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# Formulation, development and evaluation of sennoside enriched Senna extract tablets of different concentrations

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**Abstract**
Senna, a plant of ethnopharmacological importance has been traditionally used in the treatment of chronic constipation. Ancient texts from Ayurveda have mentioned the potency of Senna as a laxative. The traditional methods incorporated for formulation of Senna for the treatment of constipation, suffer from inherent drawbacks like compound-specific isolation and extraction, lack of standardisation, safety and efficacy standards and non availability in modern dosage forms. Therefore, it was proposed to prepare uncoated oral dosage form tablets of optimized and enriched extracts from dried Senna leaves. It was also envisaged to standardize the product making it acceptable for the modern generation of end users. Maceration process was used for extraction of dried Senna leaves. The physicochemical and preliminary phytochemical screening studies were performed. Hydroalcoholic extracts were prepared in concentrations of 50%, 70%, 80% and 100% ethanol. Enrichment of the Senna extracts was done using Borntrager's process. It was observed that 70% ethanol enriched Senna extract resulted in higher yield value as compared to the other extracts. The presence of Sennosides was asserted using TLC coupled UV analysis. In HPLC analysis, Sennoside B was used as the marker compound, wherein 70% ethanol enriched extract showed relatively higher concentration of Sennosides. Senna enriched and optimized extract tablets were prepared by using wet granulation method. Evaluation parameters of Senna tablets were found to be within the Pharmacopoeial limits. During dissolution studies, it was found that 70% enriched ethanolic extract of Senna resulted in higher % cumulative release of tablet ingredients at 90 minute time interval. Hence, 70% enriched Senna extract can be considered as the optimum concentration in terms of extraction, analysis, concentration of total Sennosides, formulation and evaluation, so as to achieve the desired therapeutic and pharmacological benefits of the product.

**Keywords:** formulation, sennoside enriched, extract tablets, concentrations

## Introduction
Globally the herbal extract market is expected to be driven by factors such as growing number of innovative product launches, growing number of consumers, wide range of applications of herbal extracts and rising preference for botanical flavours among consumers. However, inconsistencies in the prices of herbs and high cost of production are primary challenges hampering the growth of this market over the forecast period. The demand for herbal extracts is increasing with respect to the global market trends. The global herbal extract market is expected to grow at the rate of more than 8% over the period of 2016-2021. Use of plant extracts has significantly provided an invaluable solution for the industries like pharmaceuticals, cosmetics, food and beverage etc. (Research and Markets- "Global Spice and Herbs Extracts Market Trends analysis and forecast to 2021). The varying quality of herbal products among batches, herb-drug interactions and lack of dosage instructions are among the challenges faced by the herbal market.

Chronic constipation is one of the most common complaints in clinical medicine. It is estimated that one in every five adults worldwide suffers from constipation. Hence, the demand for use of laxatives is increasing worldwide. Constipation is a condition in which bowel evacuations occur infrequently, or in which the faeces are hard and small or where passage of faeces causes difficulty or pain. Laxative is defined as a drug used to stimulate or increase the frequency of bowel evacuation, or to encourage a softer or bulkier stool. The common laxatives are the irritants like castor oil and Jalapa, Senna and its derivatives, magnesium sulphate and other mineral salts, methylcellulose and other bulking agents (L. M. Harrison, 2010).

Senna plant (Figure 1) was selected for the study. Senna is reported to have laxative action from an ethnopharmacological point of view. Many ancient scriptures have referred

