of laxations because of the existence of anthraquinone glycosides, particularly sennosides A and B. These are common in the pharmaceutical preparations of constipation and gastro intestinal disorders. Senna leaves and pods have been employed as a purgative agent in the traditional medicine system and are regarded as significant raw materials in herbal preparations.

Plant is usually grown in the arid and semi arid areas and is well grown in the loamy and sandy soils with warm climate conditions. In India, the production of senna has been growing with the rising demands of the herbal medicines and pharmaceutical products. Vindhyan region of

Madhya Pradesh has the right climatic conditions that support the growth and cultivation of medicinal plants such as *Cassia angustifolia*.

In phytochemical research, senna has been found to have various bioactive agents like flavonoids, phenolic compounds, alkaloids and anthraquinone derivatives. These chemicals cause different biological reactions such as antioxidant, antimicrobial, anti-inflammatory as well as antidiabetic effects. Enzyme inhibitors, in particular, the enzyme alpha-glucosidase, have been of much interest within recent years due to their ability to slow down the digestion of carbohydrates and diminish the level of glucose in the blood in the aftermath of food intake.

Thus, this research paper is designed to determine the alpha-glucosidase inhibitory capability of various solvent fractions of *Cassia angustifolia* growing in the Vindhyan region of Madhya Pradesh, India.

LITERATURE REVIEW

Zeeshan, et.al. (2018). The present scenario in Pakistan is characterized by the widespread usage of herbal medicinal plants and species. Alkaloids, flavonoids, glycosides, phenols, steroids, and other specialized colors are only a few examples of the many secondary metabolites produced by plants regular metabolism. Consequently, ethnobotanical approaches are becoming more popular among botanists as a result of their cheaper cost, wider margin of safety and effectiveness, and less negative effects of plants. The unique phytochemicals found in plants have indisputable therapeutic value. A number of medicinal herbs have been touted as potential remedies for human health issues, with a focus on those involving the gastrointestinal tract (GIT), the liver, the stomach, and the colon.

Sivakumar, et.al. (2024). Among the many medicinal and therapeutic uses for plants, *Cassia angustifolia* sometimes called Indian Senna belongs to the Fabaceae (Leguminosae) family. The high concentration of sennoside anthraquinone derivatives in senna leaves makes them an effective natural laxative for promoting bowel movements and relieving constipation. When used orally, *Cassia angustifolia* alleviates indigestion, gas, and other gastrointestinal issues. Because of its significance in the pharmaceutical business, senna cultivation is on the rise in India. Thanks to senna variants, India can now supply the global pharmaceutical market. Among the many medicinal and therapeutic uses for plants, *Cassia angustifolia* sometimes called Indian Senna belongs to the Fabaceae (Leguminosae) family. The high concentration of sennoside anthraquinone derivatives in senna leaves makes them an effective natural

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Bagwe, et.al. (2019). Assessment of the Immunomodulatory Effects of *Cassia angustifolia* Vahl. *Journal of Pharmaceutical Sciences from Around the World*. An increasingly popular method of treating a wide range of illnesses is by modulating immune functioning with the use of medicinal plants and their derivatives. *Cassia angustifolia* Vahl was tested for its immunomodulatory effects on male Swiss albino mice that had been immunosuppressed by cyclophosphamide. Mice were given 2, 5, and 10 mg/kg of crude methanolic leaf extract of *C. angustifolia* orally for a duration of 14 days. Levamisole was administered to the animals as a positive control. Animals were pricked in the heart on Day 15 to draw blood, and several immunological parameters were used to assess the immune response. After being given the extract, the animals had a dramatic rise in neutrophil counts and leucocytosis. After treating with the extract, we observed an increase in the neutrophil phagocytic index and a delay in the type hypersensitivity reaction. The dosage that elicited the strongest immunological responses was 5 mg/kg. There was a dose-dependent rise in hemagglutination antibody titer levels in the rats given the extract. According to the findings, immunocompromised mice experience an increase in cell-mediated and humoral immune responses when exposed to a crude methanolic leaf extract of *C. angustifolia*.

