

Sennosides Determination of Ethiopian *Senna alexandrina* Mill Accessions

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Abstract

Senna alexandrina leaves and pods have been used in herbal medicine since ancient times. The pods and leaves extracts of this plant contains anthraquinone glycosides that have a significant laxative effect. In this study the leaf, pod and flowers of *Senna alexandrina* were collected from potential areas of Ethiopia and determined their sennosides content (hydroxyanthracene glycosides) calculated as sennoside B via spectrophotometric method. Medicinal Herbal tea preparation also standardized based on sennoside concentration. As a summary, the yields of total sennoside being to be appeared 1.08-1.76% in the leaf, 1.43-2.62% in the pod and 0.08-0.15% in the flower parts of *Senna alexandrina*, located at different areas of Ethiopia with two types of Var. For herbal tea consumption, decoction of 1.5 mg powder of senna in 300 mL water for 10 minutes (pod) and 30 minutes (leaf) were optimized for sennoside extract and Standardized according to the WHO monographs of daily intake of sennosides, twice a day for pod and once a day for leaf herbal tea.



Keywords: Ethiopia; Laxative; Optimization; *Senna alexandrina*; Sennoside; Standardization

Introduction

Constipation is a common complaint in 1-6% of the middle-aged population and 20-80% of the elderly people. One of the most commonly used groups of drugs used in the correction of functional disorders of the digestive system, are laxatives [1]. The most widely used herbal remedies are containing anthraquinone derivatives [2], and the popular source are two species of Cassia Senna (*Cassia acutifolia* Delile.), or Alexandrian senna (*senna alexandrina* Mill) and

Tinnevely senna (*Cassia angustifolia* Vahl .) with a family (Fabaceae/ Leguminosae). The active constituents in both senna leaf and fruit are dianthrone glycosides (hydroxyanthracene glycosides) principally sennosides A and B. There are also small amounts of aloemodin and rhein 8-glucosides, mucilage, flavonoids, and naphthalene precursors [3].

Senna alexandrina is originated from Mali eastwards to Somalia and Kenya, much of it from wild plants. It is also native in Asia from the Arabian Peninsula to India and Sri Lanka. Two varieties are distinguished in *Senna alexandrina*. The first is var. obtusata (Brenan) Lock, restricted to Eritrea, Ethiopia, Somalia and northern Kenya, the second is var. alexandrina, which is the more widespread variety [4]. In Sudan, Ethiopia, Somalia and Kenya both leaves and pods are used as a purgative. Decoction of the pods is drunk to get rid of intestinal worms and to cure difficulties in breathing. The infusion of the pods is recommended as a purgative for pregnant women and also to suppress fever. An infusion of the leaves is drunk to overcome flatulence and convulsions and to stop nosebleeds.

However, *Senna alexandrian* shrubs are abundant in Ethiopia, not exploit the benefit obtained due to lack of standardization and product formulation. Characterization of location based phytochemicals is stringent for the reason that quantitative and qualitative variability of secondary metabolites in the plant through cultivation conditions and

the time of harvesting [5]. Therefore, this work was performed to determine the sennosides content of *Senna alexandrina* accession. Quantitative analysis is performed by spectrophotometry. Thin-layer chromatography is employed for qualitative analysis for the presence of sennosides A and B [6].

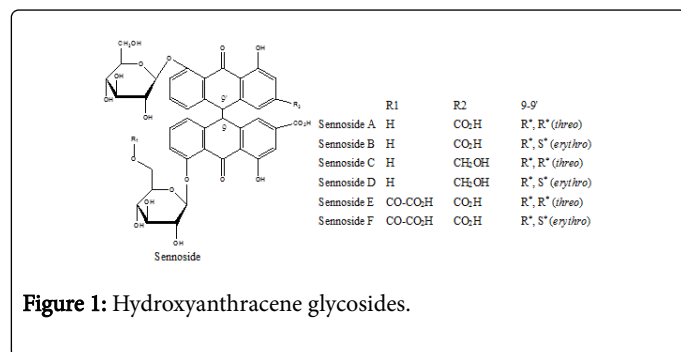


Figure 1: Hydroxyanthracene glycosides.

