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Analytical Comparison of Different Senna Extracts and Determination of Individual Ratios of Sennosides A & B in Various Senna Extracts

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ABSTRACT

Constipation is a common problem in all types of ages. Cassia angustifolia (Senna) extracts contain anthranoids that are commonly used for constipation and are well known for their laxative properties. Senna leaves or pods are highly rich with Sennosides content A, B, C & D. These Sennosides are hydroxyanthracene glycosides, which work miraculously as a laxative. In the present study, Senna extract from different vendors with variable strengths of sennosides A&B were procured and the relation between residue on ignition (ROI) with that of metals and sennosides A&B was studied. The optimized concentration of senna extracts containing different sennosides A&B content were analyzed on HPTLC to determine the ratios of sennosides A&B from the total sennosides as well as its relation with residue on ignition (ROI). Residue on ignition (ROI) was analysed gravimetrically while content of Calcium was calculated by Complexometric method. Iron content was carried out spectrophotometrically and Sennosides A&B content was carried out using HPTLC. The process control of extraction can be monitored on the basis of ROI. Specified Sennosides and ROI value together can be used in quality specification. Batch to batch variation with respect to efficacy can be avoided by determining the fixed quality norms for content of Sennosides, ratio of sennosides A&B, ROI value and content of Calcium.

Keywords: High Performance Thin Layer Chromatography (HPTLC), Senna extract, Residue on Ignition (ROI), Calcium, Iron, Sennosides A & B

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INTRODUCTION

Senna is a shrub found in India, Pakistan, South China. Botanically it is called *Cassia angustifolia* Vahl. Its name is derived from the Arabic word "sena," and it has been used in Ayurvedic and Unani medicines since the ninth century. The active substances in Senna leaves are called Sennosides. Sennosides are hydroxyanthracene glycosides. They have been used as natural, safe time-tested laxatives in traditional as well as modern systems of medicine. Senna Leaves or pods in the form of extract is used as a common effective laxative from ancient times[1]. Number of different studies are conducted on the content of sennosides present in Senna extract which plays vital role in exhibiting laxative properties.

Botanical name: *Cassia angustifolia* Vahl.

Chemical constituents of *Cassia angustifolia*[2]:

Senna leaf is a strong anthraquinone- containing purgative that is used in constipation. Senna leaf contains 1.5-3% hydroxyanthracene glycosides, mainly Sennosides A and B, which are rhein-dianthrones and smaller amounts of Sennosides C and D, rhein-8-glucoside, Rhein 8-diglucoside, Aloe-emodin 8-glucoside, Rhein Kaempferol, Aloe-emodin, Isorhamnetin, Chrysophanic Acid[1] etc.

Mode of action[3]:

Constipation has several causes, the most common ones being associated with nutritional factors such as consumption of food with a poor dietary fibre content resulting in the insufficient filling of the intestine or the intake of readily absorbed food with a reduced water binding capacity[4]. Anthraquinone laxatives are the main group plant-based drugs used in the treatment of constipation. Senna is most widely used anthranoid drug. Most of the pharmacological and clinical trials were not carried out with the plant but rather with the dianthrone .This substance has a more complex, pharmacological profile than that of the biologically formed anthraquinone derivatives which frequently act as pro-drug sennosides A, B & C. The main purgative constituents of senna i.e sennosides has a complex pharmacokinetic profile. They are not absorbed in the upper gut but are hydrolysed by the bacterial “reductase” enzymes in the colon into active rhein anthrone which in turn is not further metabolized but partially gets absorbed through epithelial cells and is thus subjected to entero-hepatic circulation. In contact with oxygen, rhein anthrones forms into rhein and sennidins. In 1934, in laboratory experiment it was found that laxatives not only stimulate intestinal peristalsis, but also initiate fluid retention in the intestinal lumen[5]. Enhanced colonic

motility and increased electrolyte and water transportation in the colon with inversion of the physiological pattern are the most crucial modes of action of anthraquinones.

