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ANTIOXIDANT POTENTIAL OF *SENNA MONTANA*-MEDIATED GREEN SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT
Antioxidants provide a critical defense against oxidative and nitrosative stress- induced cellular damage which underpins chronic disease and the aging process. While traditional antioxidants often hindered by poor stability and low bioavailability, plant mediated nanotechnology offers a promising alternative. This study evaluated the antioxidant efficacy of plant mediated Silver nanoparticles synthesized using *Senna montana* leaf extract (*Sm*L-AgNPs) was validated for their Antioxidant potential through a multi-methodological radical-scavenging assays, including DPPH, ABTS, H_2O_2 and Hydroxyl radical (OH) assays. Results demonstrated significant dose-dependent scavenging and inhibitory effect was observed when compared to all tested samples ranging from 20 and 100 µg/mL. A strong correlation was established between the percentage of radical inhibition and the calculated IC_{50}values. Our findings suggest that biogenic nano formulation (*Sm*L-AgNPs) represent a sustainable, non-toxic, and highly efficacious platform for radical neutralization in therapeutic applications.

Keywords: *Senna montana, Plant-mediated nanoparticles, Antioxidant activity, Radical scavenging effect, Silver nanoparticles*

INTRODUCTION
Oxidative stress represents a systemic homeostatic failure where the production of Reactive Oxygen and Nitrogen Species (RONS) exceeds the capacity of endogenous antioxidant defenses. While basal RONS levels are essential for cellular signaling, an excessive accumulation leads to chronic biochemical imbalance. This state induces significant structural damage including lipid peroxidation, protein carbonylation, and DNA instability serving as a primary driver in the pathogenesis of malignancies, metabolic dysregulation, and neurodegenerative disorders. (Hamzah, 2026; Daré & Lautenschlager, 2025; Arya *et al.,*2024; Halliwell, 2022; Pisoschi *et al.,*2021; Liguori *et al.,* 2018).
To encounter this degradation, human body employs multilayered “biological fortress” of antioxidants. These biological molecules function effectively as potent inhibitors capable of delaying or preventing oxidative degradation even at concentrations significantly. (Cocciolone *et al.,* 2025; Ashok *et al*., 2022; Samrot *et al.,*2022). Antioxidants are functionally classified based on their intervention in radical-mediated processes: primary (chain-breaking) antioxidants directly scavenge reactive species to terminate autoxidative chain reactions, whereas secondary (preventative) antioxidants operate through indirect mechanisms such as transition metal chelation, singlet oxygen quenching, peroxide decomposition, or the inhibition of pro-oxidative enzymes. By combining immediate radical neutralization with longterm preventive quenching, it is essential for mitigating the onset and progression of chronic human pathologies.(Alum, 2026; Uti, *et al.,*2025; Chen *et al.,*2022; Alì *et al.,* 2021).
The clinical significance of huiman antioxidant defence system is further highlighted by the application of Silver nanoparticles (AgNPs). These nanomaterials exhibit a specialized bio-activity profile characterized by remarkable biocompatibility toward eukaryotic systems, contrasted by potent cytotoxic efficacy against prokaryotic pathogens, including multidrug-resistant bacteria, viruses, and fungi (Ahmed *et al*., 2019; Keshari *et al.*, 2020; Jangra *et al*., 2025). While traditional physical and chemical synthesis

protocols have factually fastened AgNP production, they are increasingly marginalized due to suboptimal yields, high energy requirements, and the unavoidable utilization of hazardous reducing agents. Consequently, there is a critical paradigm shift toward plant-mediated green synthesis (Selvam *et al.,* 2026; Pandey*et al*.,2025; Kumari *et al*.,2025; Dubey *et al*.,2023; Kummara, 2016).

The integration of Silver Nanoparticles (AgNPs) represents a paradigm shift in therapeutic antioxidant strategies, offering a sophisticated bio-activity profile that transcends the capabilities of traditional scavenging systems. Unlike conventional antioxidants, AgNPs provide a dual-functioning defense characterized by selective cytotoxicity: they exhibit high biocompatibility toward eukaryotic cells while maintaining potent efficacy against a broad spectrum of prokaryotic pathogens, including multidrug-resistant bacteria, viruses, and fungi. By combining traditional redox-balancing properties with enhanced stability and targeted delivery, AgNPs serve as a multifunctional nanomaterial capable of simultaneously mitigating oxidative degradation and systemic infection, thereby providing a robust clinical tool for managing chronic human pathologies. (Hamzah, 2026; Alawi *et al*., 2026; Chandimali *et al*., 2025; Sadiq, 2023; Kumar *et al*., 2016).

