Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

COMPARATIVE QUALITATIVE AND QUANTITATIVE HPLC ANALYSIS OF SENNOSIDES IN TREATED AND CONTROL *SENNA ALEXANDRINA*

C. Raja Kumar
Assistant Professor, Department of Botany, Government Degree and PG College Jammikunta, Telangana, India

Abstract

The present study investigated the effect of microbial inoculation on the qualitative and quantitative accumulation of sennosides in *Senna alexandrina* using High-Performance Liquid Chromatography. A greenhouse experiment was conducted with four treatments: T1 (uninoculated control), T2 (*Azospirillum brasilense* inoculation), T3 (*Bacillus megaterium* inoculation), and T4 (co-inoculation with *A. brasilense* and *B. megaterium*). Seedlings were grown under controlled conditions for 90 days and leaf samples were collected for HPLC analysis. Crude aqueous extracts were prepared from dried leaf powder and analysed using an Agilent 1100 HP Series HPLC system equipped with a photodiode array detector. Separation was achieved on an NH2 column using acetonitrile: water (80:20, v/v) as the mobile phase, with UV detection at 210 nm. The HPLC profiles confirmed the presence of sennosides in all treatments, with minor variations in retention time reflecting changes in metabolite composition. Overall, microbial inoculation, particularly the combined application of nitrogen-fixing and phosphate-solubilizing bacteria, markedly enhanced sennoside biosynthesis in *Senna alexandrina*. These findings suggest that beneficial microbial inoculants can serve as an effective and sustainable strategy for improving the medicinal quality and phytochemical yield of *S. alexandrina*, thereby increasing its pharmaceutical value.

Keywords: *Senna alexandrina*, Sennosides, *Azospirillum brasilense*, *Bacillus megaterium,* Secondary metabolites, Microbial inoculation.

Introduction

Senna alexandrina Mill. (Fabaceae), commonly known as Alexandrian senna, is an important medicinal plant widely used for its natural laxative properties. Its therapeutic activity is mainly attributed to sennosides A and B, which are anthraquinone glycosides accumulated in the leaves and pods. These compounds are extensively used in pharmaceutical preparations for the treatment of constipation, making the enhancement of sennoside content an important objective in medicinal plant cultivation (Evans, 2009; Wang and Huang, 2005).

Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial microorganisms that improve plant growth, nutrient uptake and secondary metabolite production through biological nitrogen fixation, phosphate solubilization and phytohormone production (Vessey, 2003). Among them, *Azospirillum brasilense* acts as a nitrogen-fixing bacterium, while *Bacillus megaterium* is an efficient phosphate-solubilizing bacterium. Their combined inoculation has been reported to enhance plant growth and stimulate the biosynthesis of valuable phytochemicals in medicinal plants (Bashan and de-Bashan, 2010; Rodríguez and Fraga, 1999).

High-Performance Liquid Chromatography (HPLC) is a reliable analytical technique for the qualitative and quantitative estimation of plant secondary metabolites. It provides high sensitivity, accuracy and reproducibility for the separation and determination of sennosides A and B, making it the preferred method for quality evaluation and standardization of *Senna alexandrina* extracts (Wang and Huang, 2005).

The present study aimed to evaluate the effect of *Azospirillum brasilense* and *Bacillus megaterium*, individually and in combination, on the accumulation of sennosides A and B in *Senna alexandrina* leaves using HPLC analysis. The investigation provides insights into the potential of microbial inoculants as sustainable biofertilizers for improving the medicinal quality and phytochemical yield of this economically important medicinal plant (Pandey et al., 2017).

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

Materials and Methods:

Experimental design

The experiment was conducted in a greenhouse to determine how the *Senna alexandrina* plant responds to the inoculation of nitrogen fixer (*A. brasilense*) and PSB (*B. megaterium*). The experiment's treatments were broadly described as follows.

T1 - non-inoculated seedlings (control) treated with deionized water
T2 - Seedlings inoculated with A. brasilense (Nitrogen fixing bacteria)
T3 - Seedlings inoculated with B. megaterium (PSB)
T4 - Seedlings co-inoculated with A. brasilense and B. megaterium

Cultivation of plants

Senna alexandrina was cultivated under controlled conditions using polythene bags filled with sterile soil mixture of clay, silt and sand. The seeds were germinated under aseptic conditions and the seedlings were planted with six replications per treatment and supplemented with 20ml of A. brasilense and 20ml of B. megaterium, either single inoculum or coinoculum. Deionized water was used for periodic watering the plants. Seedlings were grown under controlled conditions for 90 days and leaf samples were collected for HPLC analysis.