Presence of Sennosides are responsible for laxative effect and laxatives containing Senna are highly demanding not only in India but globally. Number of different studies are conducted on the content of sennosides present in Senna extract which plays vital role in exhibiting laxative properties. The present study on analysis of different quality parameters conducted on different Senna extracts will help consumer to get better quality of Senna samples.

General manufacturing process of Senna extracts

Short description of process:

Alcoholic extracts of Senna leaves are collected and Calcium sennosides are precipitated by addition of Calcium Salt solution. The precipitated Calcium sennosides are centrifuged and washed with alcohol & dried under vacuum. The dried Calcium sennosides are ground, tested and packed.

MATERIALS AND METHOD

Procurement of Senna extracts:

Different samples of Senna extracts were procured from three Vendors .The criteria for selection of Vendors was based on the procurement of different percentages of Total Sennosides content mainly 10%, 15%, 20% and 60% present in the Senna samples as shown in table 1.

Table 1: Details of the Senna extract procured from Vendors

Sr No.	Vendors Name	*(Cassia angustifolia)* Sennosides content
1	Vendor 1	10%
2	Vendor 1	15%
3	Vendor 1	20%
4	Vendor 1	60%
5	Vendor 2	10%
6	Vendor 2	15%
7	Vendor 2	20%
8	Vendor 2	60%
9	Vendor 3	10%
10	Vendor 3	20%

Residue on ignition[6], metals (Ca and Fe) and HPTLC analysis:

All the 10 Senna extracts were analysed for residue on ignition, metal analysis and HPTLC analysis.

1.Residue on ignition[6]:

Method

In a previously cleaned, dried and weighed crucible about 1g of sample was weighed accurately

and was then subjected to ignition at about 550°C to 600°C for 4 to 5 hours and was allowed to attain the room temperature in a desiccator. Weighed till constant weight was obtained. Recorded the weights as mentioned below:

Observations

(i) Weight of empty crucible (A) = _____ g

(ii) Weight of crucible + sample (Before Ignition) (B) = _____ g

(iii) Weight of crucible + sample (After Ignition) (C) = _____ g

Calculation

Residue on Ignition (%w/w) = (C – A) X 100 / (B – A)

2.CONTENT OF CALCIUM AND IRON:

A).Content of Calcium[7]:

Content of Calcium was analysed by complexometric method

Chemicals and Reagents:

Dilute hydrochloric acid (10%): Dilute 10 ml of concentrated hydrochloric acid to 100 ml with distilled water.

0.05M EDTA : 18.61g EDTA (Ethylene diamine tetraacetic acid) powder ---→ 1000ml with water

8M KOH : 44.80g KOH (Potassium hydroxide) dissolve in 100ml distilled water.

P&R Indicator: 1: 99 (Taturate Patton's &Reader's indicator or calcon with anhydrous sodium sulphate)

Method:

In a previously cleaned, dried and weighed crucible about 1g of sample was weighed accurately and was then subjected to ignition for about 3 to 4 hours at 550°C till the carbon free ash is obtained. The above obtained ash was then dissolved in 10 ml dilute hydrochloric acid and the content was transferred gently in 250ml conical flask. The crucible was then rinsed with sufficient amount of distilled water (around 50ml) and all washings were collected in the same container and boiled for 3 to 4 minutes.

It was cooled to room temperature and diluted to 200 ml with distilled water. Shaken well and filtered the same through Whatman filter paper No.1. The collected filtrate was considered as a stock solution "S". Further testing was carried out by using this stock solution.

About 50 ml of the filtrate was taken from the stock solution "S" and sufficient amount of distilled water was added to it. The pH was adjusted by adding 10ml of 8M KOH and addition of Taturate calcon mixture (Patton's and Reader's indicator), was continued till wine red colour was obtained

The content was titrated against 0.05M EDTA. End point was wine red to blue. The burette reading as "A"ml was noted.