Khare, et.al. (2017). This research aims to examine the pharmacological, phytochemical, and pharmacogenetic characteristics of *C. angustifolia*. Extractive values, fluorescence studies, ash values, chemical testing, and loss on drying were all performed using the powdered medication. Research was also carried out at both the macroscopic and microscopic levels. Paralytic stomata, nonlignified unicellular trichomes with warty walls, and a fibrovascular bundle were seen in the isobilateral structure of the leaf in the transverse section (T.S.). Several reagents were used to study the fluorescence properties of leaf powder in visible light and ultraviolet (UV) light (254 nm and 365 nm). Tannins, amino acids, alkaloids, flavonoids, and carbs make up senna. According to the report, the total ash content was 11.23 ± 0.25 w/w. A value of $1.4 \pm 0.1\%$ w/w for acid insoluble ash was recorded. The extractive values of solutions including water, ethanol, methanol, petroleum ether, and chloroform were $16.6 \pm 0.26\%$ w/w, $3.7 \pm 1.75\%$ w/w, $0.83 \pm 0.05\%$ w/w, $1.6 \pm 0.1\%$ w/w, and $3.2 \pm 0.25\%$ w/w, respectively. *Bauhinia variegata* primarily fights worms, ulcers, tumors, microbes, inflammation, goitre, and

protects the liver. It also has anti-inflammatory and antidiabetic effects. This study details its pharmacological characteristics, phytochemical analysis, and pharmacogenetic background.

Kumar, et.al. (2017). This study compiles and summarizes data on *C. siamea* from every known source, dating back to 1925. There are a number of pathological issues that the *C. siamea* plant has shown promise in treating medicinally. Its nutritional and economic value are not to be overlooked either. Anthraquinones, coumarins, alkaloids, flavonoids, glycosides, triterpenoids, sterols, and other polyphenols are among the plant's primary phytochemical components. Additional *in vitro* and *in vivo* investigations on this plant have investigated its unique pharmacological characteristics, including its analgesic, antioxidant, anti-inflammatory, antimalarial, vasorelaxant, anticancer, antiarrhythmic, antiandrogen, anti-HIV, and anxiolytic effects. The potential toxicity of this plant has been raised in a small number of publications. To better understand *C. siamea* and its possible medicinal applications, this review aims to compile findings from morphological, microscopic, phytochemical, pharmacological, and toxicological investigations.

MATERIAL AND METHOD

- **Chemicals**

The following substances were procured from Sigma-Aldrich, Germany: α -Glucosidase, tyrosinase, kojic acid, gallic acid, acarbose, and catechol. Merck® of Germany supplied the following chemicals: methanol, hexane, ethyl acetate, chloroform, dimethyl sulfoxide (DMSO), $K_2HPO_4 \cdot 3H_2O$, KH_2PO_4 , and potassium hydroxide.

- **Plant Material**

In February 2018, fresh leaves of *Cassia angustifolia* were taken in the Vindhyan region of Madhya Pradesh and especially in Sidhi and Rewa district, India. A competent botanist was involved in the authentication of the plant material and a voucher specimen was placed in herbarium to use as an efficient way of reference. The leaves were washed by using distilled water to remove dust and impurities and dried by placing the leaves under shade at room temperature. The dried leaves were dried in a mechanical grinder to fine powder and stored in airtight containers to be extracted later and analyzed by enzyme inhibition.

Cassia angustifolia



- **Experimental site and soils**

The current research was carried out in the Vindhyan area of Madhya Pradesh, India especially in the districts of Sidhi and Rewa. This area is located between 24°25 N latitude and 81 82 E longitude with a tropical monsoon climate. The region has hot seasons in summer, moderate rainfall in the monsoon seasons and cold winters.

Samples of *Cassia angustifolia* were taken in plants in natural occurrences and domesticated lands of the Sidhi and Rewa district. Plant materials were authenticated by botanical experts and shade drying, powdering and extracting the leaf further was done and phytochemical analysis of the leaf extract done.

