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# Comparative In Vitro Antioxidant Evaluation of *Dendrophthoe falcata* and Its Host *Senna siamea*: Evidence of Host-Mediated Phytochemical Modulation

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*Dendrophthoe falcata; Senna siamea; antioxidant activity; free radical scavenging.*

**ABSTRACT**
**Oxidative stress, resulting from excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), plays a crucial role in the progression of chronic disorders such as cancer, diabetes, inflammation, and neurodegenerative diseases. The present study aimed to comparatively evaluate the in vitro antioxidant potential of leaf crude extracts of *Dendrophthoe falcata*, a hemiparasitic medicinal plant, and its host *Senna siamea*, and to assess the possible influence of host–parasite interaction on antioxidant activity. Crude extracts were prepared using solvents of increasing polarity and evaluated at concentrations of 50, 100, and 250 µg/mL using multiple antioxidant assays, including DPPH, hydroxyl radical, nitric oxide, superoxide anion, hydrogen peroxide, and ABTS radical scavenging assays, along with reducing power and total antioxidant capacity. Both plant species exhibited a clear concentration-dependent and solvent-dependent antioxidant response across all assays. Among the extracts, ethyl acetate and methanol fractions showed the highest scavenging activity, whereas non-polar solvent extracts exhibited negligible effects. At 250 µg/mL, the ethyl acetate extract of *D. falcata* demonstrated maximum scavenging activity of 88.1% (DPPH), 85.4% (hydroxyl radical), 82.5% (superoxide), and 81.9% (hydrogen peroxide), which was marginally higher than the corresponding *S. siamea* extracts. The methanolic extracts of both plants also exhibited strong antioxidant activity, with scavenging efficiencies comparable to the standard antioxidant ascorbic acid.The comparatively superior antioxidant potential of *D. falcata* may be attributed to its parasitic association with *S. siamea*, resulting in enhanced accumulation of phenolic and flavonoid constituents. Overall, the study highlights the significance of host-mediated phytochemical modulation and supports the potential of both plants as promising natural sources of multifunctional antioxidants for managing oxidative stress-related disorders.**



## 1. INTRODUCTION:

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are continuously generated in biological systems as by-products of normal metabolic processes as well as through exposure to environmental stressors. These reactive molecules, including superoxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide, and peroxynitrite, possess high chemical reactivity and can induce oxidative damage to cellular macromolecules such as lipids, proteins, and nucleic acids if not adequately neutralized (Apel & Hirt, 2004; Di Meo et al., 2016). Persistent oxidative stress resulting from an imbalance

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between radical generation and antioxidant defense mechanisms has been strongly implicated in the development of chronic diseases, including cancer, diabetes, cardiovascular disorders, inflammatory conditions, and neurodegenerative diseases (Di Meo et al., 2016).

Antioxidants play a critical role in maintaining cellular redox homeostasis by scavenging free radicals, chelating metal ions, and modulating oxidative signaling pathways. While endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase provide primary defense, exogenous antioxidants derived from dietary and medicinal plant sources significantly contribute to overall antioxidant protection (Apel & Hirt, 2004; Kumar et al., 2023). In recent years, plant-based antioxidants have attracted increasing scientific attention due to their structural diversity, multifunctional mechanisms of action, and comparatively lower toxicity than synthetic antioxidants (Di Meo et al., 2016).

Plant secondary metabolites, particularly polyphenols, flavonoids, tannins, terpenoids, and alkaloids, are well recognized for their antioxidant potential. These compounds exert their effects through hydrogen atom donation, electron transfer, metal ion chelation, and regulation of antioxidant enzyme activity (Rice-Evans et al., 1996; Heim et al., 2002). The antioxidant efficacy of plant extracts is influenced by solvent polarity, phytochemical composition, and extraction efficiency, with phenolic-rich extracts often exhibiting superior free radical scavenging activity in in vitro assays such as DPPH, ABTS, nitric oxide, and hydroxyl radical scavenging tests (Atun et al., 2018; Kumar et al., 2023). A strong positive correlation between total phenolic and flavonoid content and antioxidant activity has been consistently reported across diverse medicinal plant species (Atun et al., 2018).