Calculation:

Content of Calcium (%) = A x (0.0020016 / w) x (200/50) x (M /0.05) x 100

Where M was the actual molarity of the EDTA solution

B) Content of Iron[8]:

Similarly content of Iron was estimated by sphectrophotometric method

Chemicals and Reagents used:

Aqua region : HCl : HNO_3 (3 : 1)

0.1N $KMnO_4$:

3.166g of $KMnO_4$ powder → 100ml with water → warm → cool to room temperature → dilute to 1000ml with distilled water.

Dilute Sulphuric acid:

Contains approximately 10 %w/w of H_2SO_4. Dilute 57 ml of sulphuric acid to 1000 ml with water.

4M Nitric Acid: 25% v/v Nitric acid

Sample preparation:

In a previously cleaned, dried and weighed crucible about 1g of sample was weighed accurately and was then subjected to ignition for about 3 to 4 hours at 550°C till the carbon free ash was obtained. The ash was transferred into a beaker and digested in 40 ml aqua regia. Concentrated to 10ml and diluted to 50ml with distilled water. Then it was filtered through filter paper No.1. This solution was considered as STOCK SPL Solution. "A" ml.

Blank preparation:

The above procedure was repeated by omitting the sample, and the "BLANK STOCK" solution "B" ml was prepared.

Standard preparation:

Weighed accurately about 0.703g of Ferrous Ammonium Sulphate, it was dissolved in 100ml distilled water and 5ml dilute Sulphuric acid. Then 0.1N Potassium Permanganate, was added drop wise until pink colour was obtained, then the volume was made to 1000ml with distilled water. The solution was shaken well and kept for overnight. Next day again it was shaken well and accurately pipetted out 1ml (=0.1mg Fe)= 0.0001g Fe for colour development. "C"ml

Calculation:

% of Iron = (Sample Absorbance/Std. Absorbance) x (0.0001/50) x (50/wt of spl) x dilution factor of spl. x 100

3. HPTLC analysis[9]:

Chemicals and Reagents:

All the chemicals used in the experiment were of analytical grade.

Apparatus:

Sonicator (Model no. 3.7L100H)

Method:

Senna leaves extracts with sennosides content of 10%, 15%,20% and 60% were procured from approved vendors. These extracts are considered as samples.

Sample Preparation:

Vendor 1 and Vendor 3:

Each 50 mg of the extract was sonicated with 5 ml of methanol for about 10 min. Then extract was filtered through Whatman filter paper no. 1. About 2ml of this filtrate was again diluted to 5ml with methanol in a volumetric flask. The final concentration of sample was 4 mg/ml in methanol.

Vendor 2:

Each 10mg of the extract was weighed and sonicated with 5 ml of methanol for about 10 min. Then extract was filtered through Whatman filter paper no. 1. About 2ml of this filtrate was again diluted to 5ml with methanol in a volumetric flask. The final concentration of sample was 0.8mg/ml in methanol.

Standard Preparation (Total Sennosides content: 60%)

Standard solution of sennosides (0.16mg/ml) prepared in Methanol.

Chromatographic Parameters:

Chromatography was performed on 20x10 cm/ 10x10 cm, 0.2mm Precoated with silica gel 60 $F^{254;}$ Merck plates. Samples and standard of known concentrations were applied as 8mm wide bands using Linomat V Automatic sampler applicator; CAMAG, (Muttenz, Switzerland). After the completion of sample application, the plates were developed in glass twin trough chamber (20x10) and (10x10) presaturated with mobile phase of 2-propanol: ethyl acetate: water: formic acid (17:19:12:2) for 10 minutes.

Detection of Sennosides:

After development, the plates were scanned using Thin Layer Chromatography Scanner 3 with WinCATS software; CAMAG, Switzerland under 350 nm wavelength, absorption reflection scan

mode. The identification of Sennosides A and B were confirmed by superimposing the UV spectra's of Standard and Samples.

