Determination of Sennosides A and B in Sudanese Medicinal Plant *Senna alexandrina* Pods and Leaves using Reverse-Phase High Performance Liquid Chromatography

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Senna alexandrina pods and leaves have been used in herbal medicine since ancient times. The extracts of this plant contain mainly anthraquinone glycosides that have a significant laxative effect. In this study, the sennosides A and B, the major constituents of *Senna alexandrina* were calculated via a simple and accurate HPLC method. The yield of sennoside A was found 1.22 and 1.83% in the pods and leaves, respectively. Whereas the yield of sennoside B was found 2.7 and 1.83% in the pods and leaves, respectively.

Keywords: *Senna alexandrina*; anthraquinone glycosides; sennosides; laxative effect.

Introduction:

Sudan has numerous medicinal herbs and plants that have been noted for their biological activities (Ali et al., 2002; Ebtihal et al., 2017; Mohammed et al., 2017). Herbal medicine based on medicinal plants represents an integral part of the Sudanese culture and plays a crucial and indispensable role in the current public healthcare (Hussain et al., 2014; Malik et al., 2004). *Senna alexandrina* Mill (Family: Fabaceae.), locally known in Sudan as "Senna mackie" is one of the plants used to treat intestinal complications, circulatory problems, urinary stones and sexually transmitted diseases (Yagi et al., 2013). Pods and leaves of *S. alexandrina* are commercially available as a laxative drug in Sudan and other African countries such as Mali and Ethiopia (Bekri, 2017; Ghassemi-Dehkordi et al., 2014; WHO, 1999). Previous phytochemical analysis of *S. alexandrina* extracts have resulted in the isolation and characterization of sennosides A and B as the major constituents (Demirezer et al., 2011; Nasrollah et al., 2014). So, their contents should be quantified and used as an index for the valuation or even its detection in the bulk powder form.

Different techniques such as thin layer chromatography (Lemmens, 1977), column chromatography (Yagi et al., 2013), gas-liquid chromatography (Baars et al., 1976), radioimmunoassay (Atzorn et al., 1981) and high performance liquid chromatography (Erni and Frei, 1978; Ohshima and Takahashi, 1983; Srivastava et al., 1983) have been applied to sennosides analysis in *Cassia* spp worldwide. So far, very few reports published on the determination of sennosides for the Sudanese *Cassia* spp. Therefore, the aim of this study was to determine the sennosides A and B contents of Sudanese *S. alexandrina* using a reverse-phase high performance liquid chromatography (RP-HPLC) technique.

Materials and Methods:

Plant Materials

The parts of *S. alexandrina* (pods and leaves) were collected from Omdurman City, Khartoum State, Sudan and were identified by Mr. Mubark, Department of Botany, Medicinal & Aromatic Plants and Traditional Medicine Research Institute, National Centre for Research, Khartoum, Sudan. A herbarium specimen (No 1541) was deposited.

Extraction and Preparation

The extraction of the samples was done according to the method described by Ghassemi-Dehkordi et al. (2014). Briefly, in a volumetric flask, one gram (1 g) of the pods and leaves powder of *S. alexandrina* were weighed individually, and 10 mL of the ethanol: water (1:1) was added to each sample powder for 20 minutes with occasional shaking and stirring. Finally, it was extracted, filtered, and washed three times with 10 mL solvent. The extract was evaporated individually until dryness, and the percentage of each extract was calculated as shown in Table 1. The extracts that were

obtained from one gram of *S. alexandrina* dried pods and leaves powder were diluted in 20 mL methanol HPLC grade and used for HPLC analysis.

Instrumentation and Chemicals

HPLC analysis was done on the CTO-20A HPLC, 2LC-10ADvp pumps, Auto sampler Sil-10ADvp (Shimadzu, Japan). The column was a Shim-pack VP-ODS (150 mm x 4.6 mm, i.d 5 µm) (Shimadzu, Japan) and LC solution software was used for the determination of compounds and processing the data. HPLC-grade Methanol was purchased from Scharalu Company (Spain). Sennosides A and B, as analytical standards, were purchased from Aktin Chemicals Corporation, China. Water used was deionized and double distilled using a glass apparatus.