sonamukhi i.e. senna useful in the treatment of chronic constipation (Khandelwal, 2007) [21]. Hence, dried senna leaves were employed for the following study. Senna also known as Senna leaf, Sennae folium, Tinevelly senna, Cassia senna, Senai ki patti, Sonamukhi, Bombay senna, consists of the dried leaflets of *Cassia angustifolia* or *Cassia senna* Vahl belonging to family Leguminosae (Khandelwal, 2007) [21]. The Indian Senna known as Tinnevelley senna is indigenous to India. The cultivation and collection is mainly done in Tinnevelley, Madurai and Ramnathapuram districts of Tamil Nadu (Rangari, 2012) [27]. Senna contains mainly two anthraquinone glycosides called as sennoside A and sennoside B (not less than 2.5%) which account for its purgative property. Sennosides A and B are stereoisomers of each other. They are dimeric glycosides with rhein dianthrone as aglycone. Senna leaf also contains the other anthraquinone glycosides in small amounts. They are sennosides C and D, rhein 8-glucoside, aloe-emodin, anthrone diglucoside, rhein, kaempferol and iso-rhamnetin. It also contains phytosterol, mucilage, resin, myricyl alcohol, salicylic acid, chrysophanic acid and calcium oxalate. Senna also contains two naphthalene glycosides called Tinnevellin glycoside and 6-hydroxy musizin glycoside (Rangari, 2012) [27]. Senna and its preparations are used as purgative in habitual constipation. Senokot tablets®, Senna leaf extract®, Laxettes®, are the polyherbal preparations of Senna available in market. Most of the marketed formulations of Senna contain either concentrated extracts of Senna or contain powdered Senna plant parts.

It is therefore envisaged that if tablets are prepared which contain exclusively enriched amounts of Anthraquinone glycosides i.e. Sennosides, then the formulation would be pharmaceutically and pharmacologically more sound in terms of bioavailability, pharmacological action and efficacy. Thus, our objective was to develop a tablet formulation of enriched Senna leaf extract that exclusively contains more quantity of Sennosides. Also, the developed formulation must be within the standard limits as per the official standards.

## 2. Materials and Methods

The dried Senna leaves were procured from Shri Shail Medifarms, Nagpur. The authentication of the *Cassia senna* leaves was done by Dr. Nitin Dongarwar of Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur, Maharashtra (Figure 1).

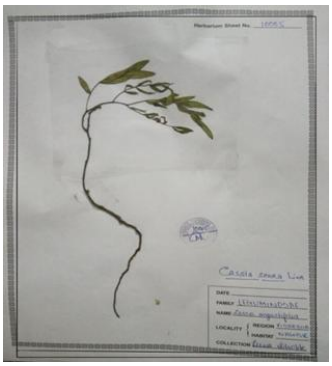

**Fig 1:** Herbarium sheet showing authenticated Senna plant

### 2.1 Physicochemical Parameters

The physicochemical tests such as loss on drying, foaming index, ash value and extractive value were carried out as per the guidelines provided by World Health Organization (WHO, 1999) [40].

### 2.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening of senna leaf extract was carried out. The screening tests were done for the following phytochemicals; proteins, carbohydrates, oils/fats, saponins, sterols, glycosides, alkaloids, tannins and flavonoids (Khandelwal, 2007) [21].

### 2.3 Extraction

The extraction was done by using maceration technique. The material to be extracted was placed in a closed vessel and suitable solvent (menstrum) was added and left for 7 days with occasional shaking. The liquid was then strained off and the solid residue (marc) was pressed to remove the solution as much as possible. The strained and expressed liquids are mixed and clarified by filtration. The product was allowed to stand for few days before use so as to allow sedimentation. Straining was done through a cloth (Rangari, 2012) [27]. Hydroalcoholic solvents were used i.e. ethanol + water. Total four extracts were obtained;

1. 50% v/v extract = 50% ethanol + 50% water
2. 70% v/v extract = 70% ethanol + 30% water
3. 80% v/v extract = 80% ethanol + 20% water, and
4. 100% ethanolic extract.

### 2.4 Enrichment

The enrichment of the extracts was done using Borntrager's method (Kalia, 2011) [20]. Extract (200-300 g) was treated with sulphuric acid. This reaction mixture was treated with lead acetate, thereby tannins were removed (pH= 3). Separation was done using chloroform. The aqueous layer was neutralized with ammonia to obtain the enriched extract of Senna. This procedure was followed for all the extracts. These enriched extracts were then analyzed using HPLC technique, wherein confirmation and quantification of sennosides was done.