Despite the therapeutic potential of antioxidants, their clinical utility is often hampered by poor bioavailability, inefficient membrane permeability, and systemic degradation. To overcome these pharmacokinetic barriers, nanomedicine has leveraged the synergistic integration of nanoparticles and bioactive plant compounds. (Daré & Lautenschlager, 2025; Shen *et al*., 2022). This overview examines the efficacy of synthetic antioxidant platforms, including inorganic nanoparticles with intrinsic catalytic properties (nanozymes) and specialized nanocarriers functionalized with natural antioxidants or enzymes. By encapsulating or tethering these compounds to nanomaterials, researchers can significantly enhance their stability and targeted delivery, offering a robust strategy for mitigating oxidative stress-related pathologies (Hu *et al*., 2025; Flieger *et al.,*2025; Suliasih *et al.,* 2024; Balkrishna *et al.,*2021; Kumar *et al.,* 2020).

Comprehensive investigations into the *Senna* genus have established a robust pharmacological foundation. Raj *et al.* (2021) conducted extensive pharmacognostic and phytochemical evaluations of *Senna* leaves, while Zibaee *et al*. (2023) provided a systematic review of the traditional applications and ethnopharmacology of *Cassia (Senna)* species. Mittoori *et al*. (2024) employed Gas Chromatography-Mass Spectrometry (GC-MS) to delineate the chemical profiles of the leaf, stem bark, and root bark of *C. montana*. Recent investigations by Sudhakar *et al.* (2026) have further substantiated the pharmacological relevance of *Senna montana*, proving through comprehensive phytochemical profiling and antibacterial screening that the pod extracts harbor a potent, multi-targeted therapeutic potential.

The current literature relies predominantly on preclinical models (*in vitro* and *in vivo*), which limits the translatability of findings regarding toxicological profiles and therapeutic indices. Adressing these limitations, this study explores the untapped potential of underutilized plant-derived compounds through green technology. To bridge the knowledge gaps, rigorous clinical validation is essential to establish human safety and efficacy. A rigorous comparative analysis of the antioxidant potency across diverse solvent extracts of *S. montana* leaves remains unexplored and has not been reported to date.

MATERIALS AND METHODS

Fresh leaves of *Senna montana* were collected from the Habitats of Tirumala Ghat roads, washed thoroughly and shade dried. Following collection, plant material was authenticated by Prof. N. Savithramma, and a representative voucher specimen (SVUTY/FAB-3454) was archived at the KNR Herbarium, Sri Venkateswara University, Tirupati.

Preparation of samples

Senna montana Leaf was subjected to maceration and subsequent extraction using a diverse polarity range of organic and inorganic solvents to optimize the yield of bioactive secondary metabolites (Sudhakar, *et al.,* 2026; Abubakar and Haque, 2020; Harborne, 1973 &1998).

Antioxidant activity studies

Pre- synthesized silver nanoparticles derived from *S.montana* (*Sm*L-$AgNO_3$) leaf extract were employed for bioactivity testing were utilised as testing material. Ascorbic acid was used as Standard Antioxidant. The comparative antioxidant activity of SmL-Aq, SmL-AgNPs, $AgNO_3$, and Vitamin C was systematically assessed through DPPH, ABTS, H_2O_2 and •OH scavenging assays. These procedures were conducted in accordance with established literature standards (Blois, 1958; Re *et al.,* 1999; Mitta *et al.,* 2014) and developed according to the methodology optimized by Ramakrishna and Savithramma (2023). Antioxidant activity (Radical scavenging activity) was expressed as a percentage of inhibition, derived from colorimetric absorbance values using the standard formula:

Inhibition % = $[(A_0-A_1) /A_0]$ X 100

(where A_0 represents the absorbance of the control and A_1 signifies the absorbance of the test sample (*Sm*L-*Aq / Sm*L-AgNp's/ AgNp).