Qualitative and Quantitative analysis of *Senna alexandrina* crude extracts (Sennosides) By HPLC method

Using a mortar and pestle, dried plant parts (leaves) from control and experimental plants on the 90th day were pulverized into a powder. In a 50 ml Erlenmeyer flask set on an orbital shaker set at 100 rpm for three days at room temperature, 500 ml of the dried material was extracted with 20 ml of water. Whatman No. 1 filter paper was then used to filter the crude extracts. The method used for the HPLC analysis was described by Wang and Huang, 2005.

Chemicals

Fine dried power of *Senna alexandrina* plant various parts (leaves - Crude extract),An amino (NH2) column (250 × 4.6mm) using acetonitrile for mobile phase, Acetic acid for pH adjustment and 70% Ethyl alcohol (w/w).

Apparatus

The diode array detector with greatest sensitivity (PDA), an injector and a quaternary pump comprised the Agilent 1100 HP Series HPLC system.

Sample preparation

The crude extract sample of 10mg was redissolved with 1 ml petroleum ether and analyzed by HPLC.

Senna crude extract

A stock solution of Senna (100 µg/ml) was prepared in deionized water and stored in a refrigerator at -20 ºC.

Chromatographic conditions

HPLC operating parameters included room temperature, an acetonitrile: water 80:20 (v/v) mobile phase, a 0.9 ml/min mobile phase flow rate, and UV detection at 210 nm.

Qualitative and Quantitative Analysis of Sennosides in Treated and Control Plants Using the HPLC Method

Measurement and comparison of Sennosides (A & B) levels in control and treated plant extracts at the 90th day of harvesting were done using HPLC analysis. In order to assess their impact on the production of sennoside in *Senna alexandrina*, the experiment included both solo and combined *Azospirillum* (Nitrogen-fixing bacteria) and Phosphate-Solubilizing Bacteria (PSB).

Results and Observations

HPLC Chromatogram of Sennosides (A & B) in Standard Sample:

The standard HPLC chromatogram of Sennosides was generated using reference Sennoside samples received from Sigma Aldrich in Germany. A 250 mg standard Sennoside sample was diluted in 5 ml of distilled water before HPLC analysis. The resultant chromatogram revealed well-defined peaks for Sennosides, with retention durations ranging from 5.743 to 8.271 minutes. The absence of interference or overlapping peaks verifies the analysis's purity and specificity (Fig.15). This standard chromatogram is used to compare the amount of Sennoside in treated and control plant extracts.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