### 2.5 Chromatographic analysis

**2.5.1 Thin layer chromatography:** The senna extracts were subjected to thin layer chromatographic studies to find out the probable number of compounds present. The samples were prepared in methanol. Solvent system used was n-propanol: diethyl ether: methanol (4:4:3). The stationary phase used was TLC Plates coated with Silica gel. Ascending chromatography was used as the technique of separation. Followed by TLC, the ultra violet analysis of the scraped spots was done at the wavelength of 276 nm. All the concentrations of enriched Senna extracts i.e. 50%, 70%, 80% and 100% v/v were analysed.

**2.5.2 HPLC analysis**

**Optimization of chromatographic conditions:** The HPLC instrument used was UFLC Shimadzu, SPD- M20A with PDA Detector (Maryam et.al, 2014). The chromatographic studies were performed on $C_{18}$ analytical column (Spinchotech Pvt. Ltd. Enable). Mobile phases were prepared in closed solvent bottles and sonicated for about 20 min. Gradient mobile phase containing methanol: water (90:10), produced the best resolution of peak at less retention time for Sennoside B with flow rate of 1.0 ml/min. The maximum absorption wavelength (λmax) of Sennoside B was found to be 276 nm, hence selected as detection wavelength for analysis. Standard solution of Sennoside B was used in the order of 10 ppm (0.1 mg/ml). 10 ppm extracts i.e. samples were prepared (0.1 mg/ml) by filtration using minipore filters (0.22 mm) and utilized for analysis purpose. The

chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. The standard solution and prepared samples were injected using Hamilton syringe and the chromatograms were recorded for the standard and the samples.

### 2.6 Tablet preparation (Lachman *et al*., 1987)

Wet granulation method was followed. The drug and the excipients were added to produce a moist, coherent mass by wet screening. The process essentially involved a series of operations such as weighing, mixing, granulation, screening of the damp mass, drying, dry screening, lubrication and finally compression.

**The procedure of wet granulation followed was**

Lactose –MCC complex (1:1 ratio) [138 mg/tab] + Senna extract [100mg/tab] + n-propanol

**Mixing**
⇩
Formation of coherent mass
⇩
**Screening** (sieve no. 8 or 10)
⇩
**Drying** at 60°C
⇩
**Dry screening** (sieve no. 20)
⇩
Remaining portion of disintegrating agent was added
⇩
**Mixing**
⇩
**Granules** ready for tablet preparation

The powder flow properties like angle of repose, Hausner's ratio and Carr's compressibility index were calculated (Lachman *et al*., 1987). The tablet preparation was done using the Clit® rotary tablet machine. Post-compression studies on compressed Senna tablets were performed which involved organoleptic parameters like colour, odour and shape of tablets, hardness, friability, dissolution, weight variation and disintegration (Lachman *et al*., 1987).

## 3. Results

The physicochemical studies revealed that the extractive value of Senna leaves was highest in hydroalcoholic solvent as compared to the other solvents i.e. absolute alcohol, water, methanol, petroleum ether and chloroform. The presence of carbohydrates, mucilage, sterols, tannins, flavonoids and glycosides was indicated by the preliminary phytochemical screening studies. The extraction by maceration revealed the maximum yield with 70% v/v hydroalcoholic extract. Borntrager's method confirmed the presence of Anthraquinone glycoside i.e. Sennosides in the extracts. The UV analysis of TLC scraped spots of enriched Senna extracts showed highest absorbance in 70% ethanolic extract sample (Figure 2, Table 2). HPLC studies confirmed the presence of Sennoside B as a marker compound i.e. the presence of Anthraquinone glycosides in the enriched extract (Figure 3, 4). HPLC analysis and quantification studies showed that the 70% v/v enriched extract produced greater concentration of Sennosides as compared to other extracts i.e. 15.87 mg/100 ml (Table 3). The organoleptic properties of tablets were studied. All tablets were round shaped with characteristic odour. Also, the colour of placebo, 50% v/v ext. tablets, 70% v/v ext. tablets, 80% v/v ext. tablets and 100% v/v ext. tablets was white, green, dark green, light green and greenish brown respectively. The dissolution studies of 70% v/v extract tablets showed the highest % cumulative release at 90 minute time interval i.e. 92.90 % (Table 6). The disintegration time of tablets was also seen to be within the standard limits (Table 5).