Materials and Methods

Sample collection

Senna (*Senna alexandrina* M.) samples were collected on mid of September 2016 from potential areas of eastern Ethiopia, particularly Dubti (410 m a.s.l, N 11°46' E 041°02"), Logia (464 m a.s.l, N 11°41' E 040°56"), Mille (502 m a.s.l, N 11°22.78' E 040°44.51"), Fentale (983 m a.s.l, N 08° 55' E 039° 50") and Shinile (1086 m a.s.l, N 09°67' E 041°09"). Different parts of the plant (leave, pod and flower) were pinched independently. Each sample was dried on sun and pulverized through grinder and packed in plastic bag until extraction.

Extraction and quantification

Powdered sample (0.5 g) was refluxed for 1 hr through superior extracting solvent (99.7% methanol) [7]. The insoluble matter were filtered through Buchner funnel and made the volume to 250 mL with methanol. The standard of sennoside B in methanol solution was scanned through Cary 100 UV-VIS spectrophotometer at wave length range of 200-800 nm and selects 276 nm as a λ max value. Samples were analyzed immediately after extraction in order to avoid possible chemical degradation by using UV-VIS spectrophotometer at 276 nm wavelength [8]. The experiments were carried out in a completely randomized design with three replications.

Detection and visualization

The presence of sennosides (hydroxyanthracene glycosides) in methanol extracts were detected by analytical grade TLC with a solvent system of N-Butanol: Ethyl acetate: Water: Glacial Acetic Acid (8:8:6:1) ratio through a slight modification of the method described by French pharmacopeia. In order to visualize the spot 20% v/v solution of nitric acid was sprayed on the plate and heated the plate for 10 min at 120°C. after cooling the plate 50 mg/L solution of potassium hydroxide in ethanol were sprayed.

Herbal tea preparation

Homogenized powder samples of leaf and pod were subjected to decoction by water with three concentrations viz. 1.5 mg/ 300 mL (sat

1), 1.5 mg/150 mL (sat 2) and 1.5 mg /100 mL (sat 3) for 10, 20 and 30 minutes. The prepared tea were filtered through muslin cloth and subjected to determine the total hydroxyanthracene glycosides in terms of sennoside B.

Statistical analysis

Significance difference of total hydroxyanthracene glycosides between different types of var., parts and location of the plant were analyzed by SAS, version 9. Statistical significance was defined as $p < 0.05$. The effects of saturation and decoction time on sennoside content of the prepared herbal tea were determined through response surface optimization method with central composite design.

Results and Discussion

The presence of sennosides in the methanol extract were detected by TLC through N-Butanol: Ethyl acetate: Water: Glacial Acetic Acid (8:8:6:1) solvent system. Sennoside B was appeared at R_f value of 0.28 next to sennoside A (0.52) followed sennoside D (0.72) and then sennoside C (0.84) bottom to top position (Figure 2). The total percent of sennoside (hydroxyanthracene glycoside) were determined by spectrophotometric method and linearity of the measurement was evaluated by analyzing different concentrations of the standard sennoside B in methanol solution at 276 nm wavelength (Figure 3). The Beer Lambert's law was obeyed in the range concentration of 184-1474 μ g/mL sennoside B and the correlation coefficient was found to be 0.999 with a regression equation $Y = 0.284 X - 0.011$ (Figure 4).

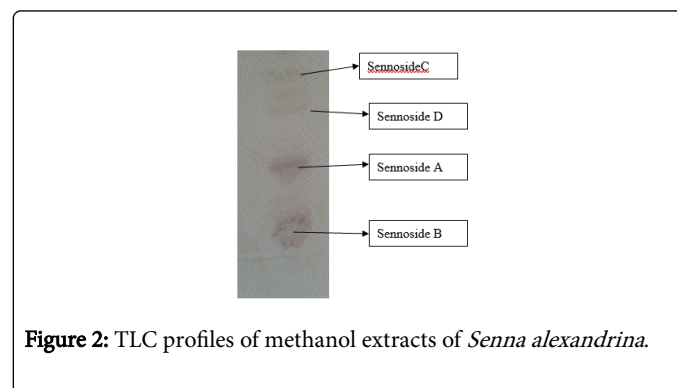


Figure 2: TLC profiles of methanol extracts of *Senna alexandrina*.