Dendrophthoe falcata, commonly known as Indian mistletoe, is a hemiparasitic plant widely distributed in South and Southeast Asia and traditionally used for wound healing, anti-inflammatory, hepatoprotective, and anticancer purposes (Pattanayak & Sunita, 2008; Kumar et al., 2023). Previous studies have demonstrated that extracts of *D. falcata* possess substantial antioxidant activity, attributed primarily to their phenolic and flavonoid constituents (Atun et al., 2018). Notably, the phytochemical composition and biological activity of hemiparasitic plants are known to vary depending on the host species, suggesting a host-mediated influence on metabolite biosynthesis and accumulation (Sharma et al., 2013; Jayaraman et al., 2020).

Senna siamea, the host plant investigated in the present study, belongs to a genus known for its rich content of phenolic glycosides, flavonoids, and anthraquinone derivatives, many of which exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Although *S. siamea* itself has received comparatively less attention in antioxidant research, related *Senna* species have been extensively documented for their redox-modulating activities. Considering the hemiparasitic nature of *D. falcata*, the possibility of phytochemical exchange or host-induced metabolic modulation cannot be excluded.

Comparative evaluation of antioxidant activity between a hemiparasitic plant and its specific host under identical experimental conditions provides a unique opportunity to elucidate host–parasite phytochemical interactions. Such studies may reveal complementary or synergistic antioxidant effects and contribute to a better understanding of metabolite plasticity in parasitic plant systems. Moreover, correlating antioxidant activity with ethnomedicinal use strengthens the biochemical rationale for traditional therapeutic claims.

Therefore, the present study aims to evaluate and compare the in vitro antioxidant potential of leaf crude extracts of *Dendrophthoe falcata* and its host *Senna siamea* using multiple ROS- and RNS-based antioxidant assays. By employing solvents of varying polarity and assessing concentration-dependent responses, the study seeks to clarify the influence of host association and extraction chemistry on antioxidant efficacy, thereby providing insights relevant to natural antioxidant discovery and pharmacognostic research.

## 2. MATERIALS AND METHODS:

### 2.1 PREPARATION OF PLANT EXTRACTS

Fresh leaves of *Dendrophthoe falcata* and *Senna siamea* were collected, washed thoroughly with distilled water, shade-dried at room temperature, and pulverized into a coarse powder. The powdered material was subjected to sequential solvent extraction using solvents of increasing polarity, namely n-hexane, petroleum ether, toluene, chloroform, acetone, ethyl acetate, and methanol. Each extraction was carried out until exhaustion, and the resulting extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The dried extracts were stored at 4 °C until further analysis. Stock solutions were prepared in dimethyl sulfoxide (DMSO), and appropriate dilutions were made to obtain working concentrations of 50, 100, and 250 µg/mL for antioxidant assays.

### 2.2 DPPH RADICAL SCAVENGING ASSAY

The free radical scavenging activity of the extracts was evaluated using the DPPH assay following the method described by Adam and Piotrowska (2006),

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with minor modifications. Briefly, 400 µL of plant extract at different concentrations was mixed with 1.6 mL of 0.5 mM DPPH solution prepared in methanol. The reaction mixture was incubated in the dark at room temperature, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference standard, while a reaction mixture containing DPPH and solvent without extract served as the control. Radical scavenging activity was calculated as percentage inhibition, and $IC_{50}$ values were determined.

### 2.3 HYDROXYL RADICAL SCAVENGING ASSAY

Hydroxyl radical scavenging activity was assessed according to the method of Klein et al. (1981), with slight modifications. The reaction mixture contained 1 mL of extract solution, 1 mL of DMSO, 500 µL of 0.018% EDTA, and 1 mL of ferrous–EDTA reagent. The mixture was incubated in a water bath at 80–90 °C for 20 min to generate hydroxyl radicals. After incubation, 1 mL of chilled trichloroacetic acid (17.5% w/v) and 3 mL of Nash reagent were added, and the mixture was allowed to stand for color development. Absorbance was measured at 412 nm, and scavenging activity was expressed as percentage inhibition.

