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EFFECTS OF SEED PRIMING ON SENNOSIDE CONTENTS IN *INVITRO* GROWN LEAVES OF *SENNA ALEXANDRINA* MILL

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ABSTRACT

Tinnevelly senna, popularly known as *Senna alexandrina* Mill, is a valuable plant drug in both the Ayurvedic and modern systems of medicine. Leaves and pods are used as natural laxatives. This plant's leaves contain sennosides A, B, C, and D, which are in high demand globally and are preferred as an ingredient in herbal tea in Asia. Sennoside is used as the most reliable and safest laxative agent. Pods and leaves of Senna plants are also used in the form of decoctions, powders, and many other herbal preparations. Senna plant is raised from seed and has a hard and tough seed coat. The sennoside contents varies among the plant parts. Poor germination of seeds affects its mass cultivation commercially. Therefore, in the present study seed priming was attempted using H_2SO_4, HCL, GA_3, BAP to induce fast germination in *in vitro* using MS Basal media. Similarly, sennoside being the important compound of this plant hence analysed in different stages of the growth using the leaf sample. The anatomical, histochemical and micromorphological features of Senna invitro leaves were evaluated to confirm the morphological integrity. The present study also examined the effects of different seed priming on *in-vitro* seed germination, seedling development and sennoside contents in different growing stages (7th day, 14th day, 21st day and 30th days) from *invitro* leaves using RP-HPLC. The priming of Senna seeds found to be effective in breaking seed dormancy and the seeds primed in GA_3 5.77 X 10^{-6} M showed the highest germination (98%) compared to all other priming treatments. Glandular and non-glandular trichomes were observed on both epidermal surfaces, although in greater number on the abaxial surface. Leaves were amphiestomatic with a single palisade layer and a slightly smaller spongy parenchyma. A comparison of all priming treatments shows no significant changes in anatomical and morphological characteristics. RP-HPLC quantification of sennoside found the highest levels of sennoside in 21st day old invitro-raised GA_3 primed leaves at 22.69 mg/g. in *Senna alexandrina*, seed priming have improved the plant growth and secondary metabolite content along with germination and seed emergence.

KEYWORD: Laxative, HPLC, Gibberellic acid.

1.0 INTRODUCTION

The *Senna alexandrina* Mill. belongs to Leguminosae family includes the genus Cassia, which has more than 500 species of flowering plants.[1] Due to the increasing demand for medicinal plants in the drug industry and there is a need to produce herbal crops in a rational manner, the study of seed germination of medicinal plant species has received special attention.[2] *Cassia angustifolia* vahl is an important herb used greatly for medicinal purposes.[3] The pods generally contain six seed and oblong in shape, measured about 2 inches long by 7/8 inch wide[4] they're extended by about a third of an inch and have four to five pairs of small pink blossoms.[5] It was prescribed by an Arabic physician to treat congestion.[6] The herb reaches a height of between two and three feet.[7] In India, Tamil Nadu, Andhra Pradesh, and Karnataka are the three states that produce this herb majorly. Gujarat (Anand), Rajasthan (Jodhpur), Maharashtra (Pune district), and Tamil Nadu are among the states where it is grown for commercial purposes. This plant is most commonly shipped to the United States, Germany, and Japan. Countries that import senna in addition to the United States include Spain, France, and China. Senna exports are the primary source of India's senna exports.[8] Senna is currently grown on around 6,000 hectares of land spread across India and the southern districts of Tamil Nadu, where it is the most popular crop for commercial purposes.[2] Additionally, Gujarat and Rajasthan are emerging as prospective senna producers in India. A number of compounds found in