Fig.1 HPLC Chromatogram of Standard Sennoside A

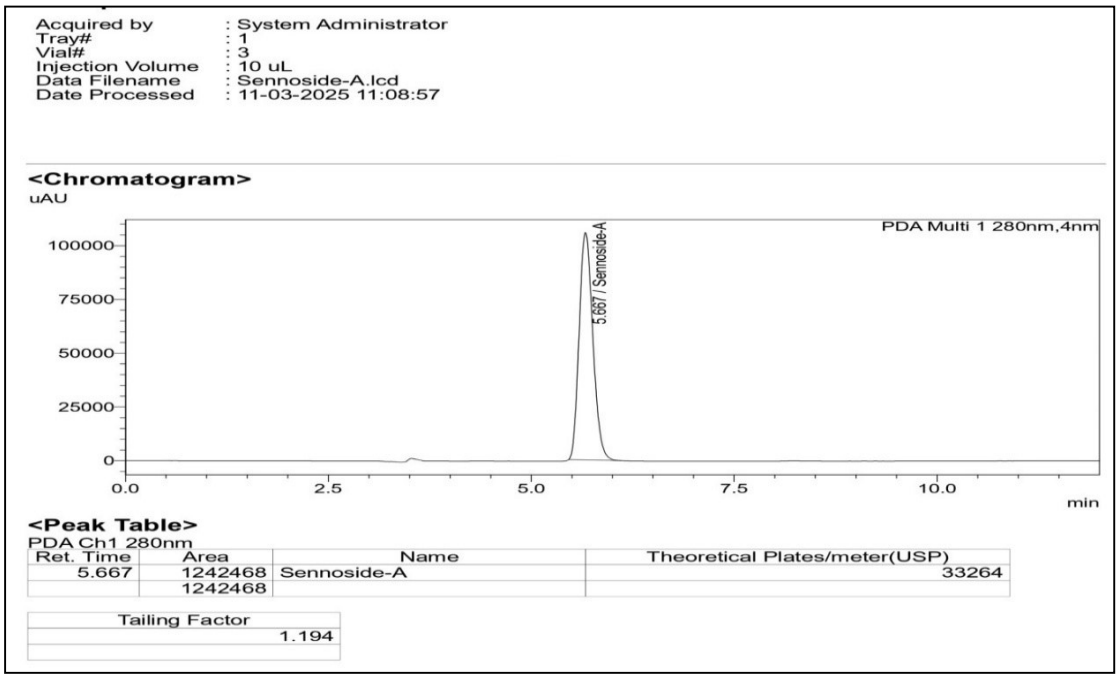
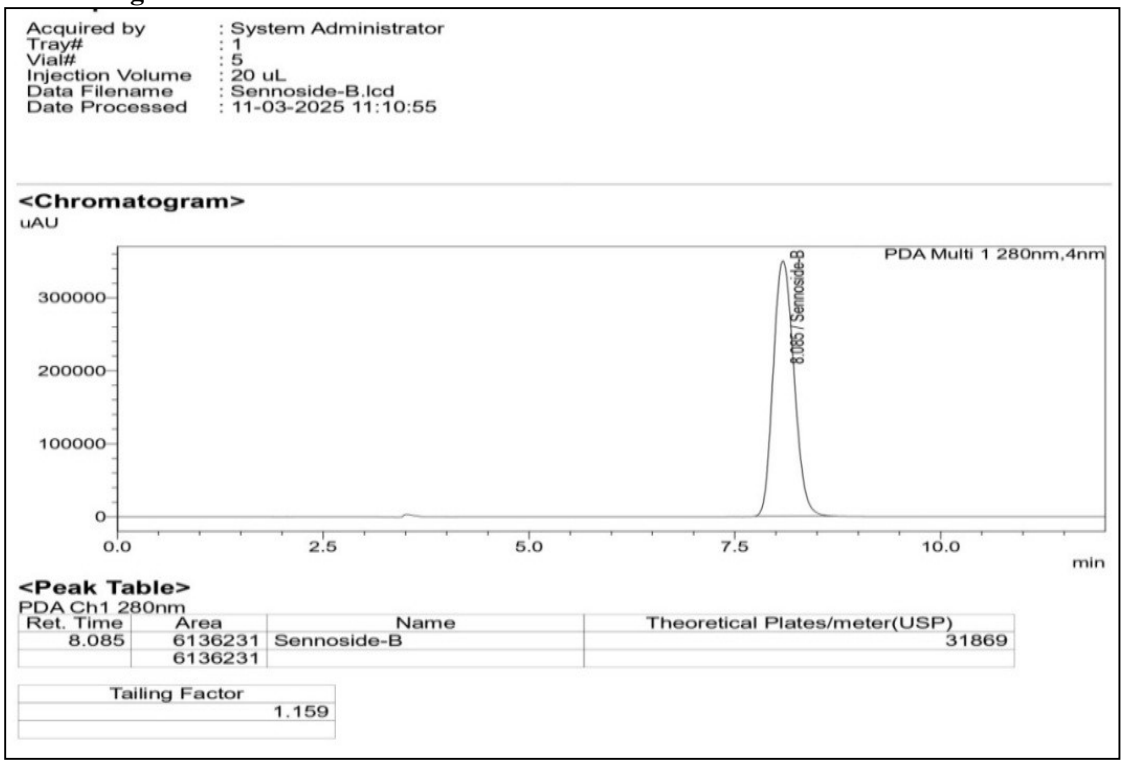