Determination of IC_{50} values

The antioxidant potency was quantified as the half-maximal inhibitory concentration IC_{50} defined as the concentration required to scavenge 50% of the free radicals under experimental conditions and calculation follows the linear equation: y = mx + c (where y = 50, and x is solved to determine the IC_{50} concentration).

Figure 1: *Senna montana* Habitat & Flowering twig

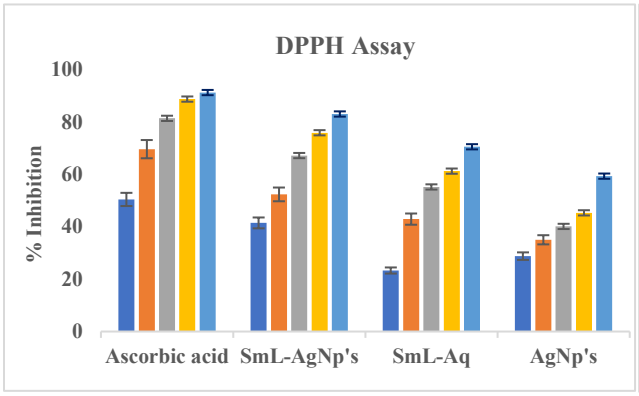
RESULTS AND DISCUSSION

Antioxidant activity

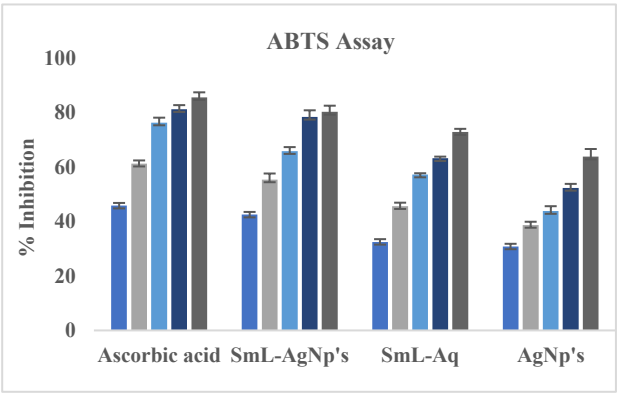
The antioxidant potential of the test samples (Ascorbic Acid (Standard), *Sm*L-AgNPs, *Sm*L-Aq, AgNPs) was assessed using across four invitro radical scavenging activity assays (DPPH, ABTS, H_2O_2, •OH). The antioxidant activities (Radical scavenging potential) of the test samples were compared with standard across a concentration range of 20 to 100 µg/mL. Values expressed as mean ± SD concentrations of (20,40,60,80.100 µg/mL).

The scavenging capacities are illustrated in Graph 1,2,3,4 depicts DPPH radical scavenging (Graph 1), $ABTS^{\bullet+}$ Decolorization (Cation radical scavenging) (Graph 2), Hydrogen peroxide (H_2O_2) scavenging (Graph 3) and Hydroxyl Radical (•OH) Inhibition (Graph 4) respectively. A significant observation is that the green-synthesized *Sm*L-AgNPs exhibited superior antioxidant activity compared to both the aqueous extract (*Sm*L-Aq) and the standard AgNPs at every concentration level in all the four assays (DPPH, ABTS, H_2O_2, •OH). The findings indicate that the green synthesis of silver nanoparticles using *Senna montana* Leaf extract (*Sm*L-AgNPs) significantly enhances the antioxidant properties of the precursor material. The dose-dependent response suggests that *Sm*L-AgNPs act as potent hydrogen donors, effectively neutralizing DPPH, ABTS, H_2O_2, •OH radicals. The increasing order of Antioxidant activity AgNPs <*Sm*L-Aq <*Sm*L-AgNPs < Ascorbic Acid The superior performance of *Sm*L-AgNPs over the pure aqueous extract confirms that the nano-formulation concentrates and stabilizes the bioactive antioxidant compounds.