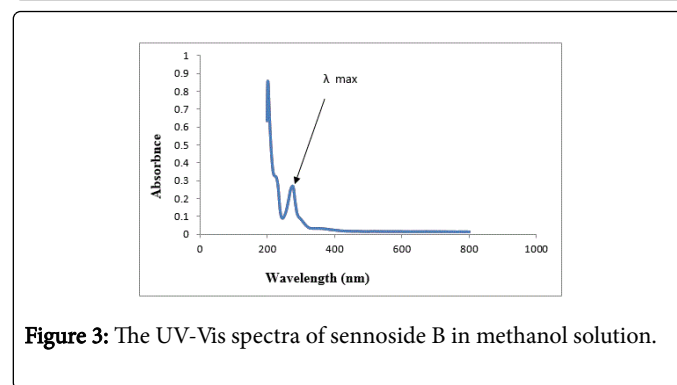


Figure 3: The UV-Vis spectra of sennoside B in methanol solution.

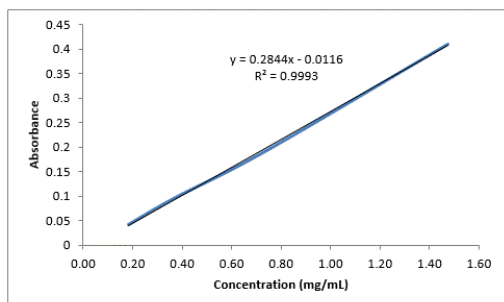


Figure 4: The plots of different concentration of sennoside B versus its absorbance.

From the analysis of variance, the types (short var. and long var.) of senna shrubs influenced the yields of sennoside in the leaf and pod part at P level <0.01 and P level <0.001 respectively. Their lower did not show a statistical significance different in total percent of sennosides content at P level <0.05. The short var. senna shrub was superior in total percent of sennosides content with a mean value of 1.7% and 2.33% in the leaf and pod part respectively. The long var. senna shrub reached a sennosides content of 1.41% and 1.72% in the leaf and pod parts respectively (Table 1). Similar results were reported by Kurkin and Shmygareva [9] on yield of total anthracene derivatives in the leaf of *Cassia acutifolia* from Russia (1.21 to 1.88%) in terms of sennoside B determined by spectrophotometric method.

Type	Total Sennosides (%)		
	Leaf	Pod	Flower
Short var.	1.70 ^a	2.33 ^a	0.13333 ^a
Long var.	1.41 ^b	1.72 ^b	0.12000 ^a
LSD at p<0.05	0.1867	0.2665	0.024

Table 1: The effect of types (var.) on sennosides content of *Senna alexandrina*.

The locations were significantly affecting the yields of sennoside content for both types of senna shrubs in the leaf, pod and flower part. The short var. senna shrub collected from Fentale gave a higher percent of sennosides content from the pod (2.62%), next to Dubti (2.30%) and followed Logia (2.06%). The sennosides content from the leaf did not show a statistical significance difference among Dubti (1.73%) and Fentale (1.77%), but Logia with minimum mean value of 1.60% (Table 2). From the long var. senna shrub accession, Shinile is superior in sennosides content in the pod (2.08%) and flower (0.15%). Mile had front from the leaf (1.61%) (Table 3).

Location	Altitude (m)	Total Sennosides (%)		
		Leaf	Pod	Flower
Dubti	410	1.73 ^a	2.30 ^b	0.13 ^a
Fentale	983	1.76 ^a	2.62 ^a	0.14 ^a
Logia	431	1.60 ^b	2.06 ^c	0.13 ^a

LSD at p<0.05	-	0.093	0.0094	0.02
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Table 2: The influence of location on the sennosides content for short var. *Senna alexandrina*.