- **Statistical analysis**

The results of the laboratory and pot culture investigations were averaged across three independent trials, and analysis of variance (ANOVA) was conducted using a fully randomized design (CRD). The mean \pm standard deviation (SD) was used to represent all evaluations, which were carried out three times. Statistical calculations were performed using Microsoft Excel to get the inhibitory concentration (IC₅₀) values.

RESULT

The main aim of the current research work was to determine the in-vitro inhibitory properties of various solvent fractions of *Cassia angustifolia* and their phytochemical properties. Besides the enzyme inhibition experiment, the physicochemical parameters of the soil, the production of bioactive compounds were also determined to gain knowledge of the environmental and

agronomic factors that affect the phytochemical accretion of the plant. The findings are consequently reported in three subsections;

- (i) physicochemical properties of the soil,
- (ii) enzyme inhibitory properties of extract fractions and
- (iii) The content and yield of bioactive compounds.

- **Soil pH, EC and SOC**

Table 1 displays the findings of the soil physicochemical parameters as they were affected by different treatments. The application of ENC and BFM resulted in notable changes to soil pH, EC (dSm^{-1}), and SOC (g kg^{-1}) when senna was harvested. Soil that was somewhat salted had a higher pH (7.85) and electrical conductivity (1.23 dSm^{-1}) compared to soil that was not slightly salted (7.67 and 0.36 dSm^{-1} , respectively). Compared to FYM and CF, the soil pH was considerably higher after applying BFM and ENC (5 t ha^{-1}) to the same area. However, regardless of the soil type, the pH values reported by chemical fertilizer (CF) applications were always the lowest. This might be because CFs are acidic. In both soils, the BFM treatment yielded the greatest electrical conductivity values, whereas the ENC application came in second. The EC values that came from CF therapy (T7) were similar to those that came from ENC application, however. Because BFM has a greater EC value than non-saline soil, its application raised the EC of non-saline soil above its starting values (Table 1). The somewhat salty soil consistently had higher pH and EC readings than the non-saline (Anand) soil because of its natural salinity. However, according to Figure 1, the treatment that received FYM had the greatest SOC content, ranging from 3.41 to 4.58 g kg^{-1} , irrespective of the type of soil. In both soils, the CF treatment led to the lowest SOC. Both soils showed a substantial improvement in SOC when ENC and BFM were applied, in comparison to control and CF ($p < 0.05$). In both soils, greater dosages of ENC (5 t ha^{-1}) and BFM (5 t ha^{-1}) resulted in considerably higher SOC levels compared to lower doses of ENC (2.5 t ha^{-1}) and BFM (2.5 t ha^{-1}).

Table 1 Soil physicochemical property and nutrient availability as influenced by application of enriched amendments and chemical fertilizer.