**Table 1:** Physicochemical Parameters of Senna leaves

| Sr. No. | Parameter | Value |
|---|---|---|
| 1. | Ash value (Total ash) | 10.2 %w/w |
| | a) Acid insoluble ash | 0.3 %w/w |
| | b) Water soluble ash | 0.8 %w/w |
| 2. | Extractive value | |
| | a) Water | 30.0 %w/w |
| | b) Ethanol | 40.0 %w/w |
| | c) Petroleum ether | 45.4 %w/w |
| | d) Chloroform | 35.5 %w/w |
| | e) Hydroalcoholic | 70.8 %w/w |
| 3. | Loss on drying | 2.68 ± 0.17 %w/w |
| 4. | Swelling index | 2.4 |
| 5. | Foaming index | less than 100 |

**Table 2:** UV absorbance and Rf values of enriched Senna extract tablets

| Sr. No. | Sample Name | U.V. Absorbance (276 nm) | Rf Value |
|---|---|---|---|
| 1. | Sennoside 50% v/v extract | 0.0866 | 0.72 |
| 2. | Sennoside 70% v/v extract | 0.2234 | 0.71 |
| 3. | Sennoside 80% v/v extract | 0.1571 | 0.70 |
| 4. | Sennoside 100% v/v extract | 0.0332 | 0.72 |

**Table 3:** Retention time, peak area and concentration of various samples using HPLC

| Sr. No. | Type of Sample | Retention time | Peak Area | Concentration (mg/100ml) |
|---|---|---|---|---|
| 1. | Standard Sennoside B | 2.645 | 7808 | ---- |
| 2. | 70 % v/v enriched extract | 2.684 | 24589 | 15.87 |
| 3. | 80 % v/v enriched extract | 2.681 | 26082 | 14.96 |

**Table 4:** Pre-compression evaluation; angle of repose, Hausner's ratio and Carr's index

| Batch type | Placebo | 50%v/v tab | 70% v/v tab | 80% v/v tab | 100% v/v tab |
|---|---|---|---|---|---|
| Angle of repose | 32.27 | 32.01 | 31.26 | 32.82 | 32.41 |
| Hausner's ratio | 1.27 | 1.16 | 1.31 | 1.12 | 1.15 |
| Carr's index | 21.70 | 14.44 | 24.13 | 11.21 | 13.04 |

**Table 5:** Hardness, friability and disintegration time of Senna tablets

| Batch No. | Hardness ($kg/cm^2$) | Friability | Disintegration time (minutes) |
|---|---|---|---|
| Pacebo | 2.8 | 0.20 | 5.7 |
| 50%v/v tab | 3.0 | 0.39 | 6.1 |
| 70%v/v tab | 3.0 | 0.31 | 6.2 |
| 80%v/v tab | 2.9 | 0.43 | 6.3 |
| 100%v/v tab | 3.1 | 0.41 | 6.5 |

**Table 6:** Dissolution studies of Senna tablets; 70% enriched Senna extract tablets

| Sr. No. | Time (min) | % cumulative release |
|---|---|---|
| 1. | 0 | 0 |
| 2. | 15 | 16.050 |
| 3. | 30 | 35.514 |
| 4. | 45 | 48.122 |
| 5. | 60 | 61.066 |
| 6. | 75 | 86.013 |
| 7. | 90 | 92.901 |

Thus, the objective of formulating optimized tablets of enriched Senna extracts and its evaluation parameters within the standard limits confirms that biomarker-enriched Senna extract tablets were successfully formulated (Figure 5).