Operating Conditions

The elution was performed by isocratic solvent systems with a flow rate of 0.8 mL/min at a temperature of 50 °C. Different compositions of the mobile phase were tested and the desired resolution of the sennosides A and B with symmetrical peaks and a stable baseline was achieved by using acetonitrile: water: acetic acid (62:38:2) as mobile phase. The mobile phase was prepared freshly, filtered through a 0.2 mm membrane filter and degassed via sonication before use. The sample injection volume was 10 µL, while the wavelength of the UV-Vis detector was set at 340 nm. Total running time was 10 min. The contents of sennosides A and B were calculated using calibration graphs of each compound.

Calibration Graphs

Exactly, 1000 µg analytical standard (Sennosides A and B) were diluted individually in 10 mL of methanol HPLC-grade to prepare the stock solution of 100 µg/mL. Different standard solutions were prepared by serially diluting the stock solution to concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL. These standards were injected into the HPLC using the above operating conditions.

Result and Discussion:

The extraction yield obtained from the pods and leaves were 23.3 % and 18.9 % (w/w., respectively. Peaks corresponding to sennoside A (Fig 1, standard), sennoside B (Fig 2, standard), sennosides A and B (Fig 3, pods sample) and sennosides A and B (Fig 4, leaves sample) were sharps and well resolved with retention times of 4.8 and 4.1 min, respectively. The area counts of peaks (Y) and the corresponding concentrations (X) were used to plot the calibration graphs.