Soil physico chemical properties	Empty Cell	Treatments							ANOVA		
		T ₁ : Control	T ₂ : FYM (5 t ha ⁻¹)	T ₃ : ENC (2.5 t ha ⁻¹)	T ₄ : ENC (5 t ha ⁻¹)	T ₅ : BFM (2.5 t ha ⁻¹)	T ₆ : BFM (5 t ha ⁻¹)	T ₇ : CF	Treatment	Soil	Interaction
pH	S ₁	7.96 ± 0.04 ^{ab}	7.70 ± 0.04 ^{efg}	7.91 ± 0.03 ^b	8.01 ± 0.05 ^a	7.79 ± 0.02 ^{cd}	7.82 ± 0.04 ^c	7.77 ± 0.03 ^{cde}	***	***	***
	S ₂	7.63 ± 0.01 ^{ab}	7.57 ± 0.01 ^{hi}	7.67 ± 0.01 ^{fg}	7.75 ± 0.01 ^{cdef}	7.71 ± 0.03 ^{defg}	7.81 ± 0.01 ^c	7.53 ± 0.01 ⁱ			
EC (dSm ⁻¹)	S ₁	1.17 ± 0.02 ^{de}	1.13 ± 0.02 ^a	1.22 ± 0.02 ^{cd}	1.24 ± 0.02 ^{bc}	1.28 ± 0.02 ^b	1.37 ± 0.02 ^a	1.23 ± 0.03 ^{bc}	***	***	**
	S ₂	0.30 ± 0.01 ^h	0.34 ± 0.01 ^{ab}	0.36 ± 0.01 ^{gh}	0.37 ± 0.01 ^{fg}	0.39 ± 0.01 ^{fg}	0.41 ± 0.01 ^f	0.35 ± 0.01 ^{gh}			
SOC (g kg ⁻¹)	S ₁	4.23 ± 0.03 ^e	4.58 ± 0.02 ^a	4.39 ± 0.02 ^c	4.50 ± 0.02 ^b	4.28 ± 0.02 ^d	4.37 ± 0.02 ^c	4.20 ± 0.02 ^e	***	***	***
	S ₂	2.96 ± 0.01 ^j	3.41 ± 0.01 ^e	3.26 ± 0.01 ^{fg}	3.34 ± 0.02 ^f	3.12 ± 0.01 ⁱ	3.20 ± 0.01 ^h	2.91 ± 0.01 ^j			
Mineral N ^a (mg kg ⁻¹)	S ₁	48.47 ± 1.64 ^{cd}	51.47 ± 1.82 ^{bc}	51.53 ± 1.17 ^{bc}	54.60 ± 1.62 ^{ab}	48.17 ± 2.20 ^{cd}	54.87 ± 1.28 ^{ab}	57.17 ± 1.56 ^a	***	***	NS
	S ₂	37.37 ± 1.13 ^f	41.37 ± 0.98 ^{ef}	43.03 ± 0.83 ^e	45.10 ± 0.79 ^{de}	41.23 ± 0.84 ^{ef}	43.73 ± 0.75 ^e	48.50 ± 0.51 ^{cd}			
Available P (mg kg ⁻¹)	S ₁	11.23 ± 0.55 ^g	13.53 ± 0.69 ^g	16.00 ± 0.93 ^f	17.77 ± 0.97 ^{def}	11.53 ± 0.69 ^g	13.57 ± 1.18 ^g	18.43 ± 1.04 ^{de}	***	***	NS
	S ₂	16.57 ± 0.55 ^{ef}	19.87 ± 0.78 ^{cd}	21.57 ± 0.63 ^{bc}	23.70 ± 0.52 ^{ab}	16.87 ± 0.38 ^{ef}	18.80 ± 0.85 ^{de}	24.77 ± 0.29 ^a			
Available K (mg kg ⁻¹)	S ₁	188.57 ± 2.60 ^c	202.93 ± 2.88 ^b	208.33 ± 2.58 ^b	216.27 ± 3.78 ^a	218.13 ± 2.98 ^a	222.50 ± 2.82 ^a	223.30 ± 3.32 ^a	***	***	NS
	S ₂	91.57 ± 1.10 ^h	105.27 ± 1.15 ^g	110.67 ± 0.90 ^{fg}	115.93 ± 1.47 ^{ef}	115.80 ± 1.16 ^{ef}	121.50 ± 1.69 ^{de}	124.97 ± 1.14 ^d			

The data is shown as the average values plus or minus the standard error from three separate tests (n = 3).

At p-values less than 0.05, 0.01, and 0.001, respectively, the significance level is *, **, and ***. The Duncan multiple mean comparison test at 5% significance indicates that there are significant differences across treatments when different letters in the same row are present at p < 0.05.