Senna's leaves and pods are used in the manufacture of laxatives and purgatives across the world. As the most dependable and safest laxative medication, the medicine is frequently prescribed to patients. An intact, viable seed that fails to germinate under favourable conditions can be classified as a seed that is dormant. Seed dormancy can be influenced by a variety of factors, including light and temperature, as well as the length of time seeds are stored.[9] Scarification, pretreatment with plant growth regulators (PGRs), and temperature shocks can be used to break dormancy depending on the plant species and kind of dormancy.[10],[11] The favourable effects of growth regulators and chemicals on breaking the seed dormancy in medicinal plants by applying PGRs externally to seeds[12]. Sandpaper was also be used for mechanical scarification.[13] Seed dormancy is the primary impediment to the proliferation of plant species. The most prevalent reason for a delay in seed germination is a blockage in the seed's water supply. In *Cassia Sp.,* seeds are thick, preventing oxygen and water from reaching and activating the embryo, causing dormancy. There is a need to remove germination-inhibitors from plants to break dormancy. A dormancy mechanism prevents seed germination in inappropriate ecological settings when the likelihood of seedling survival is very low.[14] The plant's long gestation time and cautious field management make it difficult to grow commercially.[15] Seed priming is a widely used low-cost pre-sowing strategy for improving germination[16] and the same has showed application in different crop improvement programme by altering the metabolism of the plants. The demand for the natural product compounds are increasing which seeks the alternative methods of production of the same for commercial use, *in vitro* culture has become feasible tool to produce these compounds in large scale. Similarly, *in vitro* micropropagation improves the production of bioactive metabolites in medicinal herbs, and secondary metabolite synthesis.[17] The main purgative constituents present in senna leaves are anthraquinone derivatives and their glucosides. The laxative effects are due to the presence of sennosides A and B, both anthraquinone glycosides.[18] In lieu of the above, in the present study *S. alexandrina* seed germination and *in-vitro* seedling growth and quantification of sennosides were the primary goals, which was conducted in order to determine the best conditions for these processes. There was a shortage of research on seed germination for these species, which included both conventional and modern methods. Therefore, in this study, the seed priming using different chemicals (H_2SO_4, HCL) and growth hormones (GA_3, BAP) are used for improving the seed germination efficiency under *in vitro* conditions and their influence on Sennoside contents were evaluated.

2.0 MATERIALS AND METHODS

2.1 Collection of *S. alexandrina* Mill. Seeds

Matured pods were collected during 2023 on February to May from Department of biotechnology, Bharathiar university. Coimbatore. Tamil Nadu, India. Seeds were complete dry seed were chosen for priming studies.

2.2 Seed Surface Sterilization

Seeds were washed under running tap water for 30 min to remove adherent particles and then kept in 1% (w/v) bavistin (Carbendazim Powder) a broad-spectrum fungicide, for 25–30 min followed by thorough washing with Tween - 20 solutions, a liquid detergent, by gentle shaking for 15 min. Further washing was done under laminar air flow hood with sterile double distilled water (DDW) followed by a short treatment of 40 s with 70%(v/v) ethanol. Seeds were surface sterilized with freshly prepared aqueous solution of 0.1% Sodium hypochlorite for 5–6 min and then finally rinsed 4–5 times with sterile DDW to remove excess of sterilant. Sterilized seeds were then used for priming treatments.

2.3 Seed Priming

The following priming components were used as a priming agents along with the control and designated as below

i) T1: Unprimed seeds (Control),
ii) T2: Hot water priming for 15 mints,
iii) T3: 1N Sulphuric acid in water primed for overnight,
iv) T4: 1N Hydrochloric acid in water primed for overnight.
v) T5: Gibberellic acid (1.44×10^{-6} M; 2.89×10^{-6} M; 4.33×10^{-6} M; 5.77×10^{-6} M; 7.21×10^{-6} M) in water primed for overnight.
vi) T6:6-Benzylaminopurine (2.21×10^{-6} M; 4.43×10^{-6} M; 6.65×10^{-6} M; 8.87×10^{-6}M; 11.09×10^{-6} M;) in water primed for overnight.

2.4 Seed Culture and seedling development

Murashige and Skoog (MS) basal medium were used for seed germination. The MS basal medium was added with (3%) sucrose and media were solidified with Agar (0.8%). The pH was adjusted between 5. 6 to 5. 8 before autoclaving at 121°C for 20 minutes. Seeds were washed three times with sterile water after the seed priming was complete the seeds were and inoculated into the fresh half strength Murashige and Skoog (MS) basal medium. Seed germination was carried for 30 days with five replicates for each treatment. The initiation and rate of seed germination was recorded in every 7days in total 30 days of incubation period. The entire experiment was performed in aseptic condition under laminar air flow hood to prevent contamination. The culture tubes inoculated with seeds in MS basal media were incubated at 25±2 °C for 16 hours photoperiod under a 50 $\mu M/m^2$ s intensity under cool white fluorescent lamps (Philips, New Delhi, India).