### 2.4 NITRIC OXIDE RADICAL SCAVENGING ASSAY

The nitric oxide scavenging activity of the extracts was determined using the method described by Govindarajan et al. (2003). Sodium nitroprusside (5 mM) in phosphate buffer was incubated with various concentrations of plant extracts at 25 °C for 25–30 min. Following incubation, the reaction was terminated by the addition of Griess reagent, and absorbance was recorded at 546 nm. A control containing all reagents except the extract was used for comparison. The nitric oxide scavenging activity was calculated as percentage inhibition, and $IC_{50}$ values were obtained.

### 2.5 SUPEROXIDE RADICAL SCAVENGING ASSAY

Superoxide anion scavenging activity was evaluated based on the method of Nishikimi et al. (1972) as modified by Yen and Duh (1994). The reaction mixture consisted of 1 mL of extract, 1 mL of NADH solution, and 100 µL of phenazine methosulfate (PMS). The reaction was allowed to proceed at room temperature for 10 min, and absorbance was measured at 560 nm. The decrease in absorbance relative to the control indicated the superoxide scavenging ability of the extracts.

### 2.6 HYDROGEN PEROXIDE SCAVENGING ASSAY

Hydrogen peroxide scavenging activity was measured following the method described by Ilhami et al. (2005). One milliliter of plant extract at different concentrations was mixed with 0.6 mL of 40 mM hydrogen peroxide prepared in phosphate buffer. After incubation for 15 min, absorbance was recorded at 230 nm against a blank containing phosphate buffer without extract. The scavenging activity was expressed as percentage inhibition.

### 2.7 ABTS RADICAL SCAVENGING ASSAY

The ABTS radical cation scavenging assay was performed according to the method of Arnao (2001). Pre-formed $ABTS^+$ solution was mixed with plant extracts at different concentrations, and the reaction mixture was incubated for 10–15 min at room temperature. Absorbance was measured at 734 nm, and the percentage inhibition was calculated. $IC_{50}$ values were derived from concentration–response curves.

### 2.8 REDUCING POWER ASSAY

The reducing power of the extracts was determined using the method described by Fejes et al. (2000). Extract solutions were mixed with phosphate buffer (pH 6.6) and potassium ferricyanide, followed by incubation and centrifugation. The supernatant was reacted with ferric chloride, and absorbance was measured at 700 nm. An increase in absorbance indicated greater reducing power.

### 2.9 TOTAL ANTIOXIDANT CAPACITY (TAC)

Total antioxidant capacity was assessed using the phosphomolybdenum method described by Prieto et al. (1999). Extract solutions were combined with a reagent mixture containing sulfuric acid, sodium phosphate, and ammonium molybdate, followed by incubation at 94 °C for 150 min. After cooling, absorbance was measured at 695 nm, and antioxidant capacity was expressed as tannic acid equivalents.

### 2.10 STATISTICAL ANALYSIS

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). $IC_{50}$ values were calculated using Microsoft Excel. Statistical significance between groups was evaluated using Student's *t*-test, with $p < 0.05$ considered statistically significant.

## 3 RESULTS:

### 3.1 ANTIOXIDANT ACTIVITY OF *D. FALCATA* CRUDE EXTRACTS

The antioxidant potential of *D. falcata* leaf crude extracts was assessed against a range of free radicals, including DPPH, hydroxyl radical, nitric oxide, superoxide anion, hydrogen peroxide, and ABTS. Overall, notable free radical scavenging activities were observed, particularly in ethyl acetate, methanol, and acetone extracts, with the ethyl acetate fraction showing the highest efficacy **(Table 1; Fig. 1 and 2)**.

