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UV-B Radiation effects on Phytochemical composition of *Senna auriculata* (L.) Roxb: an HPTLC Study

Selvakumar Murugan[1], Vinoth Kumar Raghunathan[2], Akila Nallusamy[3], Indira Rani Selvaraj[4], Murugesan Subbiah[5]and Shanthi Natarajan*[6]

[1]Research Scholar, PG and Research Department of Botany, Unit of Plant Stress Physiology, Pachaiyappa's College, Chennai-600030, Affiliated to University of Madras, Tamil Nadu, India.

[2&4]Assistant Professor, PG and Research Department of Botany, V.O. Chidambaram College, Tuticorin- 628008, Affiliated to Mononmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

[3]Assistant Professor, PG and Research Department of Zoology, Pachaiyappa's College, Chennai-600030, Affiliated to University of Madras, Tamil Nadu, India.

[5]Associate Professor, PG and Research Department of Botany, Pachaiyappa's College, Chennai, 600030, Affiliated to University of Madras, Tamil Nadu, India.

[6*]Assistant Professor, PG and Research Department of Botany, Unit of Plant Stress Physiology, Pachaiyappa's College, Chennai-600030, Affiliated to University of Madras, Tamil Nadu, India.

Corresponding Author: **Shanthi Natarajan**

E-mail: prithishanthi@gmail.com

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ABSTRACT

Objective: To investigate how UV-B radiation influences the phytochemical composition of *Senna auriculata*, utilizing High-Performance Thin-Layer Chromatography (HPTLC) to analyze and compare the ethanol extracts of untreated and UV-B-treated samples, thereby enhancing the understanding and standardization of the plant's medicinal properties.

Method: The study employs High-Performance Thin-Layer Chromatography (HPTLC) to analyze and compare the ethanol extracts of untreated and UV-B-treated *Senna auriculata* to investigate the influence of UV-B radiation on its phytochemical composition.

Results: The HPTLC analysis indicated that UV-B treatment results in qualitative and quantitative changes in the phytochemical composition of *Senna auriculata*, highlighting changes in the prominence and abundance of specific compounds.

Conclusion: The study concludes that UV-B radiation induces significant qualitative and quantitative changes in the phytochemical composition of *Senna auriculata*, enhancing its medicinal properties and emphasizing the importance of HPTLC in standardizing and assuring the quality of medicinal plants.

1.INTRODUCTION

Ultraviolet (UV) radiation is one of the abiotic variables that influence the concentration of secondary metabolites, thereby affecting the pharmacological qualities of medicinal plants. While UV-C is completely absorbed and scattered by ozone, UV-A and some UV-B light reach the Earth's surface. UV-B radiation, part of the ultraviolet spectrum with wavelengths between 280-320 nm, can significantly influence the growth, development, and secondary metabolite production in plants (1). UV-B radiation affects the photosynthetic rate by reducing leaf area and total canopy leaf area, modifying water loss through transpiration, and affecting leaf thickness in both medicinal and crop plants. Previous research has demonstrated that UV-B radiation can trigger secondary metabolic processes and increase the concentration of bioactive components in medicinal plants (2). Typically, UV-B exposure increases the concentration of secondary metabolites such as terpenoids, alkaloids, and phenolics (3).

In recent years, there has been increased attention from both consumers and academics on the health and nutritional benefits of metabolites such as phenolics, vitamins, and minerals. The biosynthesis of secondary metabolites is emerging as a novel approach for producing unique and valuable natural compounds. Medicinal plants, valued for their therapeutic properties due to the presence of various phytochemicals, often respond to UV-B exposure by altering their phytochemical profiles (4). Studies have shown that UV-B exposure can enhance the concentration of certain phytochemicals, potentially increasing the medicinal efficacy of the plants. Understanding these changes is crucial for optimizing the pharmacological qualities of medicinal plants and enhancing their therapeutic efficacy. Phytocompounds with high antioxidant potential, including phenols, flavonoids, steroids, alkaloids, terpenoids, anthocyanins, glycosides, ß-carotene, ascorbic acid, polyamines, synaptic esters, and volatile oils, are known to aid in treating oxidative stress-related disorders (5; 6). These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. UV-B radiation can induce the synthesis of these secondary metabolites as a part of the plant's defense mechanism against UV-induced stress. For instance, UV-B can stimulate the production of flavonoids, which serve as UV protectants by absorbing UV radiation and mitigating its harmful effects. Additionally, phenolic compounds, known for their antioxidant properties, often see an increase in production under UV-B stress in medicinal plants compared to crop plants. Increasing the stress dose can increase the concentration of desired secondary metabolites (7; 8; 9).