2.5 Anatomical and morphological characteristics of *leaves* of in vitro grown plants

The invitro raised leaf were used for anatomical studies. Leaf sections were taken from the mid portions of fully developed leaves. The leaves were hand sectioned using

a razor blade and stained with safranin for observation of cell inclusions.[19] The stained sections were mounted in glycerol on microscopic slides and observed under a BX51 light microscope. Images of the sections were photographed using a ProgRes3 camera attached to Olympus BX51 light and fluorescent microscope.

2.6 Sample preparation for Sennoside quantification

Senna leaves from different stages of growth 7th, 14th, 21st and 30th day old *in vitro* raised leaves was collected and shade-dried. They were then fine powdered using a laboratory mill and filtered (20–40 mesh).

2.7 Extraction method

The powdered senna leaves (1.0 g) from each priming experiment were weighed into a volumetric flask, and HPLC grade methanol (10 mL) was used for extraction. The flask was placed in an ultrasonic bath at room temperature (24± 2 ℃) for 30 min. The solutions were filtered through a membrane filter (0.45 µm) into vials made of glass. An aliquot of 20 µL was injected twice into the HPLC system for analysis. The standard sennoside B compounds were used to prepare the standard solutions at a concentration of 1.0 mg/mL in methanol and were used for calibration. The samples were stored at 4 ℃ before use.

2.9 Quantification of sennosides B.

Sennosides were detected using a photodiode array detector (PDA) fitted to a Waters 2998 high-performance liquid chromatography (Waters, Milford, MA) system. HPLC was employed for sample analysis, and Waters Empower2 software was used for data processing. Separation was achieved using a Symmetry® C18 column (4.6 mm × 250 mm, 5.0 cm). The gradient mobile phase consisted of methanol, water, and acetic acid (80:20:0.1; pH 4.0). The gradient mobile phases were prepared in closed solvent bottles, degassed, and sonicated for 60 min each. Sennosides B showed the best peak resolution at a lower retention time with a flow rate of 1.0 mL/min, maximum absorption wavelength of 280 nm and a temperature of 40°C. We compared the retention times of sennosides B in the samples with the sennoside standards. By comparing the standard area to the sample area, the amounts of sennosides B in each sample was calculated. Standard and sample injections were performed in triplicate. The quantification was performed using the following formula:

$$Concentration\ of\ the\ sample\ (\mu g/ml) = \frac{Peak\ area\ of\ the\ sample}{Peak\ area\ of\ the\ standard} \times 1000$$