Location	Altitude (m)	Total Sennosides (%)s		
		Leaf	Pod	Flower
Logia	464	1.55 ^b	1.64 ^b	0.08 ^c
Mile	502	1.61 ^a	1.43 ^c	0.13 ^b
Shinile	1086	1.08 ^c	2.08 ^a	0.15 ^a
LSD at p<0.05	-	0.02	0.0094	0.02

Means followed by the same letter under the same column are statistically non significant at P<0.05 according to least significant difference (LSD) test

Table 3: The influence of location on the of sennosides content for long var. *Senna alexandrina*.

Generally, the sennosides content obtained from the pod comply the WHO and European standard limit which is 2.2% calculated as sennoside B. The sennosides content in the leaf was appeared to be less than the expected range of WHO [10] monograph limit which is 2.5% calculated as sennoside B. This is occurred due to the season of sampling which was lowering season, leads to shift the secondary metabolite from leaf to lower and pod. Complimentary results were reported by Ratnayaka et al. [11] de lowering increases the sennoside A and B concentration in the leaf by 25% and also sennosides content maximize at the age of 90 days which is before lowering [12].

The herbal teas were made from leaf and pod of *Senna alexandrina* through decoction in order to set the standard dose based on daily recommended value of total sennoside content calculated as sennoside B. The interaction of decoction time (temperature effect on extracting the active constituent) and the concentration of the applied herbal matters (solvent saturation effect on extracting the active constituent) were determined. The sennoside content of the herbal tea made from senna leaf increases as decreasing saturation and increasing decoction time (Figures 5 and 6). The sennoside yield increase as decreasing saturation and decoction time of the tea prepared from senna pod (Figures 7 and 8).

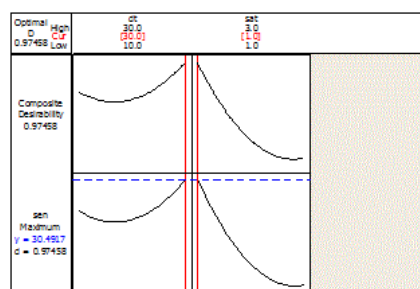


Figure 5: Optimization plots of leaf tea.

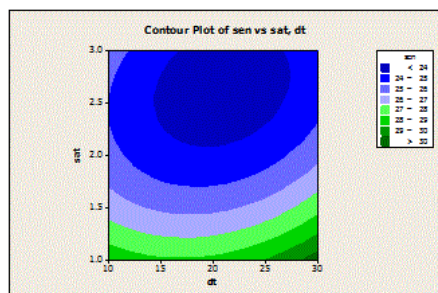


Figure 6: Contour plots of leaf tea.



Figure 7: Optimization plots of pod tea .

Sen: sennoside; sat: saturation; dt: decoction time.

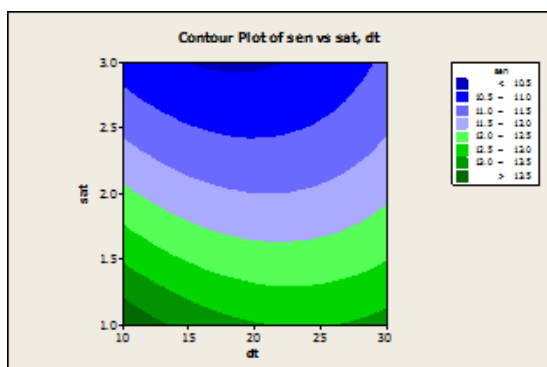


Figure 8: Contour plots of pod tea.

Sen: sennoside; sat: saturation; dt: decoction time.

Conclusion

This work was done on collecting *Senna alexandrina* leaf, pod and flower from potential areas of Ethiopia with acquiring two types (short var. and long var. shrubs) from five locations and designed to determine the sennosides content with comparing between types of var., parts and location of the plant. A spectrophotometric method was used to determine the total sennosides (hydroxyanthracene glycosides) calculated as sennoside B. As of the abundance of *Senna alexandrina* Mill in Ethiopia, the active ingredients were differs by the existing location, types of var. and parts of the plant. For herbal tea

consumption, 1.5 mg powder of senna per 300 mL water decoction for 10 and 30 minutes were set twice a day and once a day for pod and leaf respectively.

Acknowledgment

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