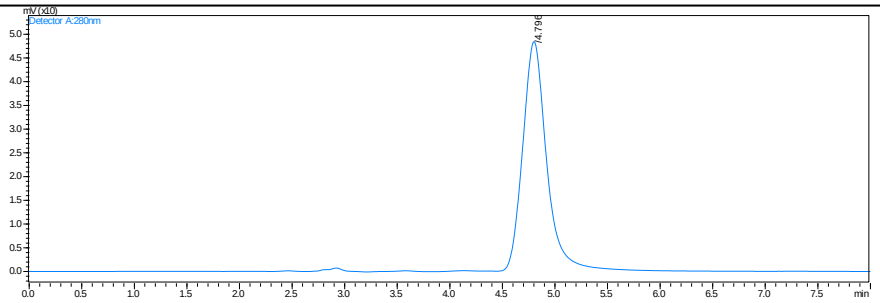

Figure 1. HPLC chromatogram of sennosides A (standard, 100 µg/ mL) at 340 nm

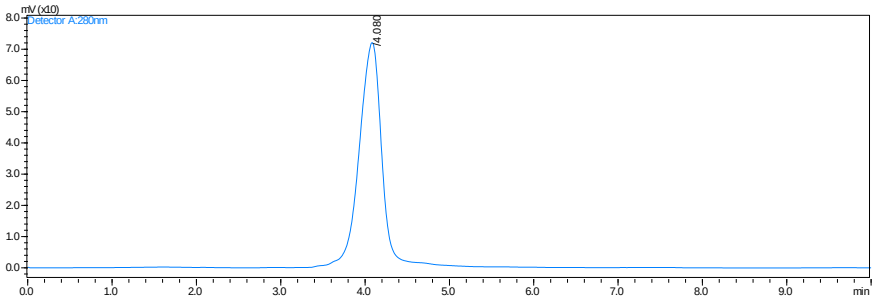

Figure 2. HPLC chromatogram of sennoside B (standard, 100 µg/ mL) at 340 nm

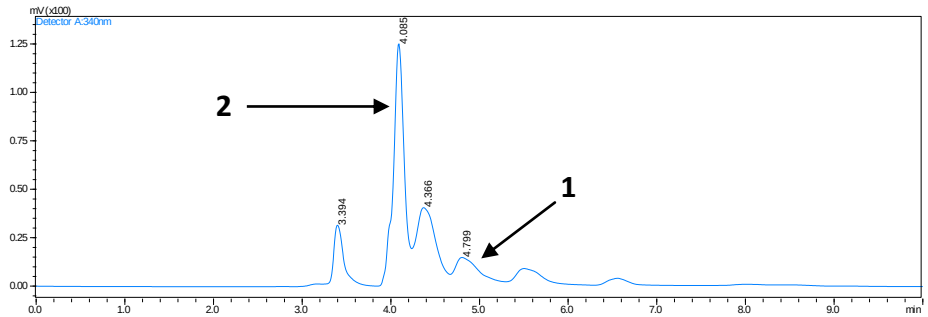

Figure 3. HPLC chromatogram of *S. alexandrina* pods methanol extract (1 g/20 mL) at 340 nm
1: sennoside A; 2: sennoside B using operation conditions mentioned in the experimental section

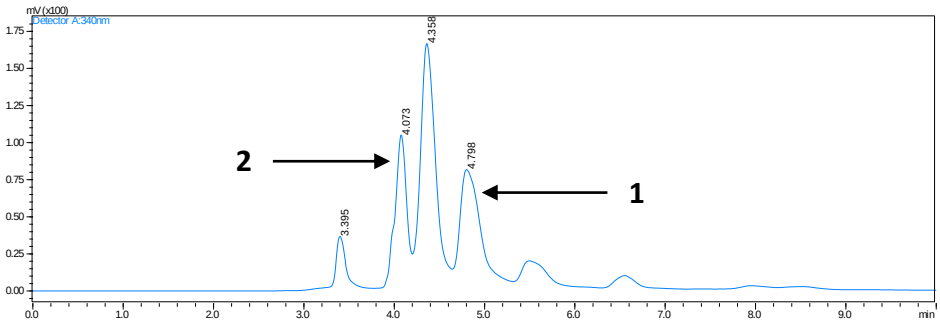

Figure 4. HPLC chromatogram of *S. alexandrina* leaves methanol extract (1 g/20 mL) at 340 nm

1: sennoside A; 2: sennoside B using operation conditions mentioned in the experimental section

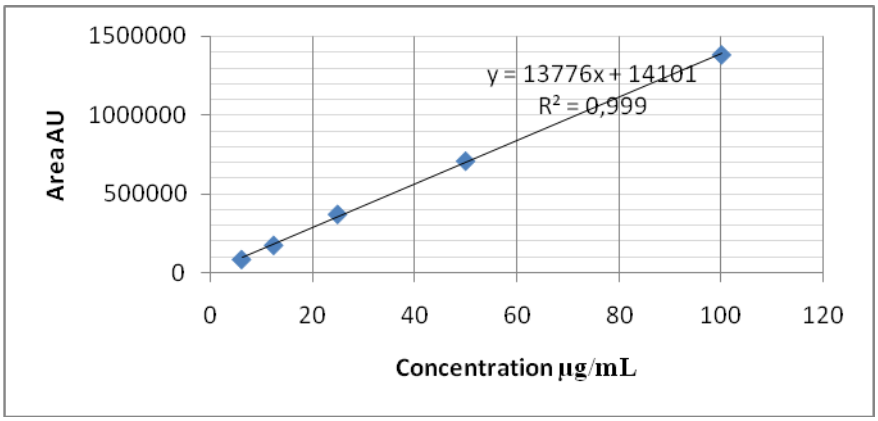

Figure 5. Calibration curve of sennoside A; the regression equation was y= 13776x+14101 with the correlation cofactor (R^2) of 0.999, where x is the concentration of standard in µg/mL.