A Mineral N (NH₄⁺-N + NO₃⁻-N)

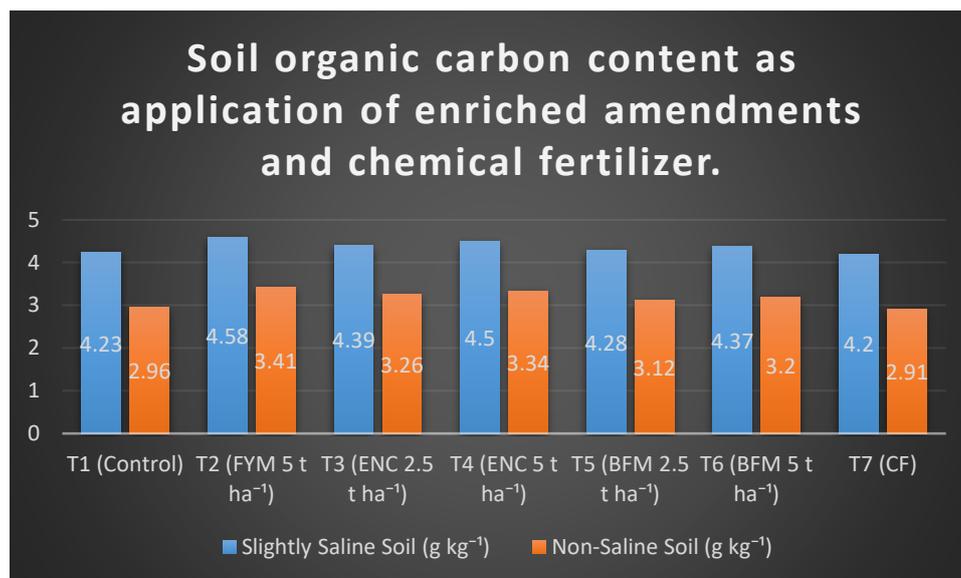


Figure 1 Soil organic carbon content as application of enriched amendments and chemical fertilizer. Bars are standard errors (n = 3).

Soil pH was reduced while EC and SOC were increased after FYM and enriched amendments (ENC and BFM) were applied. It seems that the organic additions served to stabilize the soils' pH levels. Potential causes of a drop in soil pH and EC include sodium adsorption, calcium and magnesium chelation by organic anions, and the formation of organic acids from organic matter breakdown (FYM and ENC). However, the soil EC may have been elevated due to the presence of soluble cations (Ca, Mg, and K) and resistant carbon in BFM. Past research has also shown that biochar and biochar-based products raise soil pH and electrical conductivity. A considerable increase in soil organic carbon (SOC) was observed after the application of FYM and enhanced amendments in comparison to chemical fertilizers.

The inhibitory activity of *Cassia angustifolia* extracts was evaluated to determine their potential pharmacological applications, particularly their role as natural inhibitors of carbohydrate-metabolizing enzymes.

- In-vitro α -Glucosidase and Tyrosinase Inhibitory Activity of *Cassia angustifolia* Extract Fractions**

The findings show that 80 percent of the plant's methanol-water extract (MWE) was produced, with a yield of 24.8%. During the fractionation process of the MWE, the residual crude extract (26.78g) was obtained using the following solvents: hexane (1.31g), chloroform (32.61g), and ethyl acetate (29.30g), in that order.

The hexane fraction and the remaining crude extract showed the highest inhibition at 52 and 58 $\mu\text{g/mL}$, respectively, when testing various fractions of *C. angustifolia* using the in-vitro α -glucosidase inhibitor test at a concentration of 484 $\mu\text{g/mL}$. This was more effective than the inhibition caused by acarbose. An amount of 750 μM acarbose, which is 484 $\mu\text{g/mL}$, was used. Surprisingly, the observed effects reached approximately 50%, even though the acarbose concentration used in this experiment was lower than the extract.

This research tested the inhibitory effects of *C. angustifolia* MWE fractions on the tyrosinase and α -glucosidase enzymes. According to Table 2, there was no discernible impact on the tyrosinase enzyme inhibitory action.

Table 2 Results of Tyrosinase Inhibitory, α -Glucosidase Inhibitory, Total Phenolic, and Total Anthraquinone Glycosides of *Cassia angustifolia* Fractions.