Senna auriculata, commonly known as Tanner's Cassia or Avaram, is one such medicinal plant that grows well under stress conditions and is commonly found along roadsides. It is rich in medicinal properties and contains bioactive compounds such as flavonoids, phenolic acids, alkaloids, and glycosides (10). These compounds contribute to its anti-diabetic, anti-inflammatory, antioxidant, and antimicrobial properties (10). UV-B radiation plays a significant role in influencing the phytochemical composition of *S. auriculata*. By enhancing the synthesis of key bioactive compounds such as flavonoids, phenolic acids, alkaloids, and glycosides, UV-B exposure can potentially increase the medicinal properties of this plant. Further research is needed to fully understand the mechanisms behind these changes and to optimize UV-B exposure in agricultural practices to maximize the therapeutic benefits of *Senna auriculata*.

The aim of this study is to explore the impact of UV-B radiation on the phytochemical composition of *Senna auriculata* to enhance its medicinal properties. To understand the relationship between UV-B radiation and phytochemical synthesis in *Senna auriculata*, utilizing High-Performance Thin-Layer Chromatography (HPTLC) to analyse ethanol extracts of both untreated and UV-B-treated samples. This analysis to identify and compare the distinct phytoconstituents, thereby providing insights for optimizing the cultivation of medicinal plants to maximize the production of valuable secondary metabolites and improve their therapeutic efficacy.

2.MATERIALS AND METHODS:

1.1 Plant materials

Certified seeds of *Senna auriculata* (L.) Roxb obtained commercial manufacture from Chennai. It was shown in experimental plots in the Pachaiyapppa's College Botanical Garden, Chennai. One set of plant was grown under ambient solar radiation and another set of plants grown under 10% UV-B enhanced solar radiation.

2.2. Plant growth and UV-B treatment

The seeds were soaked for 6 to 7 hours in running water. Separate soil beds were prepared for control (ambient) and UV-B treatment, and seeds were sown in the experimental plots. Four experimental plots were prepared. Two plots were used for ambient conditions, and the remaining two were used for UV-B treatment. In each plot, 20 seeds were sown. The plants were watered regularly, and care was taken to avoid microbial or pest infection during the experimental period. Plants at the first foliage leaf stage were used for UV-B treatment. UV-B treatment was given to these plants for 2 hours daily from 10 a.m. to 12 noon. Treatment was continued under ambient solar radiation and 10% UV-B enhanced solar

radiation supplemented by a Philips TL 40W/12 sunlamp (Gloelampenfabrieken, Holland). The first formed leaves were collected for HPTLC analysis.

2.3. Measurement of radiation

ALi-CorLi-188Bquantum/radio meter (Li-Cor.,Inc., USA) Here is the revised text with typographical errors corrected: A suitable photodetector was used to measure all the visible and photosynthetically active radiation. Radiation below 400 nm was determined using an IL700 radiometer with a SEE400 photodiode detector (International Light Inc., USA). This instrument was used to measure the quantity of UV-B radiation in the sunlight.

2.3.1 Extraction of sample

The dried and powdered materials (5 g) were extracted successively with 250 mL of ethanol using a Soxhlet extractor for 8 hours at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whatman filter paper (No. 1) and then concentrated in a vacuum at 40°C using a rotary evaporator. The residues obtained were stored in a freezer at -20°C until further tests.

2.3.2 High-Performance Thin-Layer Chromatography (HPTLC) Analysis

Sample Preparation for TLC:

Sample (1 g) was sonicated with 10 ml of ethanol for 15 minutes and filtered. This solution was used for TLC/HPTLC.

