# Sennosides Determination of *Senna alexandrina*

***Bekri Melka Abdo***
*Natural Product Research Laboratory, Wondo Genet Agricultural Research Center, P.O. Box 198, Shashemene, Ethiopia.corresponding author address, e-mail: bekrimelka2003@yahoo.com*

## Abstract

*In the current study, the sennoside content (hydroxyanthracene glycosides) of Senna alexandrina was investigated. Leaves, pod and flowers of Alexandrian senna were collected from potential areas of Ethiopia. After sample preparation, sennoside content of samples was calculated as sennoside B using spectrophotometric method. An effort was also made to standardize medicinal herbal tea preparation based on sennoside concentration. The total yield of sennosides (Min and Max) found in the leaf, pod and flower parts of the plant from different areas of the country were 1.08 - 1.76, 1.43 - 2.62 and 0.08 - 0.15 %, respectively. For herbal tea consumption, decoction of 1.5 g powder of senna in 300 ml water for 10 minutes (pod) and 30 minutes (leaf) were optimized for sennoside extracts. It was standardized according to the WHO monographs of daily intake of sennosides as twice a day for pod and once a day for leaf herbal tea.*

## 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 for the correction of functional disorders of the digestive system, are laxatives (Werner and Merz, 2007). The most widely used herbal remedies are containing anthraquinone derivatives (Kurkin, 2009), and the popular sources are two species of Cassia Senna (*Cassia acutifolia* Delile.) or Alexandrian Senna (*Senna alexandrina* Mill.) and Tinnevelly Senna (*Cassia angustifolia* Vahl.) within the family of 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 aloeemodin and rhein 8-glucosides, mucilage, flavonoids, and naphthalene precursors (Bruneton, 1995).

*Senna alexandrina* is originated in Mali east to Somalia and Kenya, much of it as 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, and the second is var. alexandrina, which is more widespread (Bosch, 2007). 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 to suppress fever. An infusion of the leaves is drunk to overcome flatulence and convulsions and to stop nosebleeds.

Although *Senna Alexandrina* shrubs are abundant in Ethiopia, the potential benefit from the plant has not been exploited 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 time of harvesting (Dewick, 2001). Therefore, this study was conducted to determine the sennosides content of *Senna alexandrina* accession.

## Materials and Methods

### Sample collection

Alexandrian Senna (*senna alex*andrina M.) samples were collected on mid of September 2016 from potential areas of eastern Ethiopia, particularly, Dubti (410 m a.s.l, N $11^0$46" E $041^0$02"), Logia (464 m a.s.l, N $11^0$41" E $040^0$56"), Mille (502 m a.s.l, N $11^0$22.78" E $040^0$44.51"). Furthermore, Fentale (983 m a.s.l, N $08^0$ 55" E $039^0$ 50"), and Shinile (1086 m a.s.l, N $09^0$67" E $041^0$94"). Different parts of the plant (leave, pod and flower) were pinched independently. Each sample was dried on sun, pulverized through grinder, and packed in plastic bag until extraction.

### Sample extraction

Powdered sample (0.5 g) was refluxed for 1 hr through superior extracting solvent (99.7 % methanol) (Upadhyay *et. al*., 2011) 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 wavelength range of 200-800 nm and selects 276 nm as a $\lambda$ max value. Samples were analyzed immediately after extraction in order to avoid possible chemical degradation by using UV-VIS spectrophotometer at 276 nm wavelength (Tarkase and Danve, 2015). The experiments were carried out in a completely randomized design with three replicates.

### Detection and visualization

The presence of sennosides (hydroxyanthracene glycosides) in methanol extracts were detected by analytical grade TLC with a solvent system of N-Buthanol: Ethyl acetate: Water as well as Glacial Acetic Acid in 8:8:6:1 ratio through a slight modification of the method described by French pharmacopeia (2007). 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 $^o$c. after cooling the plate 50 mg / L solution of potassium hydroxide in ethanol were sprayed.

### Herbal tea preparation

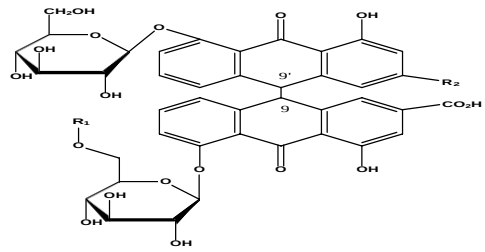
Homogenized powder samples of leaf and pod were subjected to decoction by water with three concentrations viz. 1.5 mg/ 300 mL (sat 1), 1.5 mg/150 mL (sat 2) and 1.5 mg /100 mL (sat 3) for 10, 20 and 30 minutes. The prepared tea were filtered through muslin cloth and subjected to determine the total hydroxyanthracene 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

Quantitative analysis was performed by spectrophotometry. Thin-layer chromatography was employed for qualitative analysis for the presence of sennosides A and B (European Pharmacopoeia, 1995).