Fig.2 HPLC Chromatogram of Standard Sennoside B



Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

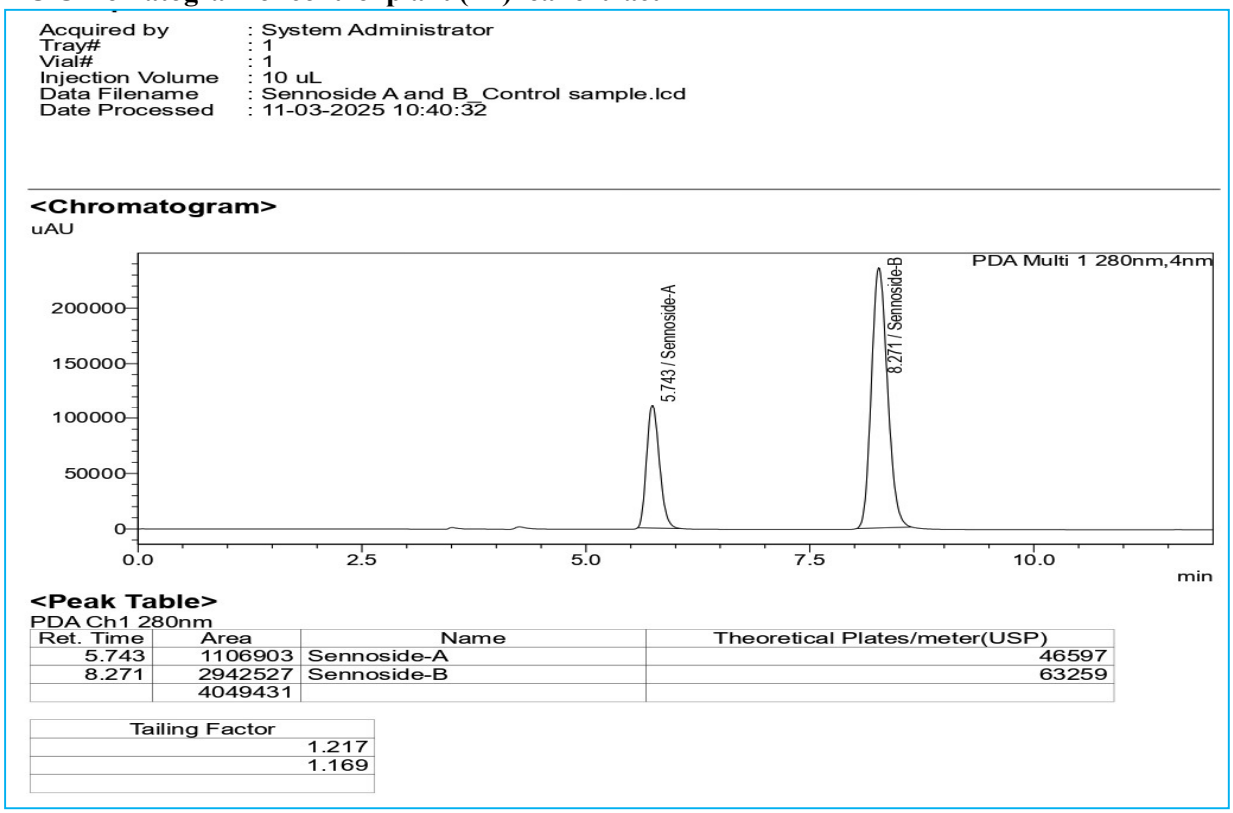
HPLC Chromatogram of Sennosides (A & B) in Control (T1) Plant Leaf Extracts:

The chromatographic examination of the T1 control plant leaf extract revealed separate peaks for Sennoside-A and Sennoside-B.

- **Sennoside-A** has retention duration of 5.677 minutes, with a peak area of 1,242,468, and a peak height of 33,264 µV.
- **Sennoside-B** has retention duration of 8.085 minutes, with a peak area of 6,136,231 and a peak height of 31,869 µV.

These data show that Sennosides are present in the control sample at baseline levels, allowing for comparison with treated samples.

Fig.3 HPLC Chromatogram of control plant (T1) leaf extract



HPLC Chromatogram of Sennosides (A & B) in Nitrogen Fixation Treated (T2) Plant Leaf Extract:

The Azospirillum inoculated (T2) plant leaf extract showed the following chromatographic characteristics:

- **Sennoside-A** has retention duration of 5.741 minutes, with a peak area of 1,108,522 and a peak height of 46,608 µV.
- **Sennoside-B** has a retention time of 8.264 minutes, with a peak area of 4,023,807 and a peak height of 63,426 µV.

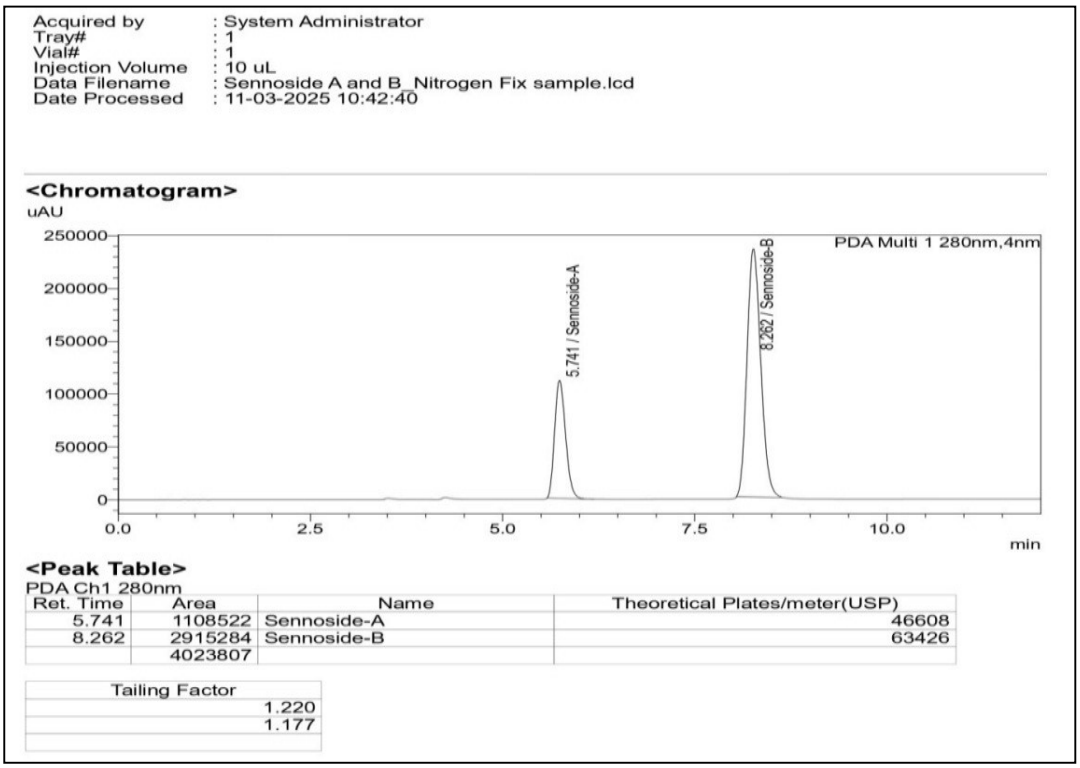
Compared to the control (T1) sample, the T2-treated sample showed a slightly shifted retention time for Sennoside-A and Sennoside-B, as well as a decrease in peak area for both compounds. This shows that nitrogen fixation treatment altered Sennoside biosynthesis or accumulation, perhaps causing metabolic changes in the plant.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