Thin-layer Chromatography Methodology:

Applied 10 µl Ethanol extract of SK control and SK treated on TLC plate using Camag'sATS4 applicator and developed by the mobile phase, Toluene: Ethyl acetate: Formic acid (4.5:1:0.5 v/v/v) up to 9 cm distance. After development, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm and then scanned using Camag's Scanner 4 at (D2 lamp/Absorption mode, Hg lamp/Fluorescent mode) fingerprint profiles of the extract were documented. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105oC till the development of colored spots. The plate was then photo-documented in white light and scanned at 520 nm for fingerprint profile.

2. RESULTS:

The chromatograms depicted in Fig.1 illustrate clear separation of all sample constituents without any tailing or diffuseness. High resolution and reproducible peaks were observed in the HPTLC analysis using a mobile phase composition of toluene: ethyl acetate: formic acid (4.5:1:0.5 v/v/v), with repeated results confirming their efficiency and accuracy.

Upon developing the ethanol extract of control *Senna auriculata* in the solvent system mentioned above, nineteen distinctive peaks were observed on the chromatograms when scanned at 520 nm (Figs.1&3). Analysis of a 10 µl portion of the ethanol extract revealed nineteen spots with corresponding *Rf* values of 0.14, 0.19, 0.21, 0.24, 0.32, 0.38, 0.42, 0.56, 0.64, 0.68, 0.84, 0.89, 0.95, 1.02, 1.08, 1.21, 1.24, and 1.32, as detailed in Table 1. The percentage area of these peaks ranged from 0.14 to 16.81% (Table 1).

Moreover, upon examination of the chromatogram and Table 1 data, it was evident that among the nineteen components, those with *Rf* values of 0.04, 0.56 (visible at 254 nm) and 0.64, 1.32 (visible at 366 nm) were more predominant, with percentage areas of 16.81%, 13.04%, 12.05%, 11.33%, 9.38%, 6.82%, 6.38%, and 5.39% respectively (Fig. 1; Table 1).
The remaining components were notably less abundant, with percentages below 4.68%.

The *Rf* values proved instrumental in standardizing the drug and providing a clearer understanding of its composition. The HPTLC analysis highlighted qualitative variations in the components of the extracts based on the number of peaks and their respective *Rf* values when scanned at 520 nm (Fig. 1). These distinct peaks, *Rf* values, and their areas collectively constitute the chemical profile of the ethanol extract of control *Senna auriculata*. Confirmation of the *Rf* values and sample purity was achieved by comparing absorption spectra at the middle and end portions of the bands (Table 1).

Table 1 Peak list and *Rf* value of the chromatogram of the ethanol extract of control *Senna auriculata*.

Peak	Start *Rf*	Start height	Max *Rf*	Max height	Max height%	End *Rf*	End height	Area	Area %	Assigned substance
1	0.01	11.4	0.04	92.5	4.78	0.07	25.4	2202.6	4.68	*Unknown
2	0.07	25.8	0.14	231.4	11.94	0.17	1.8	7909.2	16.81	*Unknown
3	0.17	0.9	0.19	103.3	5.33	0.20	1.1	844.1	1.79	*Unknown
4	0.20	2.2	0.21	11.3	0.58	0.22	0.7	63.9	0.14	*Unknown
5	0.22	0.1	0.24	23.7	1.22	0.27	0.1	302.3	0.64	*Unknown
6	0.30	0.1	0.32	12.5	0.65	0.34	0.3	134.3	0.29	*Unknown
7	0.34	0.4	0.38	217.0	11.20	0.40	22.5	3000.9	6.38	*Unknown
8	0.40	22.6	0.42	57.2	2.95	0.48	9.5	1224.4	2.60	*Unknown
9	0.48	9.6	0.56	58.4	3.02	0.62	23.9	3207.7	6.82	*Unknown
10	0.62	24.0	0.64	34.1	1.76	0.66	21.8	673.1	1.43	*Unknown
11	0.66	21.9	0.68	42.7	2.20	0.71	9.9	903.6	1.92	*Unknown
12	0.74	14.6	0.84	207.5	10.71	0.86	99.4	6135.1	13.04	*Unknown
13	0.87	100.9	0.89	194.3	10.03	0.93	77.7	5668.1	12.05	*Unknown
14	0.93	76.4	0.95	104.8	5.41	0.98	48.2	2537.7	5.39	*Unknown
15	0.98	48.5	1.02	163.3	8.43	1.05	91.4	4413.6	9.38	*Unknown
16	1.05	92.3	1.08	190.8	9.85	1.13	2.4	5330.3	11.33	*Unknown
17	1.18	0.2	1.21	23.4	1.21	1.23	10.2	454.6	0.97	*Unknown
18	1.24	10.5	1.24	11.1	0.57	1.26	0.3	127.9	0.27	*Unknown
19	1.29	1.0	1.32	158.3	8.17	1.33	28.9	1915.3	4.07	*Unknown

