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In-Vitro Analysis of Bioactive Compounds from *Senna auriculata* (L.) Roxb. (Traditional Herb) for Wound Healing Applications

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Abstract

Medicinal plants are widely recognized as natural alternatives for wound healing due to lower toxicity, safety and biocompatibility. This current study examines the comparative biological activity of *Senna auriculata* leaves, flowers and roots for wound healing properties. The ethanolic extract of *Senna auriculata* plant parts were examined for phytochemical analysis, chromatographic profiling and assessed for antimicrobial, antioxidant, and anti-inflammatory activities. Qualitative phytochemical analysis confirmed the presence of tannins, alkaloids, saponins, flavonoids, phenols, glycosides, proteins, and root-specific quinone. The TLC study represented six different Rf values with bioactive compounds, and column chromatography purified seven different fractions of extracts. HPTLC analysis revealed major bioactive compounds, flavonoids and phenols. Quantitative phytochemical analysis revealed the highest levels of phenols and flavonoids in the root extract, followed by the flower extract. The flower and root extracts demonstrated antibacterial effects even at low concentrations. In DPPH and protein denaturation assays, the root extracts exhibited stronger activity compared to the flower extract. Overall findings support that root and flower extract contains the specific bioactive compounds, flavonoids and phenols, responsible for stronger biological activities in managing skin infections and emphasize its potential as a natural agent for wound healing properties.

Key words : Phytochemicals, Chromatography, Antibacterial, Anti-inflammatory, Antioxidant activity.

Herbal medicines are generally gaining popularity among people, as they are well-tolerated and do not cause any side effects. For a significant portion of the population, herbal medicine remains a primary healthcare resource even in areas where modern medical facilities are available. The World Health Organization (WHO) reports that 80% of people rely primarily on traditional remedies to address their basic healthcare

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needs[7]. Traditional practices form the backbone of medicinal plants, offering their essential support in the prevention and treatment of various ailments. These practices are followed in rural and tribal communities where conventional healthcare services are limited. Their use is largely attributed to their affordability, accessibility and perceived safety. In India, the rural population often depends on plant-based resources for day-to-day health care needs. Among the tribal communities, traditional healing systems are deeply rooted, and they are often used as a first line of defense against illness.

Wound healing is a highly complex biological process that restores the structure and function of damaged tissues. The wound healing process involves multiple stages, such as hemostasis, inflammation, proliferation, and tissue remodeling; these are regulated by cellular and molecular mechanisms in the body. For these wound healing processes, medicinal plants like *Senna auriculata* have been utilized for centuries in traditional medicine systems, as they contain a wide range of bioactive components that are responsible for the rapeutic properties. These predominant bioactive components enhance wound repair by promoting tissue regeneration, reducing inflammation & microbial infections. As a result, in wound healing management, plant-based remedies continue to offer promising alternatives for modern medicine[18].

The medicinal plants like *Senna auriculata* (L.) Roxb has had significant importance in human health care for centuries in the traditional systems of medicine, such as Ayurveda, Siddha, and Unani. *Senna auriculata* (L.) Roxb, commonly known as Tanner's Cassia/ Avaram, belonging to the family Fabaceae, is widely recognized in the traditional medicine systems across India and Southeast Asia for its pharmacological properties[14]. In recent days, a rise in antibiotic resistance has highlighted the scientific findings that medicinal plants like *S. auriculata* have become significantly important in the search for new therapeutic agents. The plant is known to have a wide range of biological properties such as anti-inflammatory, anti-oxidant, anti-microbial, anti-pyritic, anthelmintic, and anti-diabetic, Hepatoprotective, anti-hyperlipidemic, and anti-cancer activities. *S. auriculata* plant parts are rich in bioactive constituents such as flavonoids, alkaloids, tannins, saponins, glycosides, proteins, quinone and phenolic compounds responsible for diverse pharmacological activities[5].