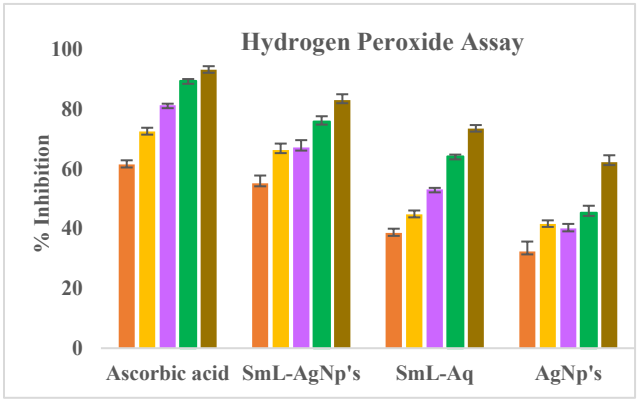
Graph 1 : DPPH Assay



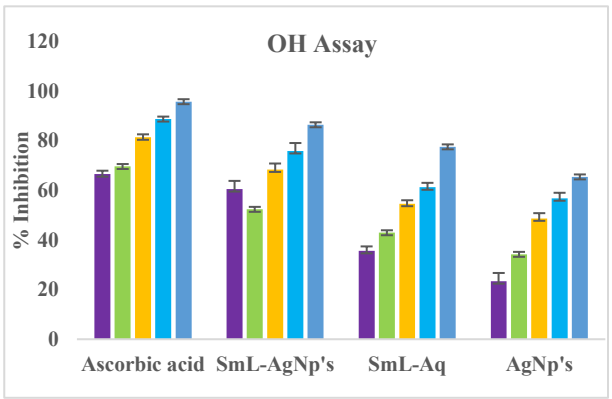
Graph 2 : ABTS Assay



Graph 3 : Hydrogen peroxide (H_2O_2) Assay



Graph 4 : Hydroxyl Radical (•OH) Assay



(Graphs 1-4: *Sm*L-AgNPs : *Senna montana* Leaf mediated silver nanoparticles; *Sm*L-Aq *Senna montana* Leaf Aqueous extract; AgNPs : Chemically synthesized Silver nanoparticles).

IC_{50} values

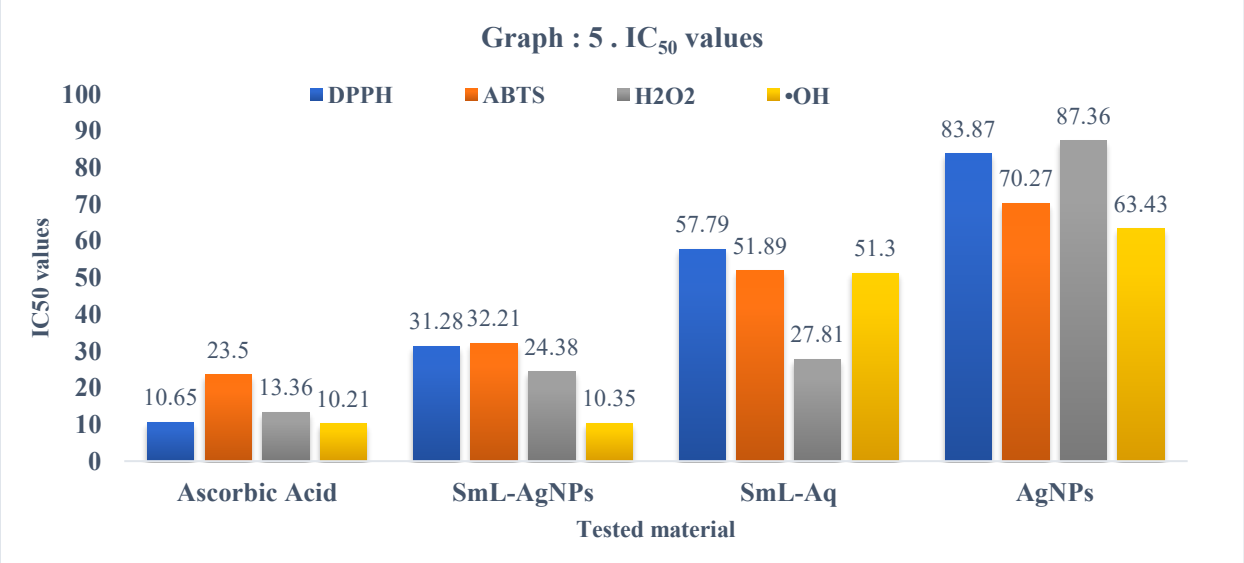
IC_{50} values were calculated via linear regression analysis by plotting the percentage of inhibition against the sample concentrations (20–100 µg/mL). All results are expressed as Mean ± Standard Deviation (SD) from triplicate measurements (n=3), and the comparative efficacy of *S. montana* extracts, AgNPs, and standards was visually established through dose-response graphical representations (Graph 5).