RESULTS AND DISCUSSION

From the analytical readings framed in table 3, we observed that Vendors with 10%, 15% and 20% Senna samples measure Residue On Ignition on higher side whereas Vendors with 60% Senna samples prominently show comparatively less Residue On Ignition. Also, in the Senna samples with 60% of total sennosides content, it was observed that as ROI was decreased , the Content of Calcium also got decreased which paves way to think about the methods of process carried out by different vendors. This further can be illustrated by observing the trend of readings obtained for Iron content for different vendors. From table. 3, for Vendor 1, the iron content which can be assumed as minor impurity in the sample was in the range of 150 ppm to 180 ppm whereas for other two vendors the iron content range starts from 300 ppm and goes upto 1020 ppm which shows that there was a vast difference in the processing methods carried out at all the three vendors.

In Table 4, we have combined the readings for ROI and derived individual ratios of Sennosides A & B from total Sennosides content by HPTLC and tried to correlate them. We found out that as we go from Senna samples containing 10% total sennosides content to Senna samples containing 60% sennosides content, the ratio of Individual Sennosides A&B in the total sennosides changes from 20:80 pattern (for 10% sennosides) to 39: 61 (60% sennosides) pattern. That means senna sample containing 60% total sennosides will be more effective as it contains both Sennosides A & B in (approximately 40:60 ratio.), where Sennoside A also increases gradually. Distinctively it was also observed that the ROI readings for Senna samples of 10% sennosides content are different from that of Senna samples with 60% sennosides. The quality and efficacy of Senna samples can be judged by Residue On Ignition readings.

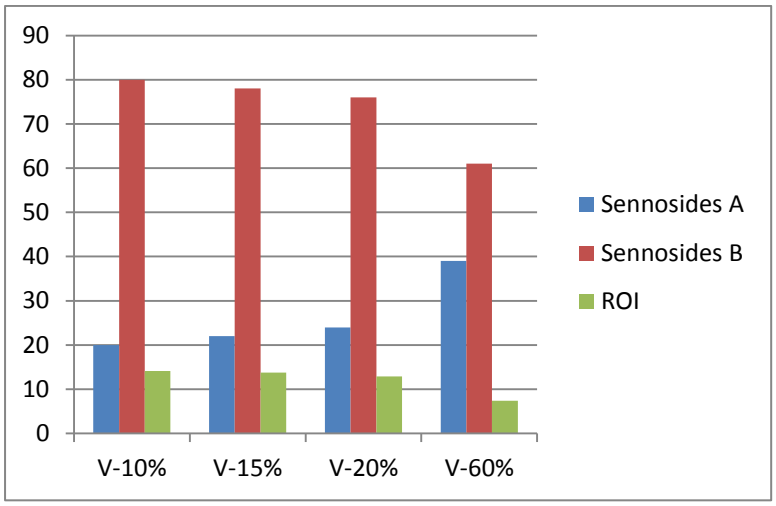
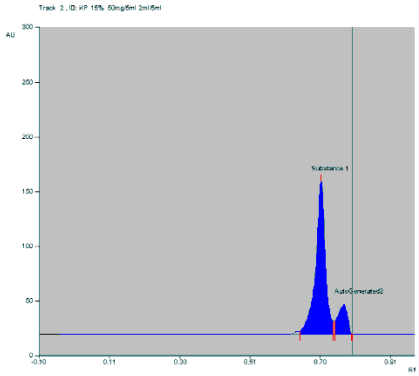
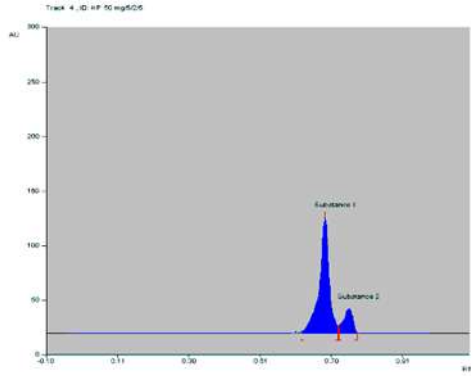