Traditionally, various parts of *S. auriculata* have been used to manage specific ailments. The bark is valued for its effectiveness in hemorrhage conditions. The roots are employed in the management of asthma, skin diseases, leprosy, tumors, urinary disorders, and other bacterial infections. The leaves are noted for their properties against alcohol induced oxidative stress, and for the treatment of liver damage in relieving constipation, while the flowers are particularly known for their role in diabetes management. In addition, *S. auriculata* leaves and flowers have long been used in the treatment of skin diseases like eczema to acne, including conditions caused by *Staphylococcus aureus* and various dermatophytes, owing to its natural antibacterial and antifungal, and anticancer effects[13]. In the study, Common techniques used in the chemical analysis of *Senna* plant parts include

the extraction of bioactive compounds using suitable solvents such as ethanol, followed by qualitative and quantitative assessments using methods like thin-layer chromatography (TLC), column chromatography, High-Performance Thin Layer Chromatography (HPTLC), spectrophotometry, agar well diffusion, DPPH assay, and protein denaturation assay. The study focuses on evaluating the bioactive compounds and biological activities of different parts of *S. auriculata* to support its role in traditional healthcare practices[12].

Collection of plant materials :

The leaves, flowers, and roots of *Senna auriculata* were selected for the present study. The plant parts were collected from the Periyaneickanpalayam area, Coimbatore district, Tamil Nadu, India. The collected plant parts were thoroughly washed with tap water to remove dust and other impurities and then shade-dried at room temperature for 10-15 days. The dried plant parts were individually ground into a fine powder using an electronic blender, and the powdered material was stored in separate air-tight containers for further analysis.

Extraction of Senna auriculata constituents:

To isolate bioactive compounds, the powdered material of *Senna auriculata* (leaves, flowers, and roots) was then separately subjected to extraction using ethanol in a Soxhlet apparatus at room temperature. Each powdered plant material was placed in the extraction chamber of the Soxhlet, where continuous extraction occurred through the solvent reflux. As the chamber filled, solvent extract was automatically siphoned into the boiling flask. The cycle repeats until complete extraction takes place. The obtained extracts from each powdered plant material were concentrated by evaporating the solvent using a rotary vacuum evaporator and stored separately in air-tight containers under refrigeration for further analysis[10].

Qualitative phytochemical analysis :

In this study, standard phytochemical tests were employed to screen each ethanolic extract of *Senna auriculata* (Leaves, Flowers, and Roots) for the following phytoconstituents[1,19,21].

Test for tannin (Ferric chloride test):

The crude extract was treated with an equal volume of 1% ferric chloride solution. Brownish-green colour formation indicates the presence of tannin.

*Test for alkaloids (*Mayer's *test) :*

To 1 mL of crude extract, a few drops of Mayer's reagent are added. Formation of a turbid or creamy yellow precipitate indicates the presence of alkaloids.

Test for saponins (Froth formation test):

Distilled (5 mL) water was added to the dry powder of crude extract and shaken vigorously. The solution was allowed to stand for 10 min. The foam formation indicates the presence of saponins.

*Test for steroids (*Salkowski's *test) :*

To the crude extract, 2 mL of chloroform was mixed with concentrated sulphuric acid and acetic acid. The presence of steroids can be indicated by the appearance of a reddish

brown colour.

*Tests for terpenoids (*Libermann- Burchard *test):*

The crude extracts were treated with chloroform, followed by 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid, shaken, and allowed to stand. A change of purple or red colour indicates the presence of terpenoids.

*Test for glycosides: (*Keller- Killiani's *Test):*

A few drops of 10% acetate, water, and chloroform were added to each crude extract. The mixture was also evaporated to dryness in a water bath, and subsequently, a few drops of concentrated sulphuric acid were added. As a result, the appearance of the Brown ring indicates the presence of Glycosides.

*Test for carbohydrates (*Benedict's *test):*

To 5 mL of Benedict's reagent, 8 drops of the crude extract were added and thoroughly mixed. The mixture was then boiled vigorously for 2 minutes and allowed to cool. The formation of a red precipitate indicates the presence of carbohydrates.