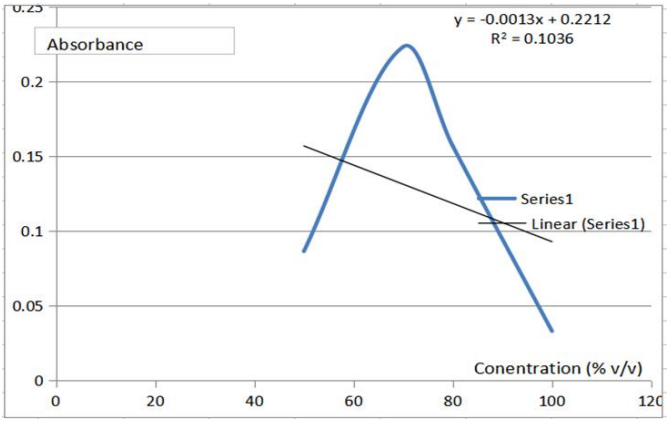


**Fig 2:** Thin layer chromatography of various enriched Senna extracts

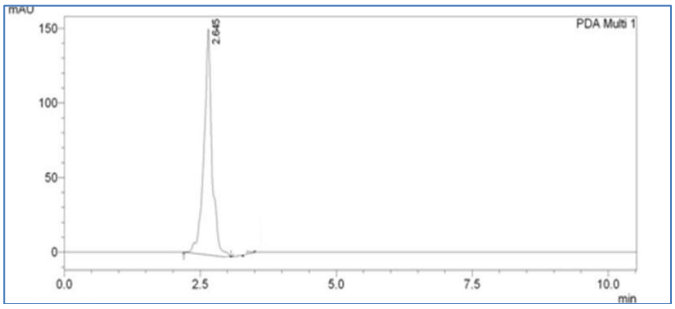


**Fig 3:** HPLC chromatogram of Sennoside B

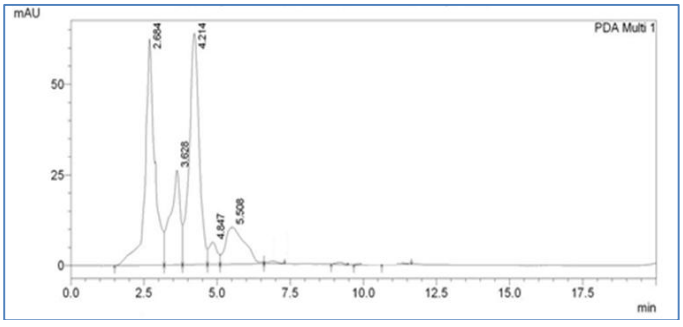


**Fig 4:** HPLC chromatogram of 70% v/v enriched Senna extract


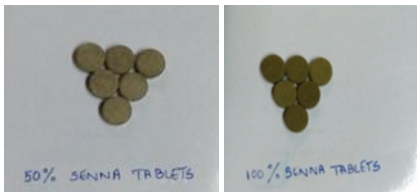


**Fig 5:** Formulated tablets; placebo, 80%, 70%, 50% and 100% v/v enriched Senna extracts.

## 4. Discussion

The present work deals with the preparation, evaluation and optimization of Senna extract tablets with respect to the standards (Sennoside A and B). The study considers the need to present natural or herbal phytochemicals in a modern dosage form. The modern dosage formulation involves the essential factors of stability, efficacy, standardization and dose uniformity (Rangari, 2012) [27].

The herbal products inherently lack in the aspect of standardization. This is the factor which contributes to the ill name of herbal remedies. As there is a lack of quality, the reliability of people on herbal products is slowly withering. The rampant use of adulterated raw materials in the formulation and development of natural/ herbal products is leading to sub-standard quality products in the market. This market pattern endangers the very trust in such kind of products, which in turn is harmful.

The present study is an attempt to overcome the above written disadvantages regarding herbal products with the insight of modern day requirements. The study throws light on quality standards right from the procurement of raw material. Also, the official guidelines and standards have been referred and seriously followed in regard with standardization (WHO guidelines 1999 [40], Indian Pharmacopoeia).

Attempts have been made to correlate the formulated tablets with respect to modern day requirements, wherein official limits of any given quality parameter exists. Also, the enriching of the active constituents has been done in the study. Formulation of dosage form using enriched fractions of particular phytoconstituents lead to the increased efficacy of their therapeutic actions. This increased efficacy would be due to the presence of mainly the active constituents (SS Agrawal and M Paridhavi, 2012) [31].