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At 250µg/mL, the ethyl acetate extract exhibited maximum scavenging percentages of 88.1%, 85.4%, 76.3%, 82.5%, 81.9%, and 78.2% against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS, respectively. The methanol extract also demonstrated strong activity, with values of 84.9%, 83.1%, 80.1%, 80.8%, 79.3%, and 76.7% against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS, respectively. The acetone extract, while comparatively less potent, still showed appreciable activity (68.1%, 70.5%, 69.9%, 71.5%, 73.0%, and 70.1%, against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS respectively). Whereas, the free radical scavenging activity percentages of chloroform leaf crude extract at 250µg/mL 60.4%, 55.9%, 59.1%, 51.3%, 53.9%, and 60.6% respectively recorded against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS. On the other hand, toluene and petroleum ether leaf crude extract at 250µg/mL showed poor activity. The free radical scavenging activity percentages of the toluene leaf crude extracts at 250µg/mL 30.1%, 35.3%, 32.7%, 30.2%, 31.8%, and 30.4% respectively recorded against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS. Whereas, the petroleum ether leaf crude extract at 250µg/mL free radical activity percentages are 25.5%, 28.0%, 22.1%, 24.8%, 21.1%, and 23.7% respectively recorded against DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS. Other extracts exhibited moderate to low scavenging capacity, while the *n*-hexane fraction displayed no detectable activity across all assays.

At 100 µg/mL, the ethyl acetate extract maintained substantial radical scavenging effects, recording 62.3%, 66.1%, 61.5%, 59.7%, 60.0%, and 65.1% inhibition for DPPH, hydroxyl radical, nitric oxide, superoxide, hydrogen peroxide, and ABTS, respectively. Methanol extract yielded similar results (63.8%, 64.6%, 60.7%, 60.1%, 58.2%, and 60.6%), followed by acetone extract (54.3%, 57.1%, 58.3%, 54.8%, 55.4%, and 57.1%). Chloroform, toluene, and petroleum ether extracts showed moderate activity, ranging from 36.8% to 50.3% across assays.

At the lowest tested concentration (50 µg/mL), radical scavenging remained measurable but reduced. Ethyl acetate extract recorded 45.3% (DPPH), 49.6% (hydroxyl), 44.5% (nitric oxide), 46.2% (superoxide), 40.0% (hydrogen peroxide), and 45.8% (ABTS). Methanol extract produced comparable values (43.2%–45.0%), while acetone extract ranged from 33.1% to 40.5%. Chloroform, toluene, and petroleum ether fractions exhibited weaker activity, with petroleum ether extract consistently showing the lowest scavenging rates (16.4%–22.2%).

Hexane leaf crude extract at all concentrations tested does not showed any free radical scavenging activity against free radicals tested.

The trend indicates a clear concentration-dependent antioxidant effect for all active extracts, with ethyl acetate and methanol consistently outperforming other solvents across all tested free radicals.

**Table 1 The trend indicates Antioxidant activity of the *D. falcata* leaf crude extract**

| Solvent Extract | Conc. (µg/mL) | DPPH | .HO | .NO | $O_2$.- | $H_2O_2$ | ABTS |
|---|---|---|---|---|---|---|---|
| **Ethyl acetate** | 250 | 88.1 | 85.4 | 76.3 | 82.5 | 81.9 | 78.2 |
| | 100 | 62.3 | 66.1 | 61.5 | 59.7 | 60.0 | 65.1 |
| | 50 | 45.3 | 49.6 | 44.5 | 46.2 | 40.0 | 45.8 |
| **Methanol** | 250 | 84.9 | 83.1 | 80.1 | 80.8 | 79.3 | 76.7 |
| | 100 | 63.8 | 64.6 | 60.7 | 60.1 | 58.2 | 60.6 |
| | 50 | 43.2 | 45.0 | 44.1 | 42.5 | 41.8 | 40.6 |
| **Acetone** | 250 | 68.1 | 70.5 | 69.9 | 71.5 | 73.0 | 70.1 |
| | 100 | 54.3 | 57.1 | 58.3 | 54.8 | 55.4 | 57.1 |
| | 50 | 40.5 | 37.1 | 38.3 | 34.2 | 35.9 | 33.1 |
| **Chloroform** | 250 | 60.4 | 55.9 | 59.1 | 51.3 | 53.9 | 60.6 |
| | 100 | 45.1 | 47.3 | 42.5 | 43.9 | 41.0 | 44.7 |
| | 50 | 29.2 | 31.8 | 28.4 | 27.6 | 26.1 | 30.5 |
| **Toluene** | 250 | 30.1 | 35.3 | 32.7 | 30.2 | 31.8 | 30.4 |
| | 100 | 24.5 | 27.8 | 25.1 | 24.3 | 22.4 | 23.7 |
| | 50 | 19.2 | 20.6 | 18.4 | 17.5 | 15.3 | 16.1 |
| **Petroleum ether** | 250 | 25.5 | 28.0 | 22.1 | 24.8 | 21.1 | 23.7 |
| | 100 | 20.3 | 22.7 | 19.8 | 18.4 | 17.6 | 18.9 |
| | 50 | 16.4 | 18.3 | 15.1 | 14.9 | 13.0 | 14.2 |
| **n-Hexane** | All conc. | – | – | – | – | – | – |
| **Ascorbic aid** | 10 | 94.8 | 93.2 | 96.7 | 92.3 | 95.1 | 91.9 |