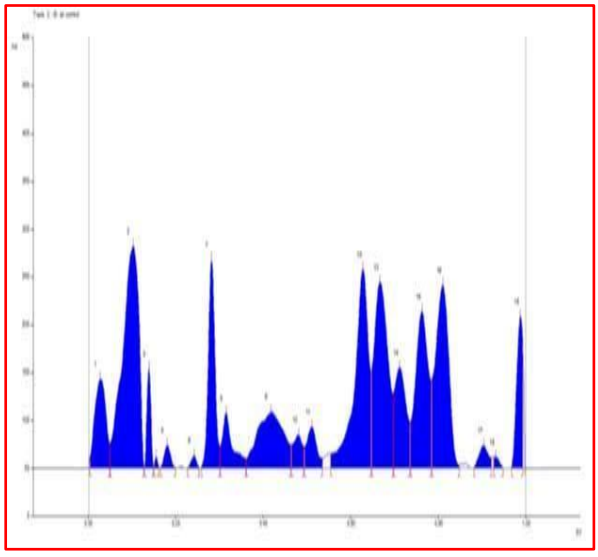
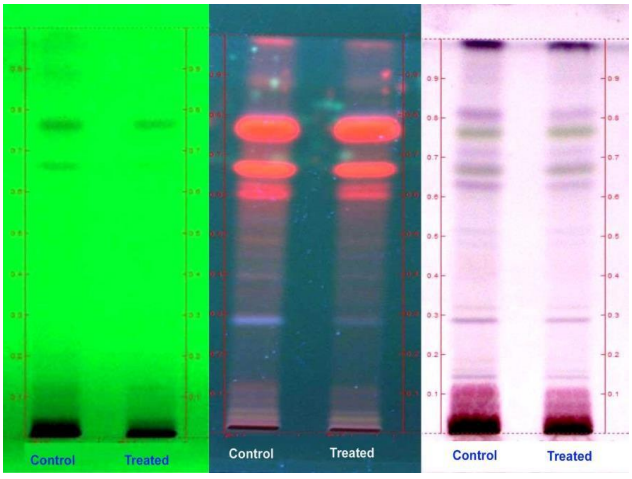


Fig.1 HPTLC Fingerprint of ethanol extract control *S.auriculata* scanned at 520 nm.



(a) (b) (c)

Fig.2a) HPTLC fluorescence image of ethanol extract of *S. auriculata* observed under short UV at 254 nm, Fig.2 b) HPTLC fluorescence image of ethanol extract of *S. auriculata* observed under long UV at 366 nm.

Fig.2 c) HPTLC white light image of ethanol extract of *S. auriculata* observed uunder white light after derivatisation at 525 nm

Table 2 Peak list and *Rf* value of the chromatogram of the ethanol extract of treated *Senna auriculata*.