Fig.4 HPLC Chromatogram of Nitrogen fixation(T2) treated plant leaf extract. (Sennosides A&B)

Peak of Sennosides



<Peak Table>
PDA Ch1 280nm

Ret. Time	Area	Name	Theoretical Plates/meter(USP)
5.741	1108522	Sennoside-A	46608
8.262	2915284	Sennoside-B	63426
	4023807		

Tailing Factor
1.220
1.177

HPLC Chromatogram of Sennosides (A & B) in PSB Treated (T3) Plant Leaf Extract:

The following chromatographic features were displayed by the plant leaf extract treated with Phosphate-Solubilizing Bacteria (PSB) (T3):

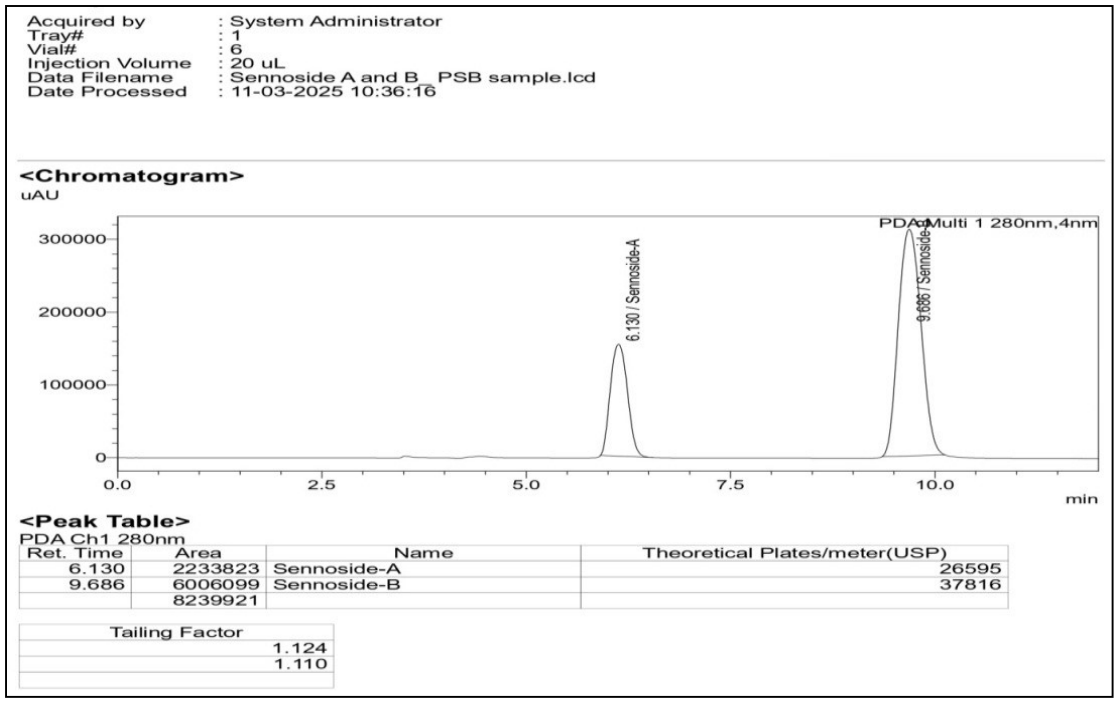
- **Sennoside-A:** peak height 26,595 µV, peak area 2,233,823, retention duration 6.130 min.
- **Sennoside-B:** peak height 37,816 µV, peak area 6,006,099, retention duration 9.686 min.