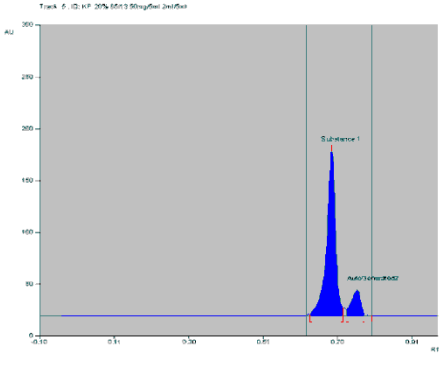
Figure 1: Statistical presentation of Table 4



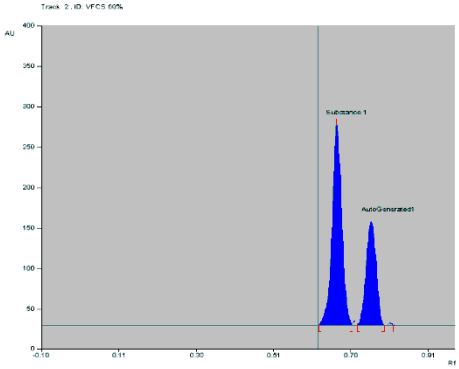
Senna samples (10% Sennosides)



Senna samples (15% Sennosides)



Senna samples (20% Sennosides)



Senna samples (60% Sennosides)

Figure 2: Graphical presentation of Individual ratios of senna samples with 10%, 15%, 20% and 60% sennosides content.

Table 2: Colour development

Sr.no.	Reagents	Blank	Standard	Sample
1	Stock Solution	1ml of sol. "B"	1ml of sol. "C"	**A
2	4M Nitric Acid	3ml	3ml	3ml
3	Distilled Water	5ml	5ml	5ml
4	0.1NKMnO4 Potassium permanganate	3-4 drops till pink color	3-4 drops till pink color	3-4 drops till pink color

5	10%Ammonium thiocyanate	10ml	10ml	10ml
6	Distilled water	Dilute up to 50ml	Dilute up to 50ml	Dilute up to 50ml

Take the reading of absorbance on suitable spectrophotometer at 480nm after 5minutes.

** Dilution Factor will differ as per the final concentration.

Table 3: **Correlation of Residue on ignition with metals such as Calcium (Ca) and Iron (Fe)**

Vendor	Residue on Ignition	Color of Ash	Content of Calcium in %w/w	Content of Iron in ppm
Vendor 1 (10%)	14.188	White	7.123	153.00
Vendor 1 (15%)	13.766	White	7.279	177.07
Vendor 1 (20%)	12.933	White	7.299	164.88
Vendor 1 (60%)	**7.492**	White	**4.465**	**98.44**
Vendor 2 (10%)	15.933	Cream	6.973	368.90
Vendor 2 (15%)	10.828	Grey	5.492	398.16
Vendor 2 (20%)	19.660	Grey	10.168	656.08
Vendor 2 (60%)	**7.726**	Grey	**4.988**	**105.50**
Vendor 3 (10%)	30.401	Grey	8.995	599.29
Vendor 3 (20%)	27.805	Grey	9.596	1012.75

Table 4: **Correlation of Residue on ignition with Sennosides A & B**

Vendor	Residue on Ignition	Individual ratios of Sennosides A and B from total Sennosides		Average of the Ratios (A:B)
		Sennosides A	Sennosides B	
Vendor 1 (10%)	14.188	19	81	**20:80**
Vendor 2 (10%)	15.933	19	81	
Vendor 3 (10%)	30.401	22	78	
Vendor 1 (15%)	13.766	22	78	22:78
Vendor 2 (15%)	10.828	22	78	
Vendor 1 (20%)	12.933	24	76	24:76
Vendor 2 (20%)	19.660	22	78	
Vendor 3(20%)	27.805	25	75	
Vendor 1 (60%)	7.492	42	58	**39:61**
Vendor 2 (60%)	7.726	36	64	

CONCLUSION

The process control of extraction can be monitored on the basis of ROI. Specified Sennosides and ROI value together can be used in quality specification. Batch to batch variation with respect to efficacy can be avoided by determining the fixed quality norms for content of Sennosides, ratio of sennosides A & B, ROI value and content of Calcium.

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