3.0 RESULTS AND DISCUSSION

3.1 Priming and *Invitro* seed germination

The demand for sennoside is increasing as it is being the one of the prime drug molecules used as a effective laxative compound in different formulas in allopathy medicine. Sennoside still being isolated from the Senna species, among the senna species, *S. alexandrina* is consider as a major source of sennoside and they were commonly cultivated in and around India and leaves are exported for the purification of sennoside. Hence, any attempt to increase the production of Sennoside will be more useful for the industries to isolate the sennosides from the Senna (Sing et al., 2018). Seed dormancy is the major constraint in the Senna species which limits the cultivation irrespective of all climatic changes. Seed priming is the recent hit in the crop improvement programme where different priming agents are used to increase the germination uniformity in many economically important plants. Apart from their influence on germination, the priming also influences the metabolism of the plant metabolites. In the present study also, the primed seeds showed high percentage of germination and similarly an increase in accumulation of sennosides also recorded. Among the treatment viz., T1: Unprimed seeds (Control), T2: Hot water priming in 15 mints, T3: 1N Sulphuric acid in water scarification for overnight, T4: 1N Hydrochloric acid in water scarification for overnight., T5: Gibberellic acid: (1.44 x10^{-6} M; 2.89 x10^{-6} M; 4.33 x 10^{-6} M; 5.77 x 10^{-6} M; 7.21 x 10^{-6} M) in water primed for overnight. T6:6-Benzylaminopurine (2.21x10^{-6} M; 4.43x10^{-6} M; 6.65x10^{-6} M; 8.87x10^{-6}M; 11.09x10^{-6} M;) in water primed for overnight. It has been shown that GA_3 5.77x10^{-6} M concentration has led to maximum seedling length, frequency of germination, speed of germination, germination vigor index, fresh weight (g), and dried weight (g) when compared to non-primed treatments. (Figure. 2). Similarly next to GA_3 the seeds primed with BAP 11.09x10^{-6} M concentration showed the maximum length of the seedlings and the highest germination rate 86%. The primed treatments of 1N H_2SO_4 (27%) and 1N HCL (23%) showed average germination rates and slow germination speed. The germination of seeds was lowest with the hot water primed treatment (19%). Earlier mechanical injury of the seed coat or chemical treatment has been used for breaking the seed dormancy of certain cultivated medicinal plants *Gloriosa superb*, *Echinacea purpurea*, *Atropa belladonna,*[9][20][8] The present study is first in *S. alexandrina* attempted to compare different priming on seed germination. Sulfuric acid and hot water priming treatment have been reported that to improve the 96% of seed germination and seedlings growth of *Cassia fistula.*[21] In contrast, the present study shows treatment of 1N Sulfuric acid (27%), 1N HCL (23%) and hot water (19%) priming treatments lowered germination percentage compared to the hormones priming GA_3 (95%) and BAP (86%). Primary exogenous dormancy due to physical factors present outside the embryo is present in most of the Fabaceae plants in Senna seeds mechanical dormancy is reported to break the dormancy this may leads to damage of the seed. Seed coats are too hard to allow the embryo to expand during germination. The outer macrosclereid and mucilaginous cell layer becomes impermeable to water a hardened endocarp are the reasons that make seed coats impermeable to water. Such seed coats develop during the last stages of seed development. Whereas in *S. alexandrina*, hormones break the seed layers which are impermeable and helps in germination. *S. alexandrina* seed when primed with

different concentrations of GA_3 (1.44 x10^{-6} M; 2.89 x10^{-6} M; 4.33 x 10^{-6} M; 5.77 x 10^{-6} M; 7.21 x 10^{-6} M) and BAP (2.21x10^{-6} M; 4.43x10^{-6} M; 6.65x10^{-6} M; 8.87x10^{-6}M; 11.09x10^{-6} M;) for overnight at 24±2°C. Seeds primed with GA_3 5.77 x 10^{-6} M concentration for overnight were found to be effective with 95% seed germination.

GA_3 a growth promoting hormone acts as stimulant for embryos also confirmed in our findings in *S. alexandrina* as similar to results propounded by several researchers.[22],[23],[24] Seeds primed with 11.09x10^{-6} M BAP for 24 hrs showed higher germination percentage (86%). When the concentration of GA_3 and BAP is increased or decreased or if the priming period is increased, the germination percentage increases. Gibberellin is a signalling substance produced during seed germination.[25] Several studies have shown that exogenous GA_3 treatment aids seed germination and dormancy.[26] It was also shown by the seed analysis rules[27] that gibberellic acid could be used to break the dormancy of several species that are physiologically dormant.[28] Among the Poaceae family genus, *Avena sativa*, *Hordeum vulgare*, *Secale cereale* and *Triticum aestivum* have been shown to require dormancy-breaking through the use of gibberellic acid. This research hypothesized that gibberellic acid also acts as dormancy-breaking promoter in senna. In addition, soaking seeds in warm water has the effect of softening the seed coat and increasing the permeability of the seed coat. Therefore, warm water soaking at a suitable temperature is beneficial for seed germination.[29],[30] This study confirmed that priming with GA_3 5.77x10^{-6} M concentration are beneficial to the dormancy breaking and germination of *S. alexandrina* seeds. According to[31], the germination rate of seed over 80% occurred at 150 mg·L^{-1} GA_3 solution. Germination initiates a variety of metabolic activities inside seeds. Reactive oxygen species are produced by these metabolic processes, which can damage cells and tissues via oxidative stress.[32],[22] The different concentration of hormones priming was effective for breaking strong seed dormancy and impermeable seed coat which is the major cause low germination frequency in *S. alexandrina*. It is an effective, reliable and reproducible method to get high frequency seed germination in *S. alexandrina*, when compared to any other priming treatments, seeds damaging and breaking possibilities are very low at this hormone priming. Thus, this method can be adopted as a good alternative than the other treatments for higher percentage of seed germination since the aseptic seedlings were used as explants in large number of *in vitro* studies.