The sample peak area (y) obtained for sennoside A in pods was 8420523.
From the regression equation $y = 13776.x + 14101$

$$\text{Concentration of senoside A in pods} = \frac{8420523 - 14101}{13776} = 610\ \mu g/mL$$

Ethanol:water (1:1) extract obtained from 1 g of *S. alexandrina*pods dried powder diluted to 20 mL with methanol was standardized to contain 12.2 mg/mL sennoside A.

$$\text{Senoside A\% in dry pods} = \frac{12.2}{1000}\ x\ 100 = 1.22\%$$

The sample peak area (y) obtained for sennoside A in leaves was 12630785.

$$\text{Concentration of senoside A in leaves} = \frac{12630785 - 14101}{13776} = 916\ \mu g/mL$$

Ethanol: water (1:1) extract obtained from 1 g of *S. alexandrina* pods dried powder diluted to 20 mL with methanol was standardized to contain 18.3 mg/mL sennoside A.

$$\text{Sennoside A\% in dry leaves} = \frac{18.3}{1000}\ x\ 100 = 1.83\%$$

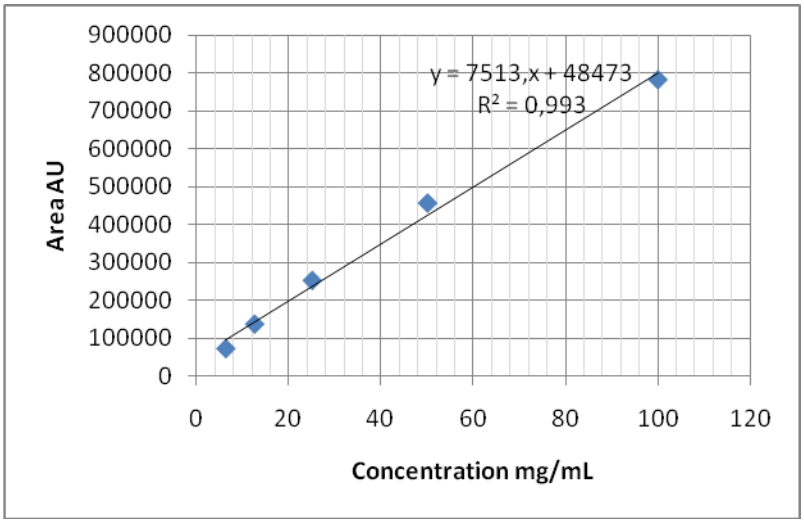

Figure 6. Calibration curve of sennoside B; the regression equation was y= 7513.x+48473 with the correlation cofactor (R^2) of 0.993, where x is the concentration of standard in µg/mL.

The sample peak area (y) obtained for sennoside B in pods was 10153109.

From the regression equation $y = 7513.x + 48473$

$$\text{Concentration of sennoside B in pods} = \frac{10153109 - 48473}{7513} = 1345\ \mu g/mL$$

Ethanol: water (1:1) extract obtained from 1 g of *S. alexandrina* leaves dried powder diluted to 20 mL with methanol was standardized to contain 27.0 mg/mL sennoside B.

$$\text{Sennoside B\% in dry pods} = \frac{27.0}{1000} \text{ x } 100 = 2.7\%$$

The sample peak area (y) obtained for sennoside B in leaves was 7988296 .

$$\text{Concentration of sennoside B in leaves} = \frac{7988296 - 48473}{7513} = 1057\ \mu g/mL$$

Ethanol: water (1:1) extract obtained from 1 g of *S. alexandrina* leaves dried powder diluted to 20 mL with methanol was standardized to contain 21.1 mg/mL sennoside B.

$$\text{Concentration of sennoside B\% in dry leaves sample} = \frac{21.1}{1000} \text{ x } 100 = 2.1\%$$

Table 1. Contents of sennosides A and B in *S. alexandrina* pods and leaves

Plant part	Content of sennoside A%	Content of sennoside B%
Pods	1.22	2.7
Leaves	1.83	2.1

Conclusions:

The HPLC method reported here is simple and efficient for the isolation of sennosides (A and B) in plant extracts. The method is developed over previous methods (Verma et al., 1996), since the retention times of both compounds is reduced, with excellent resolution, which is desirable for column-life and for assay efficiency.

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