Test for flavonoids (Lead acetate test) :

The crude extract was treated with a few drops of lead acetate solution. The formation of yellow precipitate indicates the presence of flavonoids.

Test for phenol (Ferric Chloride Test) :

To 2 mL of the crude extract, a few drops of 5% ferric chloride (FeCl*f*) solution were added. The development of a green colour indicates the presence of phenolic compounds.

Test for proteins (Ninhydrin test) :

A few drops of 2% ninhydrin solution were added to the crude extract and boiled for 1-2 min. The formation of a blue violet colour indicates the presence of amino acids and proteins.

Chromatographic analysis :

Thin-layer chromatography, column chromatography, and high-performance thin-layer chromatography analysis were performed using each extract of *S. auriculata* to identify the bioactive compounds.

Thin layer chromatography analysis :

Thin Layer Chromatography (TLC) analysis of each *Senna auriculata* extract was performed using standard methodology to identify the presence of individual phytochemical constituents[22]. In this analysis, the stationary phase contains the TLC plate (10 cm × 10 cm) coated with silica gel, while the mobile phase is hexane and ethyl acetate, used as a solvent system in a 9:1 ratio. A small amount of concentrated sample was carefully spotted on the baseline of the TLC plate using a fine capillary tube. The plate was then placed in a development chamber containing the mobile phase, and the solvent front allowed the compounds to separate based on their polarity. After the solvent front had moved an appropriate distance, the plate was removed and air-dried. The spots representing different compounds were visualized, and their relative movement was quantified using the Retention factor (Rf), calculated with the equation (1) below.

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent.} \quad (1)$$

Partial purification by column chromatography:

Column chromatography is a highly effective method for separating plant extracts into their pure components. In this method, each crude extract solution, called the mobile phase, is passed through a column packed with silica gel, which serves as the stationary phase. Compounds in the mixture travel at different rates depending on their interactions with the stationary phase. This process enables the purification of extracts by removing impurities[3]. The purified extracts are then collected as they elute from the column in separate fractions and subsequently analyzed using HPTLC.

High Performance Thin Layer Chromatography (HPTLC) analysis :

Each fractions of purified ethanolic extracts of *S. auriculata* were applied on silica gel 60 F_{254} precoated HPTLC plates using an applicator. Chromatographic separation was performed in a twin-trough chamber with hexane: ethyl acetate (9:1) as the mobile phase. Developed plates were air-dried, visualized under UV light at 365 nm, and densitometric scanning was performed to obtain fingerprint profiles of phytoconstituents[2,17].

Quantitative phytochemical analysis :

Total phenolic content :

The phenolic content present in each ethanolic extract of *Senna auriculata* plant parts (leaf, flower, and root) was determined by the spectrophotometer method. To 100 mg of each extract, 100 mL of distilled water was added. About 1 mL of the sample was treated with 0.5 mL of 2N Folin-Ciocalteu reagent and 1.5 mL of 20% Na_2CO_3. The volume of the solution was made up to 10 mL. The solution was shaken vigorously and allowed to stand for 2 hrs. The phenolic content was calculated using a standard calibration curve obtained from Gallic acid at the absorbance of 765 nm[15].

Estimation of flavonoids :

Total flavonoid content determination of each ethanolic extract of *Senna auriculata* plant parts was assessed using aluminium chloride using the colourimetric method, and quercetin was used as a standard solution. About 1 mL of each extract sample was mixed with 4 mL of water, 0.3 mL of 5 % sodium nitrate, and 0.3 mL of Aluminium chloride. The mixture was then stirred well and incubated at room temperature for 10 min. After the incubation, 2 mL of 1M sodium hydroxide was added to the solution. Absorbance of the sample compound was measured at 510 nm using- Vis spectrophotometer, and results were expressed as quercetin equivalents[20].

Antibacterial activity of Senna auriculata extracts :

The antibacterial activity of the ethanolic extract of leaves, flowers, and roots of *Senna auriculata* was evaluated against the test organisms *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. Extracts (10–100 µg/mL) were loaded into wells, inoculated with test organisms, and incubated at 37°C for 24 hrs. Antibacterial activity was determined by measuring the zone of inhibition in diameters (mm)[16].