3.2 Microscopic studies

As a taxonomic consideration, epidermal characteristics are recognized as being important in identifying both genus and species levels.[5] The dorsiventral characters of *Senna alexandrina in vitro* leaves in the transverse section. There is no roundness or straightness to the dorsal side, but the ventral side is convex. In comparison with the lower epidermis, the upper epidermis has larger and more distinct cells. In shape, the bundle is hemispherical, single, small, collateral, and small in size. Thin cells form the phloem and a thick band forms the xylem. Between the vascular bundle and the lower epidermis lies the collenchyma, a small rounded cell layer. In the mesophyll tissues, there are palisade and spongy parenchymas. Piths are oval-shaped and located in the center (Figure 3). On a tertiary level, the veins aren't as prominent. As far as directions and dimensions are concerned, vein islets are polyhedral. There is a good deal of prominence and thickness to the vein islets. Across all priming treatments, no significant differences in anatomical or morphological characteristics have been observed.

3.3 Effect of priming on Sennoside content

Maceration was used as the extraction technique for extracting *invitro* leaves. Percentage of the extract recovery was determined for different stages of *invitro* leaves. With methanol as the extraction solvent, we were able to recover the most amount of extract yield: 14.28 mg/g on the 7th day, 13.46 mg/g on the 14th, 15.88 mg/g on the 21st, and 14.83 mg/g on the 30th day GA_3 5.77x10^{-6} M concentration primed seeds, compare to the other priming treatments (data not shown). Various chemical compositions of plant metabolites may have contributed to differences in extract yields in the present study. There are many compounds with different chemical properties that may or may not be soluble in a specific solvent. Therefore, the polarity of the extraction solvent plays a significant role in extraction efficiency and biological activity. Maceration was used in our study as an extraction technique, since it is the first step in discovering new drugs from plants.[33] It has been found that the extraction yields of herbal medicines are higher when they are optimized.[34]

3.4 High-performance liquid chromatography for Sennoside analysis

This study primarily focused on quantification of sennosides B, from different stages of invitro seedlings grown after different priming treatment. The sennosides contents of *in vitro*-grown leaves 7th day, 14th day, 21st day, 30th day showed Sennoside contents in varied concentrations. The standard chromatograms of sennosides B displayed peaks at 5.075 min, respectively, in all samples, with varying content. Various stages of senna leaves underwent HPLC analysis for sennosides from *in vitro* seedlings of senna. As indicated by the respective chromatograms, the content of SB significantly varied between the different stages of *S. alexandrina* (Figure 4). In previous studies, water or aqueous methanol (70%) have been commonly used as solvents for sennoside extraction.[35] Comparing GA_3 5.77x10^{-6} M concentration with the other priming treatments, it shows the highest germination percentage and leaves of the germinated plant showed maximum sennoside content than the other priming. The HPLC analysis of sennoside content 7th day SB 1.98 mg/g; 14th

day SB 7.33mg/g; 21st day SB 24.05mg/g; 30th day SB 15.47mg/g (Table.1). Similarly, according to[36], sennoside concentration in 90 days old field grown plants was maximum with a slight decline thereafter. According to our results, compared to previous reports, 21 days old *invitro* raised leaves of Senna showed the highest sennoside content, followed by 7th, 14th, and 30th day old leaves. According to[37], sun drying results in sennosides being lost due to daily temperature variations. The highest yield of sennoside B was recorded in summer harvested leaves which were significantly higher than the rest of the harvest dates.[15] In the present study the results showed that the GA_3 priming not only improved the seedling growth and also increases the sennoside content. Further studies are needed to confirm the same.

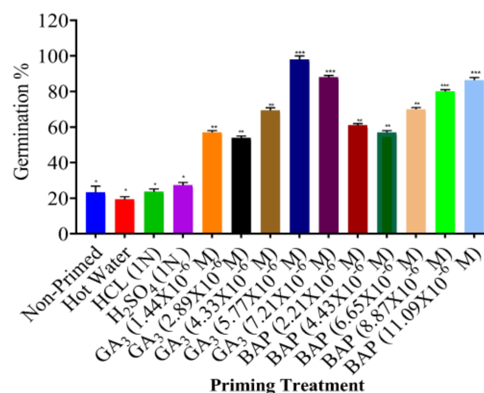


Figure 1: Effect of priming treatment on invitro seed emergence parameters of Senna alexandrina.