Peak	Start *Rf*	Start height	Max *Rf*	Max height	Max height%	End *Rf*	End height	Area	Area %	Assigned substance
1	0.00	4.8	0.03	78.6	4.44	0.06	0.7	1784.5	4.33	*Unknown
2	0.07	0.9	0.13	189.0	10.66	0.16	1.2	4991.7	12.12	*Unknown
3	0.17	0.1	0.19	93.3	5.26	0.22	0.9	1119.8	2.72	*Unknown
4	0.22	0.5	0.24	15.3	0.86	0.26	0.3	182.6	0.44	*Unknown
5	0.35	0.1	0.38	172.3	9.75	0.40	12.1	2313.1	5.62	*Unknown
6	0.41	12.3	0.42	48.9	2.76	0.46	13.4	849.1	2.06	*Unknown
7	0.49	8.4	0.55	35.7	2.02	0.61	18.3	1931.0	4.69	*Unknown
8	0.62	18.6	0.64	31.7	1.79	0.66	23.8	709.3	1.72	*Unknown
9	0.66	24.0	0.68	37.5	2.12	0.71	12.5	867.5	2.11	*Unknown
10	0.71	12.6	0.84	145.9	8.23	0.86	75.2	5034.1	12.22	*Unknown
11	0.87	77.0	0.89	189.7	10.70	0.93	64.2	5414.8	13.15	*Unknown
12	0.94	64.9	0.95	78.7	4.44	0.98	38.0	1835.9	4.46	*Unknown
13	0.98	38.4	1.02	173.2	9.77	1.05	83.3	4286.8	11.72	*Unknown
14	1.05	84.1	1.08	132.4	7.47	1.13	0.0	3377.1	8.20	*Unknown
15	1.17	0.1	1.20	19.2	1.08	1.24	2.4	365.0	0.89	*Unknown
16	1.27	0.1	1.32	331.0	18.67	1.33	0.0	5588.7	13.57	*Unknown

The ethanol extract of treated *S. auriculata*, when developed using the solvent system toluene: ethylacetate: formic acid (4.5:1:0.5 v/v/v), revealed sixteen distinctive peaks on the chromatograms upon scanning at 520 nm (Fig.1). The HPTLC images presented in Figs.2 indicate clear separation of all constituents without any tailing or diffuseness.

The developed chromatogram of the ethanol extract of treated *S. auriculata*, scanned at 520 nm, displayed 16 spots at specific *Rf* values: 0.13, 0.19, 0.24, 0.38, 0.42, 0.55, 0.64,
0.68, 0.84, 0.89, 0.95, 1.02, 1.08, 1.20, and 1.32. The percentage areas of these peaks ranged from 0.44 to 13.57%, indicating the presence of at least sixteen different components (Table 2).

Further examination of Table 2 and the chromatogram in Fig.3 reveals that among the sixteen components, those with *Rf* values of 0.04, 0.64 (visible at 254 nm), and 1.02 (visible at 366 nm) were more predominant, with percentage areas of 13.57%, 13.15%, 12.22%,
12.12%, 8.20%, 5.62%, and 4.69% respectively (Fig.3; Table 2). The remaining components were found in significantly lower quantities, with percentages below 4.33%.

The chromatogram obtained through this specific solvent system, along with its precise *Rf*value, serves as a valuable tool for the standardization of extracts. HPTLC fingerprinting of a plant provides crucial insights into isolating, purifying, characterizing, and identifying chemical compounds unique to that species. This process significantly aids in accurately identifying and controlling the quality of a specific plant species. The present study furnishes ample data for the identification, standardization, and quality assurance of *S. auriculata*, a medicinal plant, while enhancing our understanding of the phytoconstituents present in its ethanol extract.