Sennoside

| R2 | 9-9' | | |
|---|---|---|---|
| Sennoside A | H | $CO_2H$ | $R^*, R^*$ (*threo*) |
| Sennoside B | H | $CO_2H$ | $R^*, S^*$ (*erythro*) |
| Sennoside C | H | $CH_2OH$ | $R^*, R^*$ (*threo*) |
| Sennoside D | H | $CH_2OH$ | $R^*, S^*$ (*erythro*) |
| Sennoside E | CO-$CO_2H$ | $CO_2H$ | $R^*, R^*$ (*threo*) |
| Sennoside F | CO-$CO_2H$ | $CO_2H$ | $R^*, S^*$ (*erythro*) |
| R1 | R2 | 9-9' | |
| Sennoside A | H | $CO_2H$ | $R^*, R^*$ (*threo*) |
| Sennoside B | H | $CO_2H$ | $R^*, S^*$ (*erythro*) |
| Sennoside C | H | $CH_2OH$ | $R^*, R^*$ (*threo*) |
| Sennoside D | H | $CH_2OH$ | $R^*, S^*$ (*erythro*) |
| Sennoside E | CO-$CO_2H$ | $CO_2H$ | $R^*, R^*$ (*threo*) |
| Sennoside F | CO-$CO_2H$ | $CO_2H$ | $R^*, S^*$ (*erythro*) |

Figure 1. Hydroxyanthracene glycosides

The presence of sennosides in the methanol extract were detected by TLC using N-Buthanol: Ethyl acetate: Water: Glacial Acetic Acid (8:8:6:1) as a solvent system. Sennoside B was appeared at R*f* value of 0.28 next to sennoside A (0.52), followed by sennoside C (0.84) and then sennoside D (0.72) (Figure 1). 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 2). The Beer Lambert's law was followed in the range concentration of 184-1474 µg / ml sennoside B and the correlation coefficient was found to be 0.999 with a regression equation Y= 0.284 X - 0.011 (Figure 3).

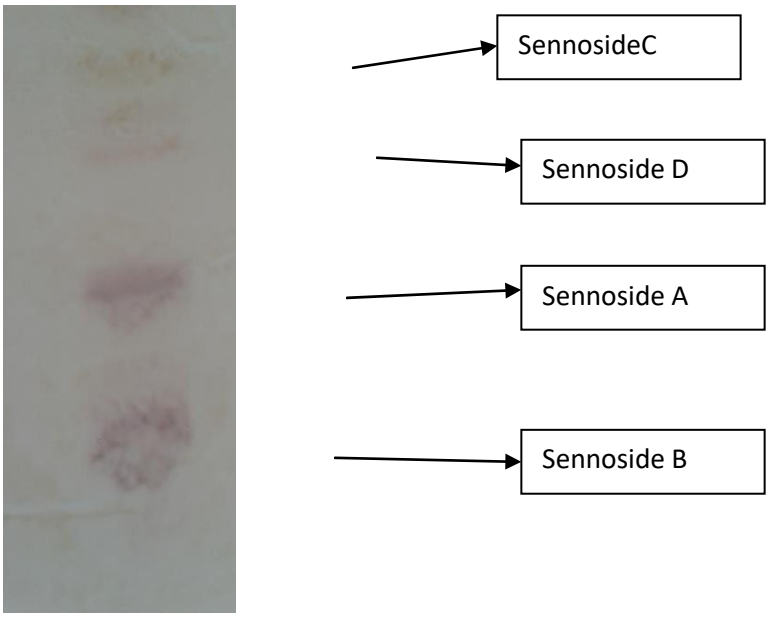



Figure 1. TLC profiles of methanol extracts of *senna alexandrina*

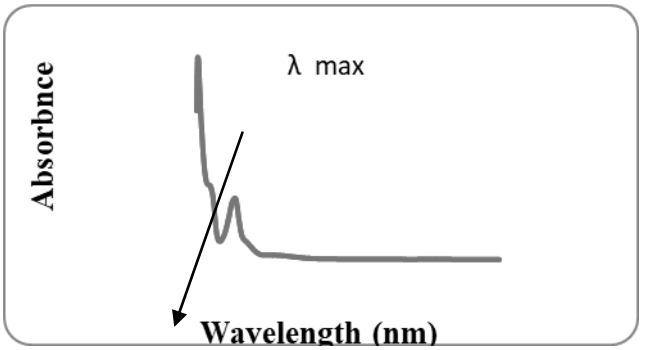



Figure 2. The UV-Vis spectra of sennoside B in methanol solution.