**DPPH** (2,2-diphenyl-1-picrylhydrazyl), **HO (**Hydroxyl Radical), **.NO** (Nitric Oxide), **$O_2$.-** (Superoxide), **$H_2O_2$** (Hydrogen Peroxide), **ABTS** (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

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### 3.2 ANTIOXIDANT ACTIVITY OF *SENNA SIMENTIA* CRUDE EXTRACTS

The antioxidant potential of *Senna siamea* leaf crude extracts was evaluated at three different concentrations (50, 100, and 250 µg/mL) using multiple in vitro free-radical scavenging assays, including DPPH, hydroxyl radical, nitric oxide, superoxide anion, hydrogen peroxide, and ABTS assays. The results demonstrated a clear concentration-dependent and solvent-dependent antioxidant response across all assays (Table 2; Fig 3 and 4).

At the highest concentration (250 µg/mL)**,** the ethyl acetate extract exhibited the strongest antioxidant activity among all solvents, showing maximum radical scavenging percentages in DPPH, hydroxyl, nitric oxide, superoxide, hydrogen peroxide, and ABTS assays. This was closely followed by the methanolic extract, which also displayed high scavenging efficiency across all tested radicals, indicating the presence of abundant phenolic and flavonoid constituents with strong hydrogen-donating and electron-transfer capabilities. Acetone extracts showed moderate antioxidant activity at this concentration, whereas chloroform extracts demonstrated comparatively lower but still measurable scavenging effects. In contrast, non-polar solvents such as toluene and petroleum ether exhibited weak antioxidant activity, suggesting limited extraction of polar antioxidant compounds. The n-hexane extract showed no detectable activity at any concentration tested.

At the intermediate concentration (100 µg/mL)**,** a marked reduction in radical scavenging activity was observed for all solvent extracts; however, the overall trend remained consistent with that seen at higher concentration. Ethyl acetate and methanol extracts continued to exhibit significantly higher antioxidant activity compared to acetone and chloroform extracts. The reduction in activity at this concentration confirms a dose-dependent antioxidant behavior of the extracts. Toluene and petroleum ether extracts showed minimal scavenging activity, further supporting the influence of solvent polarity on the extraction of bioactive antioxidant compounds.

At the lowest concentration (50 µg/mL)**,** all extracts displayed reduced antioxidant activity across the assays. Nevertheless, ethyl acetate and methanol extracts retained appreciable scavenging potential, indicating their effectiveness even at lower doses. Acetone and chloroform extracts exhibited modest activity, while toluene and petroleum ether extracts showed very low radical scavenging effects. The absence of activity in the n-hexane extract at this concentration reinforces the conclusion that non-polar solvents are inefficient in extracting antioxidant constituents from *S. siamea* leaves.

The results clearly demonstrate that the antioxidant activity of *Senna siamea* leaf crude extracts is both concentration-dependent and solvent-dependent, with polar solvents, particularly ethyl acetate and methanol, being more effective in extracting antioxidant phytochemicals. The superior performance of these extracts across multiple radical systems suggests the presence of diverse antioxidant compounds capable of neutralizing both reactive oxygen and nitrogen species.