Sennoside-A and Sennoside-B peak areas in the PSB-treated sample (T3) were significantly higher than in the Nitrogen Fixation (T2) treatment, suggesting that PSB may have enhanced Sennoside biosynthesis. The somewhat longer retention period points to small changes in the way the compounds interact inside the column matrix.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

Fig.5 HPLC Chromatogram of PSB treated(T3) plant leaf extract. (Sennosides A&B)



HPLC Chromatogram of *Azospirillum*+PSB Treated (T4) Plant Leaf Extract: (Sennosides A&B)

The following chromatographic characteristics were shown by the double inoculation treatment (T4), which combined *Azospirillum* and PSB:

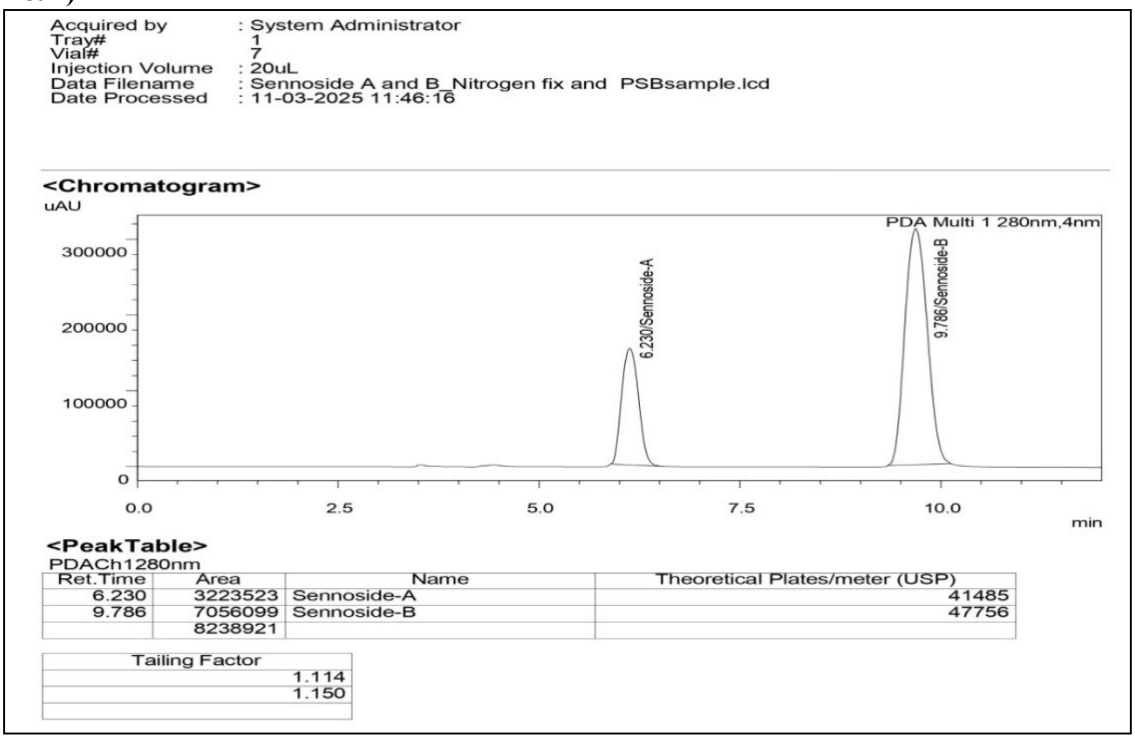
- **Sennoside-A:** peak height 41,485 µV, peak area 3,223,523, retention duration 6.230 min.
- **Sennoside-B:** peak height 47,756 µV, peak area 7,056,099, retention duration 9.786 min.

For both Sennoside-A and Sennoside-B, this treatment produced the largest peak area, suggesting that Azospirillum and PSB work in concert to promote the synthesis of Sennosides. The idea that simultaneous microbe inoculation promotes bioactive component accumulation in *Senna alexandrina* is further supported by the retention time shift and elevated peak intensity.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

Fig.6 HPLC Chromatogram of Nitrogen fixer + PSB treated (T4) plant leaf extract. (Sennosides A&B)



Comparative Perspectives:

- When compared to the Nitrogen Fixation (T2) treatment, the PSB (T3) treatment by itself considerably raised the Sennoside concentration.
- The highest Sennoside levels were seen in the *Azospirillum* + PSB (T4) treatment, indicating that the two microbial inoculants worked well together to promote the manufacture of secondary metabolites.
- The gradual rise in peak area from control **(T1) - T2 - T3 - T4** indicates a clear link between increased Sennoside production and microbial inoculation.