Determination of anti-oxidant activity :

The ability of the plant extracts to scavenge free radicals was determined by the DPPH radical scavenging assay. The total free radical scavenging capacity of the plant extracts with the solvents was estimated using this assay. The free radical solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. 5 µL of plant extract solution was added to 3.994 mL of methanolic DPPH. The mixture was shaken vigorously and incubated in the dark for 30 minutes. The absorbance was measured at 517nm [11]. The ability to scavenge the radical was calculated using the following equation (2) below.

$$\%\ Inhibition\ = \frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs Control}} \qquad (2)$$

Determination of anti-inflammatory activity:

The anti-inflammatory activity of the plant extracts was determined by the Protein denaturation assay. In this assay, 100 µL of the plant extract was mixed with 100 µL of 5% aqueous solution of Bovine serum albumin (BSA). The mixture was incubated at 37/ °C for 20 minutes, and the temperature was increased to 70 °C in the water bath for 10 minutes. After cooling the mixture under tap water for 10 minutes, the turbidity and absorbance were measured at 660nm [4]. The percentage inhibition of protein denaturation was calculated by the following equation (3) below.

$$\%\ Inhibition\ = \frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs Control}} \qquad (3)$$

Qualitative phytochemical screening of Senna auriculata:

The preliminary phytochemical screening analysis of ethanolic extracts of *Senna auriculata* leaves, flowers, and roots revealed the presence of essential phytochemical compounds such as tannins, alkaloids, saponins, flavonoids, phenols, and proteins. Quinones were detected exclusively in roots, and their anti-inflammatory, antimicrobial and cell growth-promoting properties can support wound healing[6]. The results were represented in Table-1.

Table-1. Qualitative screening of phytochemicals in ethanolic extracts of *S. auriculata.*

S. No	Phytochemicals	Ethanolic Extract		
		Leaves	Flower	Root
1	Tannin	Present	Present	Present
2	Alkaloids	Present	Present	Present
3	Saponins	Present	Present	Present
4	Steroids	Not present	Not present	Not present
5	Terpenoids	Not present	Not present	Not present
6	Glycoside	present	Present	Not present
7	Phenol	Present	Present	Present
8	Quinone	Not present	Not present	Present
9	Protein	Present	Present	Present
10	Carbohydrate	Not present	Not present	Not present
11	Flavonoids	Present	Present	Present

Thin layer chromatography :

The ethanolic extracts of the leaves, flowers, and roots of *Senna auriculata* revealed the presence of six distinct biomolecules, as indicated by their separation on TLC plates. The compounds exhibited different Rf values, as represented in Table-2. Thin-layer chromatography analysis demonstrated the presence of various functional groups, with each compound exhibiting a unique Rf value. The separation patterns observed in the TLC profiles of leaf, flower, and root extracts are correlated with distinct bactericidal zones, suggesting that the bioactive compounds are phenolic and flavonoid compounds that are responsible for antibacterial activity.

Table-2. Bioactive compounds present in *S. auriculata* extracts

Rf Value					
0.18 (Leaf)	0.25 (Leaf)	0.31 (Leaf)	0.66 (Leaf)	0.73 (Flower)	0.73 (Root)

Partial purification by column chromatography :

Based on the polarity of *S. auriculata,* partial purification through column chromatography separated each of the ethanolic extracts into distinct fractions: three fractions from the leaf extract, two fractions from the flower extract, and two fractions from the root extract Figure 1. Each fraction exhibited distinct colours, indicating varied phytochemical constituents. As a result, column chromatography effectively purified and concentrated the bioactive components for further HPTLC evaluation.