**Table 2 Antioxidant activity of the S. simentia leaf crude extracts against stable free radicals**

| Solvent Extract | Conc. (µg/mL) | DPPH | ·HO | ·NO | $O_2^{\cdot -}$ | $H_2O_2$ | ABTS |
|---|---|---|---|---|---|---|---|
| **Ethyl acetate** | 250 | 84.0 | 81.7 | 72.8 | 78.9 | 78.2 | 74.6 |
| | 100 | 58.6 | 62.4 | 57.8 | 55.9 | 56.3 | 61.4 |
| | 50 | 41.6 | 45.8 | 40.7 | 42.5 | 36.4 | 42.1 |
| **Methanol** | 250 | 81.3 | 79.2 | 76.5 | 77.1 | 75.6 | 73.0 |
| | 100 | 59.7 | 60.8 | 56.9 | 56.4 | 54.5 | 56.8 |
| | 50 | 39.5 | 41.3 | 40.2 | 38.6 | 37.9 | 36.8 |
| **Acetone** | 250 | 64.5 | 66.8 | 66.1 | 67.4 | 69.2 | 66.5 |
| | 100 | 50.8 | 53.6 | 54.9 | 51.2 | 52.1 | 53.4 |
| | 50 | 36.7 | 33.9 | 34.8 | 31.5 | 32.6 | 29.8 |
| **Chloroform** | 250 | 56.9 | 52.4 | 55.6 | 47.8 | 50.4 | 56.8 |
| | 100 | 41.3 | 43.6 | 38.9 | 40.2 | 37.4 | 41.1 |
| | 50 | 25.8 | 28.1 | 24.9 | 23.4 | 22.0 | 26.7 |
| **Toluene** | 250 | 26.8 | 31.7 | 29.2 | 26.5 | 28.1 | 26.9 |
| | 100 | 21.2 | 24.3 | 21.6 | 20.9 | 19.1 | 20.4 |
| | 50 | 16.0 | 17.3 | 15.2 | 14.1 | 12.7 | 13.5 |
| **Petroleum ether** | 250 | 22.1 | 24.6 | 18.9 | 21.2 | 18.5 | 20.3 |
| | 100 | 17.6 | 19.9 | 16.3 | 14.8 | 14.2 | 15.6 |
| | 50 | 13.2 | 14.8 | 12.0 | 11.5 | 10.3 | 11.6 |
| **n-Hexane** | All conc. | – | – | – | – | – | – |
| **Ascorbic aid** | 10 | 94.8 | 93.2 | 96.7 | 92.3 | 95.1 | 91.9 |

**DPPH** (2,2-diphenyl-1-picrylhydrazyl), **HO (**Hydroxyl Radical), **.NO** (Nitric Oxide), **$O_2$.-** (Superoxide), **$H_2O_2$** (Hydrogen Peroxide), **ABTS** (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

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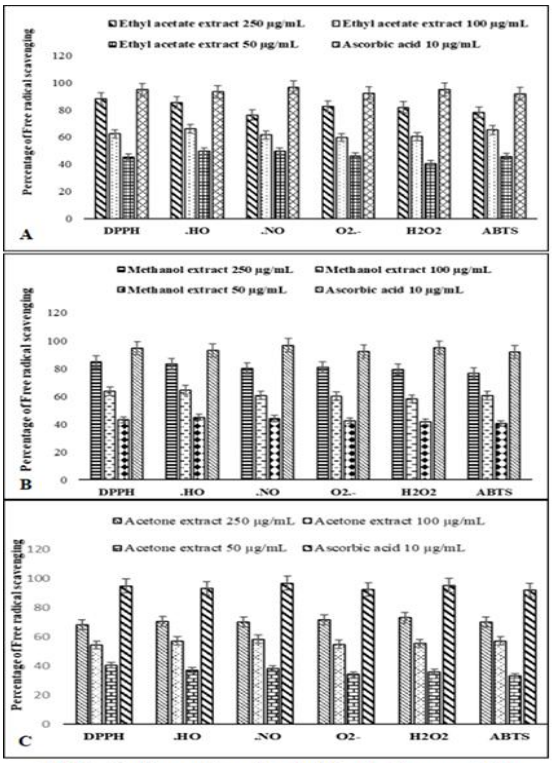


**Fig 1** Antioxidant activity of the ***D. falcata*** leaf extracts; **A**-Free radical scavenging activity of the Ethyl acetate extract, **B**- Free radical scavenging activity of the Methanol extract, **C**- Free radical scavenging activity of the Acetone extract