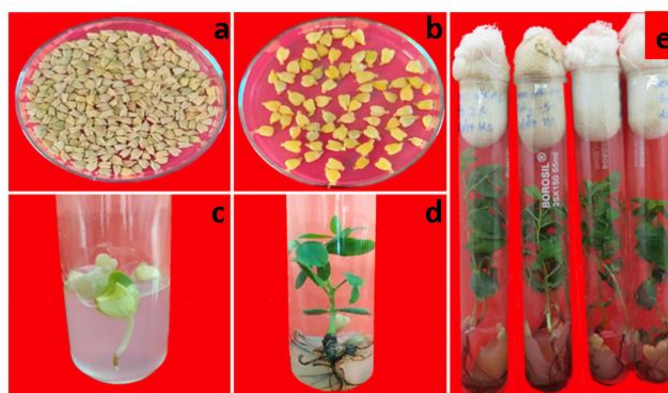


Figure 2: *In vitro* seed germination and seedling development of *Senna alexandrian* Mill. (a) Mature senna seed; (b) Scarification in Gibberellic acid 5.77×10^{-6} M conc. for overnight seeds after Sterilized seeds; (c)Seeds after 6th day of culture on (Half strength) MS medium containing 3% sucrose; (d) After 21 days of culture; (e) Showing shoot apex and Complete seedlings on 30th day.

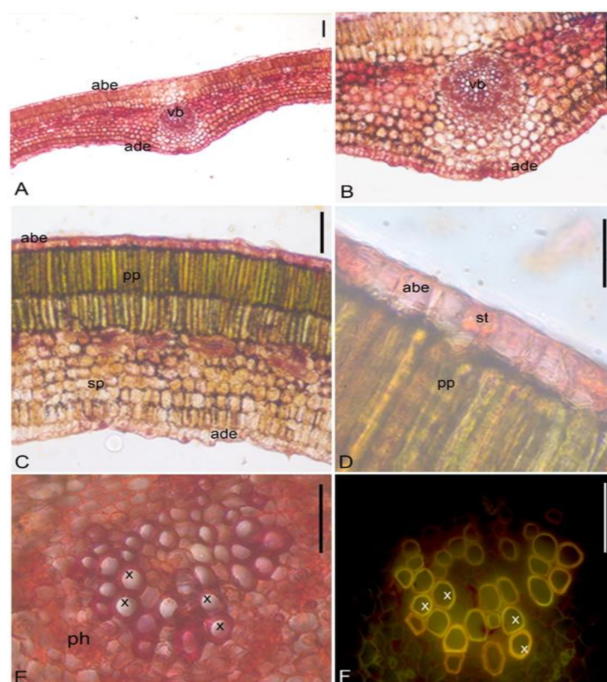
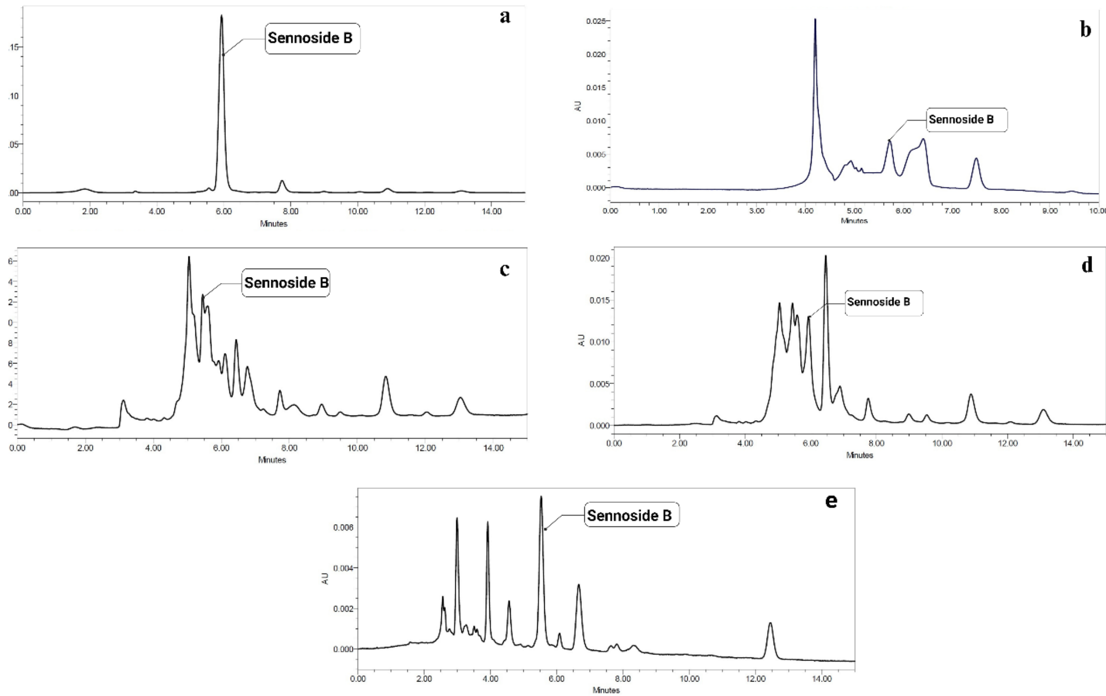