The linear regression analysis (y=mx+c) confirms that the *Sm*L-AgNPs act as high potency antioxidants with a predictable dose-response profile (Table: 2). The significant reduction in IC_{50}values compared to the crude aqueous extract and chemically synthesized AgNPs highlights the role of *Senna montana leaf extract* capping agent in enhancing radical scavenging showing antioxidant potency. The near unity R^2 values (R^2 > 0.87 to 0.99) further substantiate the reliability with high linearity relationship between concentration and antioxidant activity suggesting dose dependent. (Table: 1).

The superiority of *Sm*L-AgNPs consistently exhibited lower IC_{50} values for all the assays experimented.Ascorbic Acid remained the most potent inhibitor across all tests. This demonstrates that the biological capping agents (Polyphenols, Flavonoids) may provide a functional antioxidant shield that metallic silver alone cannot provide . The IC_{50} values for *Sm*L-AgNPs (<20 µg/mL) for H_2O_2 & •OH assays indicate high sensitivity to oxygen based radicals, making them excellent candidates for preventing oxidative stress in biological systems.

Table 1: IC_{50} values of the samples in DPPH, ABTS, H_2O_2, •OH radical scavenging assay

Assay	Ascorbic Acid	*Sm*L-AgNPs	*Sm*L-Aq	AgNPs
DPPH	10.65	31.28	57.79	83.87
ABTS	23.5	32.21	51.89	70.27
H_2O_2	< 20.00	< 20.00	27.81	87.36
•OH	< 20.00	< 20.00	51.3	63.43



Graph 5: IC_{50}values of AgNPs, *Sm*L-Aq, *Sm*L-AgNPs comparision with Standard (Ascorbic acid)

Similar studies by Prof. Savithramma Nanomaterial Laboratory by Yugandhar *et al.* (2018), Yugandhar *et al.* (2017), Kumar *et al.* (2016), Yugandhar & Savithramma (2016), Sasikala *et al.* (2015), Bhumi & Savithramma (2014), Yugandhar & Savithramma (2013), Savithramma *et al.* (2012), Savithramma *et al.* (2011), Rao & Savithramma (2011), Ankanna *et al.* (2010)) revealed that Green synthesized outperformed chemically synthesized AgNPs proving that biological capping provides additional antioxidant functional groups while Ascorbic acid served as the most inhibitor. *Sm*L-AgNPs showed comparable activity that approached the efficacy of the standard.

The potent scavenging of DPPH, ABTS, H_2O_2, •OH suggests that the *Sm*L-AgNPs act as effective primary antioxidants. To further elucidate the specific chemical moieties responsible for this stabilization and enhanced activity, subsequent Fourier-Transform Infrared (FT-IR) spectroscopy studies are essential.

CONCLUSION

The investigation established a robust linear correlation between dose-dependent radical inhibition and the calculated IC_{50} values, with biogenic *Senna montana* silver nanoparticles (*Sm*L-AgNPs) demonstrating superior antioxidant kinetics across the 20–100 µg/mL range. Compared to the aqueous leaf extract, Silver nanoparticles and the reference standard, Ascorbic acid, the SmL-AgNPs exhibited markedly lower IC_{50} threshold, confirming an enhanced stoichiometric efficiency in neutralizing reactive oxygen species. Consequently, this enhanced bioactivity is ascribed to the interfacial synergy between the metallic silver core and the bioactive phytochemical capping agents, which optimizes radical quenching at minimal concentrations. This capped *Senna montana* leaf extract *Sm*L-AgNPs emerged as a stabilized, sustainable, and highly efficacious alternative to traditional antioxidants, providing a multifunctional platform for mitigating oxidative stress-mediated pathologies.

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