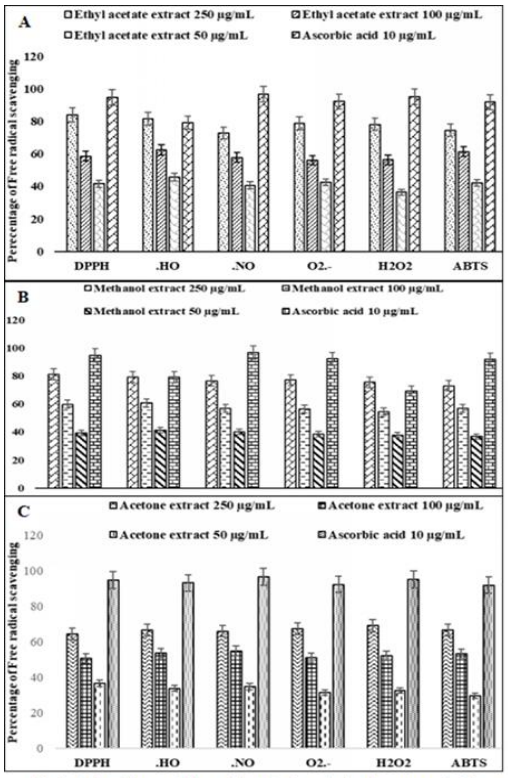


**Fig 3** Antioxidant activity of the ***S. simentia*** leaf extracts; **A**-Free radical scavenging activity of the Ethyl acetate extract, **B**- Free radical scavenging activity of the Methanol extract, **C**- Free radical scavenging activity of the Acetone extract

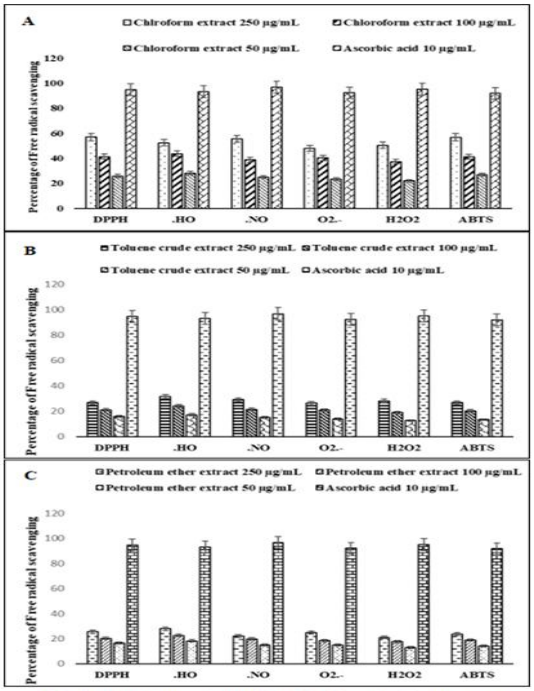


**Fig 2** Antioxidant activity of the ***D. falcata*** leaf extracts; **A**-Free radical scavenging activity of the Chloroform extract, **B**-Free radical scavenging activity of the Toluene extract, **C**- Free radical scavenging activity of the Petroleum extract