Extract	Percent of Tyrosinase Inhibitory Effect (500 $\mu\text{g/mL}$)	Percent of α -Glucosidase Inhibitors (484 $\mu\text{g/mL}$)	Total Phenolic Content ($\mu\text{g/mL}$)	Percent of Total Anthraquinone Glycosides (%)	
				Drug	Extract
Hexane fraction	10	94	372.36	0.577	0.447
Chloroform fraction	34	9	368.64	0.425	0.318
Ethyl acetate fraction	22	6	404.88	1.379	1.127
Remaining crude extract	36	58	374.04	0.438	0.688
Acarbose (750 μM)	-	50	-	-	-
Kojic acid (82 μM)	50	-	-	-	-

The tyrosinase enzyme was not significantly inhibited by the MWE fractions, according to the data. However, the hexane fraction and the rest of the crude extract might be useful for future research into α -glucosidase enzyme inhibitors.

The total amount of phenolic compounds was estimated to be around 382.25 $\mu\text{g}/\text{mg}$ for the ethyl acetate fraction, 290.58 $\mu\text{g}/\text{mg}$ for the remaining crude extract, and 288.08 $\mu\text{g}/\text{mg}$ for the chloroformic fraction, according to absorbance values obtained from the reaction of the MWE fractions with the Folin-Ciocalteu reagent and their comparison with the absorbance values of gallic acid as a standard.

- **Bioactive compound content and yield**

Figure 2 displayed the altered sennoside content and yield in two distinct soil types as a result of chemical fertilization and different amendments. The leaf sennoside concentration was greater in the treatments that received FYM and enriched amendments (ENC and BFM) compared to the control. Regardless of the fertilizer treatments applied to the two soil types, the sennoside content of the pods remained same. The overall sennoside content of senna was unaffected by several treatments in slightly salty soil. In non-saline soil, the total sennoside levels were considerably greater ($p < 0.05$) when ENC and BFM were applied compared to CF and FYM treatments (Fig. 24a). Under non-saline soil, the BFM treatment (5 t ha^{-1}) exhibited the greatest sennoside concentration (3.13%), which is 21.5 percent greater than FYM and 29.2 percent higher than CF, respectively. Fig. 2b shows that total sennoside production changed considerably by treatment in both soils, in contrast to sennoside content. In non-saline soil, the treatment that got 5 t ha^{-1} or BFM had the greatest total sennoside production at $108.46 \text{ mg plant}^{-1}$, whereas the treatment that received 5 t ha^{-1} of ENC had the second highest yield at $101.23 \text{ mg plant}^{-1}$. Lower doses of ENC (2.5 t ha^{-1}) and BFM (2.5 t ha^{-1}) produced lower sennoside yields in both soils compared to high dosages (5 t ha^{-1}) and BFM (2.5 t ha^{-1} , respectively). Nevertheless, ENC treatment yielded the greatest total sennoside production in slightly saline soil, followed by CF treatment. In general, soil that was not slightly salty yielded more sennosides than soil that was somewhat salty.