Optimization of the whole process increased the probability of obtaining correct results. Analysis of the enriched extracts and formulations have also been done using sophisticated chromatographic techniques. Accuracy and precision of results was of high priority. Thus, the trueness and the repeatability of the results were obtained.

The formulation and development of tablets essentially involved the follow up of various parameters of tablet processing and evaluation. It was made sure that the obtained results are within the prescribed limits of official standards.

The work commenced by first preparation of Senna extract using a suitable method. The Senna extract was prepared using the maceration method of extraction, which was found to be the optimum method as far as the extraction procedure is considered. The solvents used were ethanol and water, resulting into different concentrations of hydroalcoholic extracts. Ethanol was chosen as the solvent on the basis of its extractive value studies. The extractive value studies revealed that the extractive value of hydroalcoholic (ethanolic) solvent was highest. Thus, total of four concentrations differing in percentage of ethanol were prepared.

The next step was that of enrichment of anthraquinone glycosides (sennosides). It consisted of removing the sugar

moiety (glycone), so as to yield only the non-sugar (aglycone) part. The Borntrager's method was followed for the enrichment. (Kalia, 2009) [20].

Physicochemical studies and preliminary phytochemical tests were further performed. Followed by these studies, HPLC analysis was done using Sennoside B as the marker compound and it was confirmed that the enriched extracts contained Sennosides i.e. Sennoside A, B, C and D. It was also observed that the 70% v/v hydroalcoholic enriched extract gave the highest concentration as compared to the 80% v/v enriched extract. Thus, enriched 70% hydroalcoholic extract can be considered as the extract with maximum concentration of Sennoside B.

TLC studies coupled with the UV analysis of the TLC spots revealed that the maximum absorbance was that of 70% v/v enriched extract. Also, the analyses were done at the wavelength of 276 nm, same as that of HPLC analysis. Hence, the optimum wavelength for Sennoside detection is 276 nm.

Followed by the analysis part, tablet formulation and development was done. It consisted of processes like preparation of granules, drying, tablet punching and evaluation of tablets. Wet granulation process was chosen for granulation because of the nature of the enriched extracts. These extracts showed shear thinning property after application of stress through grinding in a mortar and pestle. Due to this property the extract was mixed uniformly with the tablet excipients. Also, as the Senna leaf enriched extracts contained mucilage as observed in preliminary phytochemical screening, hence the amount of binder used reduced significantly. The mucilage acted as a natural binder in the process of wet granulation of enriched Senna extract tablets. (K. Pundarikakshudu, 2005) [19].

Complex of lactose and MCC was used for the preparation of Senna tablets. The co-crystals of lactose and MCC improved the dissolution and disintegration characteristics of Senna extract tablets. It can be pointed that the co-crystallization technique reduces the disintegration time of the tablets and also enhances the dissolution properties (K. Pundarikakshudu, 2005) [19]. Iso-propyl alcohol was used as the binding (granulating) agent, in a small quantity, due to its volatile nature. During the mixing of all the tablet ingredients, iso-propyl alcohol was slowly incorporated drop wise into the mortar. The grinding action of the pestle led to the evaporation of iso-propyl alcohol, thus binding the whole ingredients into a wet mass i.e. granules. Tableting/ punching process was followed. Talc was used as polishing agent and was applied to the face of the punches in order to get the expected polish on the tablet surface.

The dissolution study was done for 4 batches of Senna tablets. The % cumulative release at 90 minute time interval of 50%, 70%, 80% and 100% ext. tablets was found to be 50.320%, 92.901%, 85.582% and 47.908% respectively. This revealed that the 70% tablet batch resulted in the highest amount of dissolution value amongst other tablet batches. Disintegration tests revealed that the disintegration time of Senna extract tablets was less than six minutes in an average, this showed that the prepared Senna tablets were in- line with the modern conventional tablets.

Other parameters of tablet evaluation confirmed that the formulated tablets were within the prescribed limits of the official standards. Thus, the Senna extract tablets can be seen in the form of modern dosage form and standardized tablets. Last but not the least it can be said that this work incorporated and achieved all the requirements needed for entitling Senna tablets at par with the standards and requirements of modern dosage formulations.

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