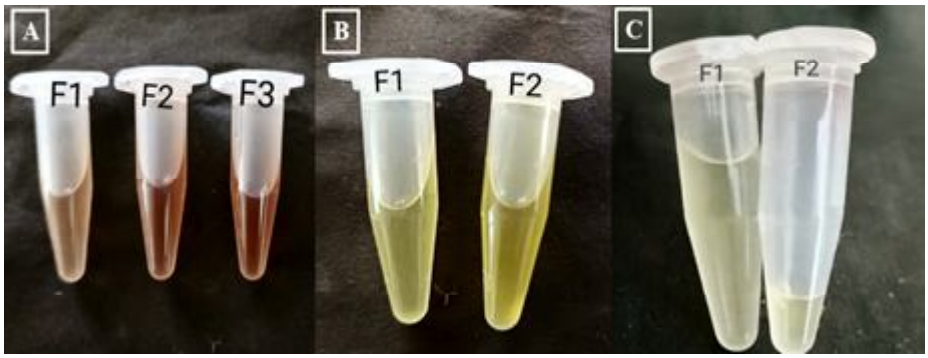

Figure 1. Column chromatographic extracts of *Senna auriculata*
(A) Leaf (B) Flower (C) Root.

High-performance thin layer chromatography :

HPTLC analysis of each fraction of purified ethanolic extracts of *Senna auriculata* plant parts (leaves, flowers, and roots) was performed and the corresponding chromatograms are shown in Figure 2. According to an earlier reference[2,9], flavonoids typically exhibit Rf values ranging from 0.15 - 0.79, while polyphenolic compounds range between 0.40 - 0.95. In the present study, nearly identical Rf values were observed in the leaf (0.863,

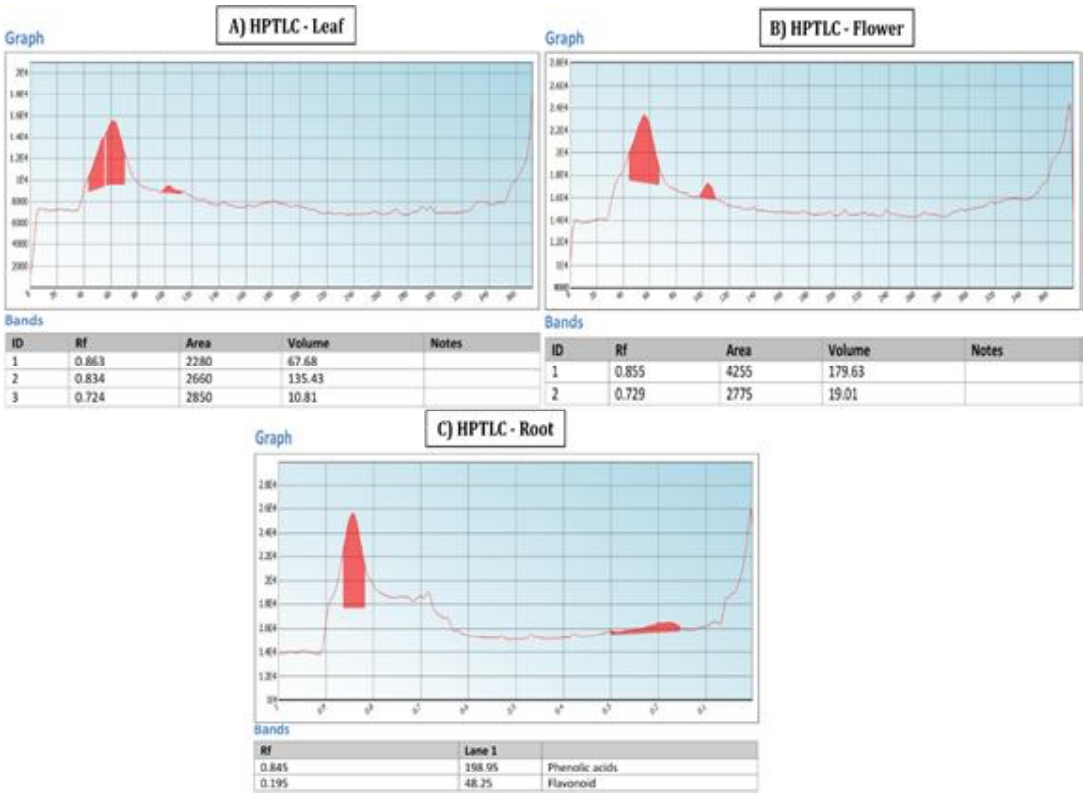

Figure 2. HPTLC fingerprint profile of ethanol extracts of *Senna auriculata* observed at 365 nm (A) Flower extract, (B) Root extract, and (C) Leaf extract.