Figure 3: Anatomy of *Senna alexandrina invitro* leaf surface; (a,b) Abaxial epidermis, Adaxial epidermis surface of the leaf stomata and vascular bundle in *Senna alexandrina invitro* leaf surface; (c) Uniseriate upper and lower epidermis enclosed by cuticle, palisade parenchyma 3–4 layer and 5–6 layer of spongy parenchyma present in *Senna*

alexandrina invitro leaf. (d) Stomata with guard cells possessing on the straight epidermal cell wall in abaxial surface of *Senna alexandrina invitro* leaf. (e,f) *Senna alexandrina invitro* leaf midrib Well-developed structural xylem and phloem. Scale bar- 50um, PP- palisade parenchyma, sp- Sponge parenchyma, st- stomata, X- xylem, ph- phloem, Ade- adaxial epidermis, abe- abaxial epidermis, vb- vascular bundle.



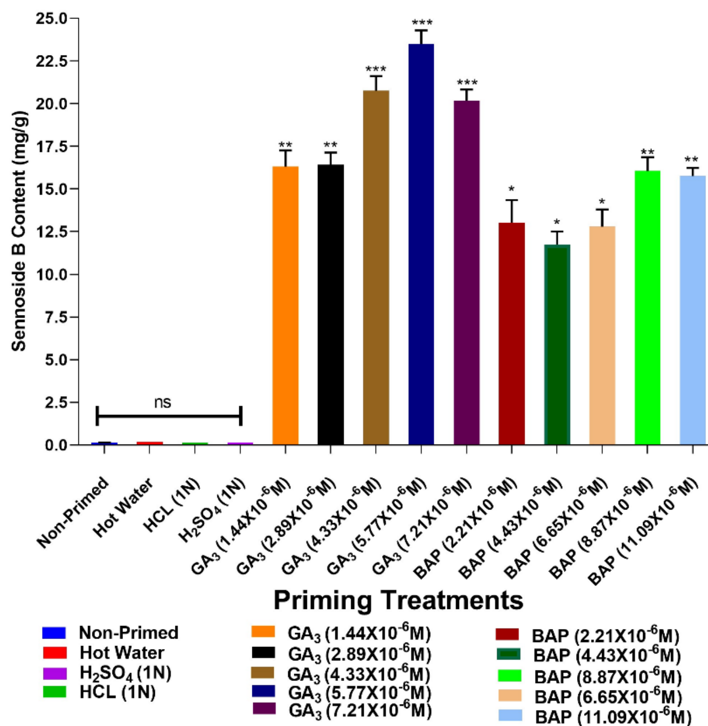


Figure. 4a. Quantitative determination of sennosides B in different stages of *invitro* leaf of *Senna alexandrina* using HPLC analysis. HPLC profile (RP-C18) of sennoside B in senna *invitro* leaf, with sennoside B (Rt: 5.122 minutes) standard at 280nm. chromatogram labels **a)** Sennoside B standard; (**b)** Methanolic extract of 7th day leaf. (**c)**

Methanolic extract of 14th day leaf. (**d**) Methanolic extract of 21st day leaf. (e) Methanolic extract of 30th day leaf. 4b. Bar graph represent 21st day old invitro leaves sennoside B RP-HPLC quantifications.