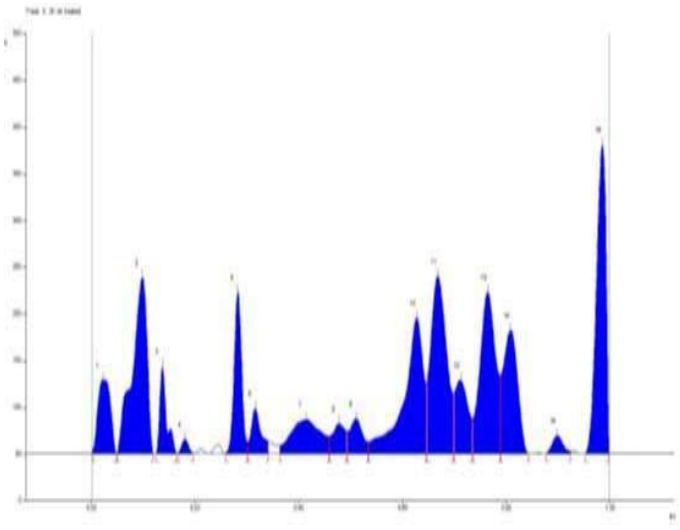


Fig.3 HPTLC Fingerprint of ethanol extract treated *S.auriculata* scanned at 520 nm.

3.DISCUSSION

UV-B radiation plays a significant role in modulating the phytochemical composition of *S. auriculata*. The exposure to UV-B can induce changes in the synthesis and accumulation of various bioactive compounds, enhancing the medicinal properties of this plant.The study presents a comprehensive analysis of the phytochemical composition of *Senna auriculata* focusing on its ethanol extract. Through a meticulous process involving High-Performance Thin-Layer Chromatography (HPTLC), the research identifies and quantifies various bioactive compounds present in both control and treated samples of *S. auriculata*.The clear separation of constituents without tailing or diffuseness indicates the reliability and precision of the analysis. Moreover, the identification of distinctive peaks with specific Retention Factor (*Rf*) values provides a standardized approach for assessing the composition of the extracts. A recent study revealed the existence of several phytoconstituents in *sennaauriculata* plants, which are responsible for the plants' therapeutic effect, was revealed by HPTLC fingerprint analysis. The existence of polyphenols in varying amounts was confirmed by the UV spectrophotometer evaluation of the phenol content, which may contributeto the chosen plant materials' anti-diabetic effect (11).

The comparison between control and treated samples reveals qualitative differences in the phytochemical composition, with variations in the number of peaks and their respective *Rf*values. This suggests that the UV-B treatment of *S. auriculata* may influence the concentration or presence of certain bioactive compounds. UV-B radiation is a significant abiotic elicitor that can induce stress reactions and increase the production of secondary metabolites in plants (12). Many studies have shown that UV-B treatment has a substantial impact on the accumulation of phenolic compounds, which are vital in protecting plants from potential damage caused by UV-B radiation penetration (12;13; 14). According to a prior study, UV-B radiation significantly sped up the accumulation of seven different chemicals in newly harvested *Lonicera japonica* Thunb flower buds (15). Similar research revealed that postharvest fresh leaves of *Ginkgo biloba* were exposed to UV-B radiation, which encouraged the production of secondary metabolites (16). According to a different study, UV-B radiation dramatically raised the amount of bioactive ingredients in fresh medicinal chrysanthemum flowers that were separated using different techniques (17).A number of phytochemical and pharmacological research have been done in the species of *Senna Mill*. To study the bioactive components and clinical activities (18;19), because the seeds of several species of *Senna Mill*. are utilized as an important raw medicine in many traditional formulations. Doshi et al. (20) observed High Performance Thin Layer. Chromatographic analysis of the extract indicated the presence of rutin in minute levels, demonstrating its antioxidant and anti-inflammatory activity.

Furthermore, the discussion emphasizes the significance of HPTLC fingerprinting in the standardization and quality assurance of medicinal plants like *S. auriculata*. By providing valuable data for the identification and characterization of phytoconstituents, HPTLC serves as a crucial tool in ensuring the consistency and efficacy of herbal remedies derived from these plants.

4. CONCLUSION

The results suggest qualitative variations in the phytoconstituents of *S. auriculata,* indicating potential differences induced by external factors such as UV-B radiation exposure. These findings underscore the significance of further research to elucidate the mechanisms behind these variations and optimize agricultural practices to enhance the therapeutic benefits of *S. auriculata*. Additionally, the study provides valuable insights into the chemical composition of *S. auriculata* ethanol extract, facilitating its identification, standardization, and quality assurance in medicinal applications.

5.CONFLICT OF INTEREST:

No conflict of interest was disclosed.

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