0.834 & 0.724), flower (0.855 & 0.729) and root (0.845) extracts, indicating the presence of similar phytochemicals, mainly polyphenols, which appear at the higher Rf range. Whereas the root extract presented distinct bands, notably at Rf 0.195, signifying the presence of unique flavonoids, which were not observed in the aerial parts. The presence of these unique and concentrated compounds in the root probably contributes to its distinct biological activity. This analysis supports previous phytochemical screening results and highlights the plant's potential in pharmacological applications.

Quantitative phytochemical analysis :

The distinct polyphenolic and flavonoid compounds observed in the HPTLC profile were further validated by quantitative phytochemical analysis using spectrophotometric methods. The analysis revealed that root and flower extract carried higher flavonoid content compared to leaf extract, which can contribute to the antibacterial property against a wide range of microorganisms. Whereas the phenolic content associated with microbial enzyme inhibition and disruption of bacterial metabolism was found to be greater in root

Table-3. Quantitative analysis of Plant extract

S.No	Phytoconstituents	Leaves	Flower	Root
1	Flavonoids (mg/g)	15 mg	28 mg	30 mg
2	Phenols (mg/g)	0.17 mg	0.21 mg	0.25 mg

extract, followed by flower and leaf extract[8]. The quantitative analysis of flavonoids and phenols present in the plant extract is represented in Table-3.

Anti-Bacterial activity :

In this study, leaf extract showed anti-bacterial activity at 80 µg/mL against *S. aureus and E. coli*. The flower extract exhibited anti-bacterial activity against both organisms, with activity against *E. coli* starting at ≥50 µg/mL concentrations. In contrast, the zone of inhibition for root extract started at lower concentrations (30 µg/mL) against *S. aureus* and consistent activity was observed against both test organisms from 40 µg/mL. The flower and root extract exhibited a dose-dependent inhibitory effect against. *S. aureus* and *E. coli* even at lower concentrations, supporting the rationale for further analysis. The results of the antibacterial activity of the ethanolic extracts of leaves, flowers and roots of *Senna auriculata* are represented in Table 4.

Antioxidant activity :

A dose-dependent increase in the radical-scavenging activity was observed in both root and flower extracts as shown in the figure 3. However, root extract demonstrated consistently higher % inhibition at all concentrations compared to flower extract, indicating a stronger antioxidant activity.

Table-4. Anti-Bacterial activity of *Senna auriculata* plant parts against *E. coli* and *S. aureus*

S. No.	Concen-tration (µg/mL)	Leaf Extract (*E. coli*)	Leaf Extract (*S. aureus*)	Flower Extract (*E. coli*)	Flower Extract (*S. aureus*)	Root Extract (*E. coli*)	Root Extract (*S. aureus*)
1	10	-	-	-	-	-	-
2	20	-	-	-	-	-	-
3	30	-	-	-	-	-	6 mm
4	40	-	-	-	-	8 mm	8 mm
5	50	-	-	10 mm	-	10 mm	8 mm
6	60	-	-	11 mm	-	12 mm	10 mm
7	70	-	-	11 mm	-	13 mm	12 mm
8	80	12 mm	13 mm	10 mm	11 mm	13 mm	12 mm
9	90	13 mm	10 mm	12 mm	11 mm	14 mm	13 mm
10	100	16 mm	11 mm	16 mm	12 mm	16 mm	13 mm