Table 1: HPLC Quantification sennoside B from different stages *invitro* leaves of *Senna alexandrina.*

Priming treatments	Quantification of Sennoside B (mg/g)			
	7th day	14th day	21st day	30th day
Non-priming	0.013±7.88^{i}	0.041±0.14^{h}	0.12±0.17^{i}	0.098±0.24^{h}
Hot water	0	0.024±0.23^{i}	0.186±0.68^{g}	0.163±0.47^{f}
1N HCL	0	0.011±0.21^{j}	0.131±0.25^{i}	0.088±0.21^{h}
1N H_2So_4	0	0.029±0.19^{i}	0.156±0.61^{h}	0.139±0.25^{g}
1.44x10^{-6} M GA_3	1.30 ±0.32^{d}	2.76 ±2.89^{g}	15.64 ±0.18^{d}	12.16±0.07^{e}
2.89x10^{-6} M GA_3	1.76±0.14^{c}	4.11 ±7.50^{d}	15.91±0.47^{d}	13.75 ±0.31^{d}
4.33x10^{-6} M GA_3	1.79 ± 6.29^{b}	4.85 ±2.50^{c}	20.14 ±0.12^{b}	14.33 ±0.29^{bc}
5.77x10^{-6} M GA_3	1.98± 2.77^{a}	7.33 ±0.18^{a}	24.05±0.16^{a}	15.47± 0.18^{a}
7.21x10^{-6} M GA_3	1.33±8.51^{d}	5.62±0.39^{b}	20.63 ±0.32^{b}	14.19 ±0.36^{c}
2.21x10^{-6} M BAP	0.28 ±6.12^{g}	2.29 ±0.41^{g}	12.10 ±0.45^{f}	11.57±0.0^{9}
4.43x10^{-6} M BAP	0.40 ±9.02^{f}	3.80 ±0.22^{f}	12.28 ±0.09^{f}	13.23 ±0.21^{de}
6.65x10^{-6} M BAP	0.19±0.21^{h}	4.24 ±0.24^{cd}	13.50 ±0.17^{e}	13.99 ±0.17^{d}
8.87x10^{-6} M BAP	1.04±0.26^{e}	5.89 ±0.15^{b}	16.62 ±0.03^{c}	14.85 ±0.14^{b}
11.09x10^{-6} M BAP	0.39±0.31^{f}	3.56 ±0.93^{f}	16.09±0.01^{c}	13.41± 0.02^{d}

Mean values of three independent experiments (N=3/replicate) with standard deviations and standard errors.

4.0 CONCLUSION

Understanding germination requirements of medicinal plant species is one of the most important steps in the survival of these species. Findings of our study revealed that seed dormancy of all medicinal species under study is caused by hard and water impermeable seed coat. A high level of germination was observed by using traditional scarification technique of seed coat and making it permeable to water though various methods. In the present study it was found that seeds primed with gibberellic acid 5.77x10^{-6} M concentration was effective in dormancy breaking in *S. alexandrina.* It was found that *invitro* leaf tissues have large intercellular air spaces and are generally unorganized and poorly differentiated cells. Acclimatizing the *invitro* plants resulted in complete differentiation of leaf tissues. According to HPLC analysis, Sennoside B content was maximum (24.05 mg/g) in 21 days old in vitro grown leaves of GA_3 primed seeds. It was revealed that seedling production of *S. alexandrina in in vitro* was most effective under *in vitro* culture conditions. In order to reproduce this plant in mass, farmers can adopt this priming method and similarly the *invitro* grown plants can also be used for the Sennoside purification as evident from our data.

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Author contribution

Gowtham Kumaraguru: Conceptualization, Methodology, Data curation, Writing – original draft. Malaiyandi Jayanthi: Data curation. Girija Shanmugam: Conceptualization, Supervision, Writing – review & editing.

Conflict of interests

The authors declare no conflict of interest.

5.0 REFERENCE

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