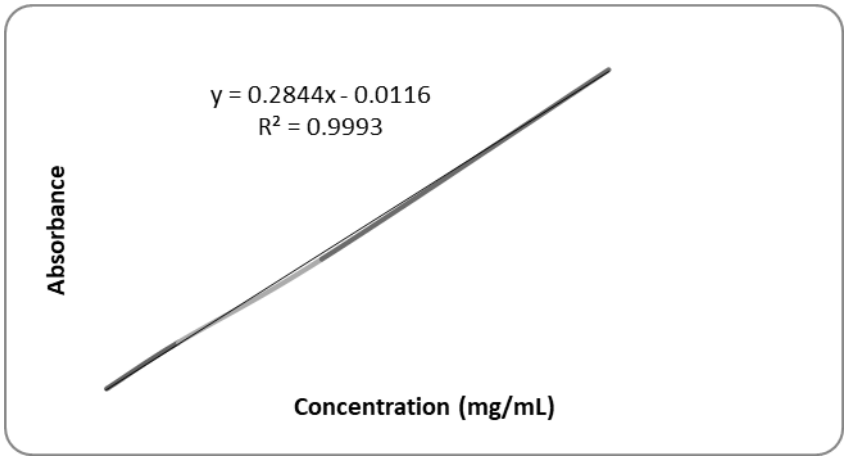



Figure 3. The plots of different concentration of sennoside B versus its absorbance.

From the analysis of variance, the types (short var. and long var.) of senna shrubs significantly influenced the yields of sennoside in the leaf ($P < 0.01$) and pod ($P < 0.001$) part. Their flowers did not show significant difference in the total sennoside percentage. The short var. senna shrub was superior in total percent of sennoside with a mean value of 1.7 % and 2.33 % in the leaf and pod parts, respectively. The long var. senna shrub exhibited 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 Anna (2014) on yield of total anthracene derivatives in the leaf of *Cassia acutifolia* from Russia (1.21 to 1.88 %) interms of sennoside B determined by spectrophotometric method.

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 from the pod (2.62 %), next to Dubti (2.30 %) and followed by Logia (2.06 %). The sennosides content from the leaf did not show a significant difference among Dubti (1.73 %) and Fentale (1.77 %) samples, but Logia samples exhibited lower mean value (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 %), while Mile had higher value for the leaf (1.61 %) (Table 3).

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 appeared to be less than the expected range of WHO (1999) monograph limit, which is 2.5 % calculated as sennoside B. This occurred due to the season of sampling which was during flowering, leading to shift of the secondary metabolite from leaf to flower and pod. Complimentary results were reported by Ratnayaka *et al* (2002), indicating that deflowering increases the sennoside A and B concentration in the leaf by 25% and also sennosides content increases at the age of 90 days, which is before flowering (Upadhyay *et al*., 2011).

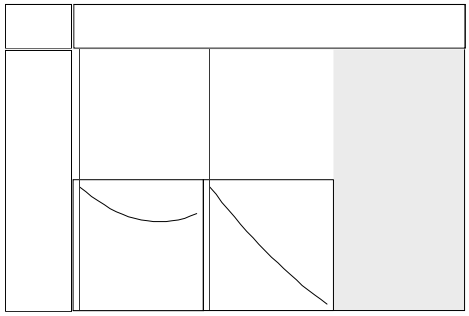
Table 1. The effect of types (var.) on sennosides content of Senna alexandrina

| 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 |

*Means followed by the same letter within a column are not significantly different at P<0.05. .*

Table 2. The influence of location on the sennosides content for short var. Senna alexandrina

| Location | Altitude (m) | Total Sennosides (%) | | |
|---|---|---|---|---|
| | | Leaf | Pod | Flower |
| Dubti | 410 | 1.73 [a] | 2.30 [b] | 0.13 [a] |



European Pharmacopoeia.1995. Strasbourg, Council of Europe. (2nd edn).
Upadhyay A., Nayak P.S., Dwivedi S.K. and Rao S. (2011) HPTLC densitometric quantification of Sennosides from Cassia angustifolia. Gene Plant Physiol 1: 1-2.
Tarkase KN and AV Dan.2015. Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Aloin and Sennoside in Suppository Dosage Form. Int J Pharm Sci Rev Res 31: 195-199.
Kurkin VA and AS Anna.2014. The development of new approaches to standardization of Cassia acutifolia leaves. J Pharmacog Phytochem 3: 163-167.
World Health Organization. 1999. WHO monographs on selected medicinal plants. World Health Organization.
Ratnayaka HH, B Meurer-Grimes,and D Kincaid.2002. Sennoside yields in Tinnevelly senna affected by deflowering and leaf maturity. Hort Sci 37: 768-772.
Upadhyay A, PS Nayak,and NA Khan 2011. Sennoside contents in Senna (Cassia angustifolia Vahl.) as influenced by date of leaf picking, packaging material and storage period. J Stored Prod Postharv Res 2: 97-103.