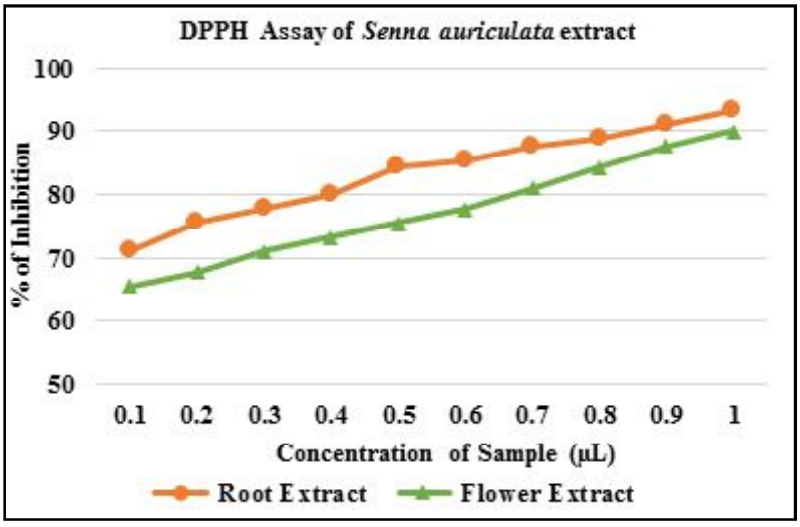

Figure 3. DPPH Assay of *Senna auriculata* extract.

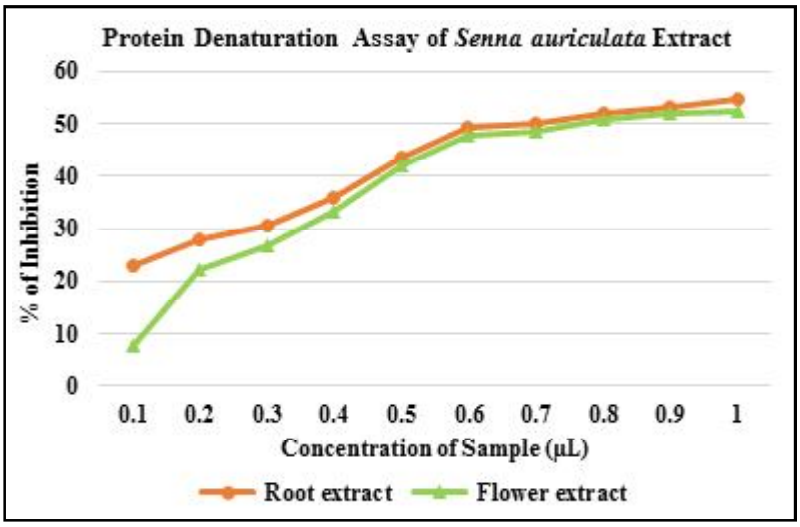

Figure 4. Protein Denaturation Assay of *Senna auriculata* Extract.

Anti-Inflammatory Activity :

A dose-dependent increase in protein denaturation inhibition was observed in both root and flower extracts as shown in figure 4. However, in comparison to flower extract, root extract consistently showed slightly higher inhibition across all concentrations, indicating a stronger anti-inflammatory effect.

In the present comparative study of *S auriculata* extracts, qualitative phytochemical

screening revealed the presence of predominant bioactive compounds. Chromatographic and quantitative phytochemical analysis confirmed and validated that the flower and root extracts were rich in flavonoids and phenolic compounds. Further, bioactivity studies demonstrated that plant extracts exhibited promising antibacterial, anti-inflammatory, and antioxidant activities, with root extracts showcasing superior activity. Overall, the study findings highlight that the root and flower extracts of *Senna auriculata* are rich in diverse bioactive compounds, supporting its strong therapeutic value in healthcare, particularly as a natural remedy for wound healing applications.

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Author Contributions

D S Srimathi: Conceptualisation, Methodology, and Writing – original draft; Dr R Manju: Supervision, investigation, formal analysis, data curation, writing – review & editing**.**

Conflict of interest

The authors declare that they have no conflict of interest.

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