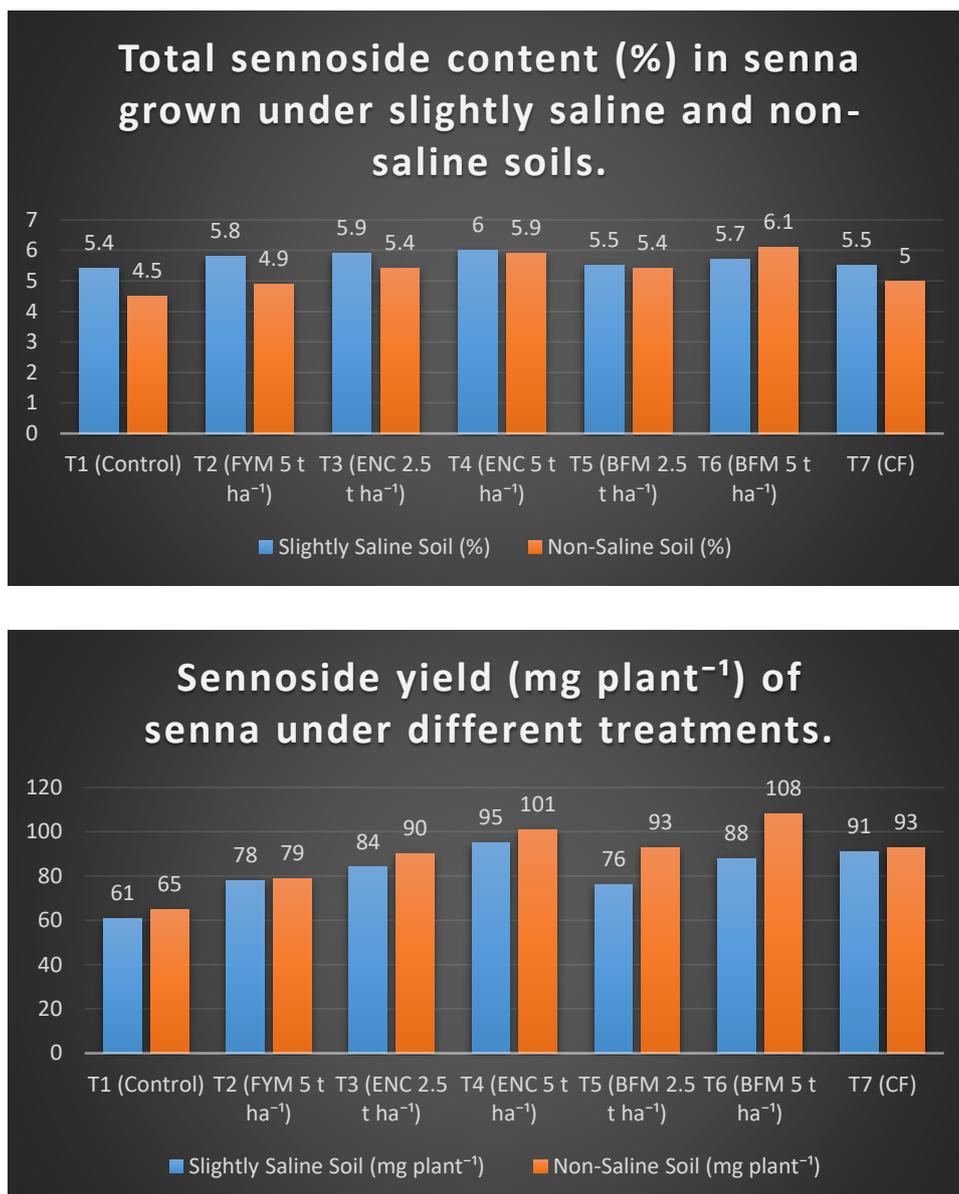


Figure 2 Bio active compound content (a) and yield (b) of senna as influenced by application of enriched amendments and chemical fertilizer. Bars are standard errors (n = 3).

Overall, the results indicate that different solvent fractions of *Cassia angustifolia* exhibit varying levels of enzyme inhibitory activity and phytochemical composition. Among the tested fractions, the hexane fraction showed the highest α -glucosidase inhibition, suggesting the presence of non-polar bioactive compounds responsible for antidiabetic potential. The study also demonstrated that organic amendments improved soil properties and enhanced bioactive compound production in senna plants. These findings highlight the relationship between soil fertility, phytochemical composition, and biological activity of *Cassia angustifolia*.

CONCLUSION

The findings of this initial study will be considered in future research, which suggests that the hexane extract is quite an effective inhibitor of the 1, 5-glucosidase enzyme. Nevertheless, the phytochemical studies are necessary to identify the specific elements of this extract that can be considered as α -glucosidase inhibitors. Chemical fertilizer was established as the most overall herbage produce of Senna when used on slightly salty soil, and the augmented amendments (ENC and BFM) showed to be more effective than fertilizer when it comes to the concentration of bioactive substance (sennoside) and yield. The study findings confirmed our conception of improved amendments as an alternative fertilizer that could be used instead of chemical fertilizers. When it comes to herbage and sennoside yields, overall, the use of enriched amendments (ENC and BFM) is better than using ordinary FYM in Senna farming. More significantly to sennoside yields in Senna farming, these improved supplements can be used in the future to substitute the costly chemical fertilizers.

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