Treatment	RetentionTime (RT) (min)	Sennoside-A (Area, µV)	Sennoside-B (Area, µV)
Control (T1)	5.677 / 8.085	1,242,468	6,136,231
Nitrogen Fixation (T2)	5.741 / 8.264	1,108,522	4,023,807
PSB (T3)	6.130 / 9.686	2,233,823	6,006,099
Azospirillum + PSB (T4)	6.230 / 9.786	3,223,523	7,056,099

Discussion

The present study demonstrated that microbial inoculation significantly influenced the accumulation of sennosides A and B in *Senna alexandrina*, as confirmed by HPLC analysis. The chromatographic profiles revealed the presence of both sennosides in all treatments, while variations in peak areas indicated that the bacterial inoculants affected their biosynthesis and accumulation.

The control plants (T1) exhibited normal levels of sennosides, whereas *Azospirillum brasilense* inoculation (T2) resulted in lower peak areas for both Sennoside-A and Sennoside-B. Although *Azospirillum* is known to promote plant growth through

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

nitrogen fixation and phytohormone production, enhanced vegetative growth may reduce secondary metabolite accumulation because metabolic resources are preferentially utilized for primary growth (Bashan and de-Bashan, 2010). Plants inoculated with the phosphate-solubilizing bacterium *Bacillus megaterium* (T3) showed higher sennoside content than the nitrogen-fixing treatment, particularly for Sennoside-A. Increased phosphorus availability enhances ATP production, enzyme activity and metabolic processes associated with secondary metabolite biosynthesis, thereby promoting sennoside accumulation (Rodríguez and Fraga, 1999).

The combined inoculation of *A. brasilense* and *B. megaterium* (T4) produced the highest peak areas for both sennosides, indicating a synergistic effect of the two beneficial microorganisms. Improved nitrogen availability, phosphorus solubilization and phytohormone production may have enhanced nutrient utilization and stimulated the biosynthetic pathways responsible for sennoside production. Similar synergistic effects of PGPR on phytochemical accumulation have been reported in several medicinal plants (Pandey et al., 2017).

Slight differences in retention times among treatments may be attributed to variations in metabolite concentration or sample composition rather than changes in compound identity. The well-resolved chromatographic peaks confirmed the sensitivity and reliability of the HPLC method for qualitative and quantitative analysis of sennosides (Wang and Huang, 2005).

Overall, the study indicates that microbial inoculation positively influences secondary metabolite production in *S. alexandrina*. Among all treatments, co-inoculation with *Azospirillum brasilense* and *Bacillus megaterium* was the most effective in enhancing sennoside accumulation, suggesting that the combined use of nitrogen-fixing and phosphate-solubilizing bacteria is a promising and sustainable approach for improving the medicinal quality of this important herbal plant.

Conclusion

The present study demonstrated that microbial inoculation significantly influenced the production of sennosides A and B in *Senna alexandrina*, as confirmed by qualitative and quantitative HPLC analysis. Among the different treatments, co-inoculation with *Azospirillum brasilense* and *Bacillus megaterium* resulted in the highest accumulation of both sennosides, indicating a synergistic effect of nitrogen-fixing and phosphate-solubilizing bacteria on secondary metabolite biosynthesis. The phosphate-solubilizing bacterial treatment alone also enhanced sennoside production compared with the nitrogen-fixing treatment, highlighting the importance of phosphorus availability in phytochemical synthesis.

The HPLC method proved to be accurate, reliable and effective for the identification and quantification of sennosides A and B in *S. alexandrina* leaf extracts. Overall, the findings suggest that the combined application of beneficial microbial inoculants is an eco-friendly and sustainable approach for improving the medicinal quality and pharmaceutical value of *S. alexandrina*. This strategy may be adopted for the commercial cultivation of medicinal plants to enhance the yield of valuable bioactive compounds while reducing dependence on chemical fertilizers. Further field-based investigations and molecular studies are recommended to understand the mechanisms underlying microbial-mediated enhancement of sennoside biosynthesis.

Acknowledgements: The author expresses his gratitude to Head of the Department of Botany Professor T.Vijaya, Sri Venkateshwara University in Tirupati (AP) for providing the required facilities for this work.

Conflict of Interest: None

References:

1. Adesemoye, A.O., Torbert, H.A. and Kloepper, J.W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology*, 58, 921–929.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

2. Ahmad, F., Ahmad, I. and Khan, M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities. *Microbiological Research*, **163**, 173–181.
3. Bashan, Y. and de-Bashan, L.E. (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—A critical assessment. *Advances in Agronomy*, **108**, 77–136.
4. Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*, **28**, 1327–1350.
5. Bottini, R., Cassán, F. and Piccoli, P. (2004). Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Applied Microbiology and Biotechnology*, **65**, 497–503.
6. Cappuccino, J.G. and Sherman, N. (2014). *Microbiology: A Laboratory Manual*. 10th Edition. Pearson Education, New York.
7. Cassán, F., Vanderleyden, J. and Spaepen, S. (2014). Physiological and agronomical aspects of phytohormone production by *Azospirillum* spp. *Plant and Soil*, **378**, 1–15.
8. Dobbelaere, S., Vanderleyden, J. and Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences*, **22**, 107–149.
9. Egamberdieva, D. (2007). The effect of plant growth-promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology*, **36**, 184–189.
10. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, **4**, 177.
11. Evans, W.C. (2009). *Trease and Evans Pharmacognosy*. 16th Edition. Elsevier, London.
12. Glick, B.R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, **2012**, 963401.
13. Harborne, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edition. Chapman and Hall, London.
14. Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology*, **60**, 579–598.
15. Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice Hall of India, New Delhi.
16. Karthikeyan, B., Joe, M.M. and Islam, M.R. (2009). Biofertilizer influence on growth, biochemical changes and yield of medicinal plants: A review. *International Journal of Plant Production*, **3**, 139–150.
17. Kloepper, J.W., Lifshitz, R. and Zablotowicz, R.M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology*, **7**, 39–44.
18. Kumar, A., Maurya, B.R. and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat. *Biocatalysis and Agricultural Biotechnology*, **3**, 121–128.
19. Lucy, M., Reed, E. and Glick, B.R. (2004). Applications of free-living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek*, **86**, 1–25.
20. Maheshwari, D.K. (Ed.). (2011). *Bacteria in Agrobiology: Plant Nutrient Management*. Springer, Berlin.
21. Pandey, P., Irulappan, V., Bagavathiannan, M.V. and Senthil-Kumar, M. (2017). Plant growth-promoting rhizobacteria enhance growth and secondary metabolite production in medicinal plants. *Biocatalysis and Agricultural Biotechnology*, **12**, 156–165.
22. Patel, D.K., Kumar, R., Laloo, D. and Hemalatha, S. (2012). Natural medicines from plant source used for therapy of inflammatory bowel disease: An overview. *Journal of Ethnopharmacology*, **140**, 1–11.
23. Patten, C.L. and Glick, B.R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, **68**, 3795–3801.
24. Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*, **17**, 362–370.
25. Richardson, A.E., Barea, J.M., McNeill, A.M. and Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, **321**, 305–339.
26. Rodríguez, H. and Fraga, R. (1999). Phosphate-solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, **17**(4–5), 319–339.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :10.16(2026); IC VALUE:5.16; ISI VALUE:2.286
Peer-Peer Reviewed, Refereed & Open Access International Journal - As per UGC Norms
(Fulfilled Suggests Parameters of UGC by IJMER)
Volume:15, Issue:7(1), July 2026
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed: Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available: www.ijmer.in

27. Ryan, D., Robards, K., Prenzler, P.D. and Antolovich, M. (1999). Applications of HPLC in phytochemical analysis. *Analyst*, **124**, 1193–1204.
28. Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A. (2013). Phosphate-solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, **2**, 587.
29. Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–158.
30. Spaepen, S., Vanderleyden, J. and Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism–plant signaling. *FEMS Microbiology Reviews*, **31**, 425–448.
31. Taiz, L. and Zeiger, E. (2015). *Plant Physiology and Development*. 6th Edition. Sinauer Associates, Sunderland, MA.
32. Vance, C.P., Uhde-Stone, C. and Allan, D.L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a non-renewable resource. *New Phytologist*, **157**, 423–447.
33. Vanderleyden, J. and Spaepen, S. (2013). Plant growth promotion by rhizobacteria. *Advances in Botanical Research*, **60**, 205–238.
34. Verma, J.P., Yadav, J., Tiwari, K.N. and Kumar, A. (2010). Impact of plant growth-promoting rhizobacteria on crop production. *International Journal of Agricultural Research*, **5**, 954–983.
35. Vessey, J.K. (2003). Plant growth-promoting rhizobacteria as biofertilizers. *Plant and Soil*, **255**, 571–586.
36. Wang, C.C. and Huang, Y.L. (2005). Determination of sennosides in *Senna* species by high-performance liquid chromatography. *Journal of Chromatographic Science*, **43**, 201–206.
37. Wink, M. (2010). *Annual Plant Reviews, Volume 40: Functions and Biotechnology of Plant Secondary Metabolites*. Wiley-Blackwell, Oxford.
38. Zahir, Z.A., Arshad, M. and Frankenberger, W.T. Jr. (2004). Plant growth-promoting rhizobacteria: Applications and perspectives in agriculture. *Advances in Agronomy*, **81**, 97–168.