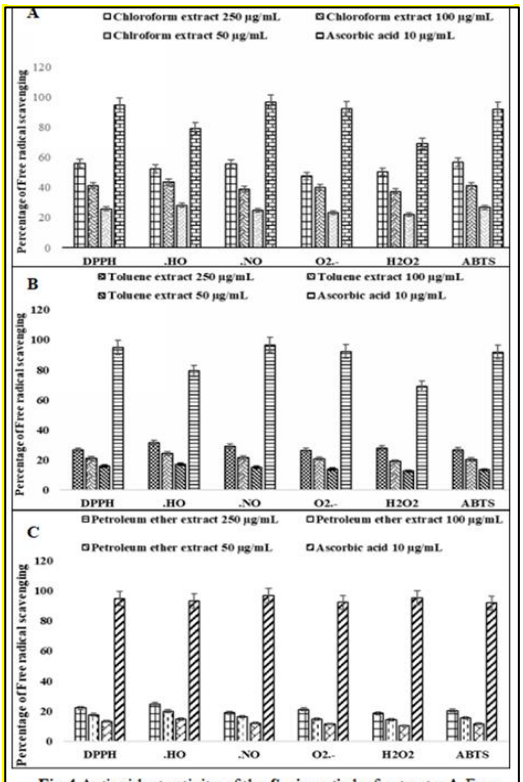


**Fig 4** Antioxidant activity of the ***S. simentia*** leaf extracts; **A**-Free radical scavenging activity of the Chloroform extract, **B**- Free radical scavenging activity of the Toluene extract, **C**- Free radical scavenging activity of the Petroleum extract

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## 4 DISCUSSION

Oxidative stress, resulting from excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), is widely recognized as a key biochemical factor contributing to the development and progression of chronic pathological conditions such as cancer, diabetes, cardiovascular disorders, neurodegenerative diseases, and inflammatory ailments. In this context, medicinal plants have gained considerable attention as sources of natural antioxidants due to their broad-spectrum redox-modulating activity and comparatively lower toxicity than synthetic antioxidants.

In the present study, leaf crude extracts of *Dendrophthoe falcata* and *Senna siamea* demonstrated pronounced antioxidant activity across all evaluated free radical scavenging assays, including DPPH, hydroxyl radical, nitric oxide, superoxide anion, hydrogen peroxide, and ABTS assays. The antioxidant response was clearly concentration-dependent and strongly influenced by solvent polarity. Among the solvents tested, ethyl acetate and methanol extracts consistently exhibited superior scavenging activity, followed by acetone extracts, whereas chloroform, toluene, petroleum ether, and n-hexane extracts showed comparatively low to negligible activity.

This solvent-dependent trend is in strong agreement with earlier reports indicating that phenolics, flavonoids, tannins, and other redox-active secondary metabolites are preferentially extracted by medium-to-polar solvents such as ethyl acetate and methanol (Harborne, 1998; Sultana et al., 2009). Previous phytochemical investigations on *D. falcata* have reported the presence of polyphenols, flavonoids, triterpenoids, and glycosides, compounds well known for their electron-donating and hydrogen-transfer properties (Pattanayak & Mazumder, 2009; Karthikeyan et al., 2016). Likewise, *S. siamea* leaves have been documented to contain anthraquinones, flavonoids, phenolic acids, and saponins, all of which contribute to antioxidant and related pharmacological activities (Somchit et al., 2003; Jothy et al., 2011).

The observation that ethyl acetate and methanol extracts exhibited scavenging efficiencies approaching those of the standard antioxidant ascorbic acid, particularly at higher concentrations, underscores the high antioxidant density of these extracts. Similar findings have been reported in other medicinal plant studies, where ethyl acetate fractions demonstrated enhanced DPPH, ABTS, and hydroxyl radical scavenging activities due to enrichment of phenolic constituents (Prior et al., 2005; Cai et al., 2010).

The application of multiple antioxidant assays in the present study provides a comprehensive understanding of the antioxidant behavior of the extracts through different mechanistic pathways. While DPPH and ABTS assays primarily reflect electron-transfer and hydrogen-donation mechanisms, hydroxyl and superoxide radical scavenging assays indicate metal-chelating ability and effective quenching of highly reactive oxygen species. Nitric oxide and hydrogen peroxide scavenging further reflect the capacity of the extracts to neutralize reactive nitrogen species and prevent the formation of secondary, more damaging radicals.

The consistently high scavenging activity observed for ethyl acetate and methanol extracts across all radical systems suggests the presence of a diverse pool of antioxidant molecules capable of acting through multiple complementary mechanisms rather than a single dominant pathway. Such multifunctional antioxidant behavior is a characteristic feature of plant-derived polyphenolic systems and is often attributed to synergistic interactions among different phenolic constituents (Rice-Evans et al., 1997; Shahidi & Ambigaipalan, 2015).

In contrast, the poor antioxidant performance of non-polar solvent extracts (toluene, petroleum ether, and n-hexane) can be explained by their limited ability to solubilize polar antioxidant compounds. This observation supports earlier findings that lipophilic fractions contribute minimally to free radical scavenging unless specific lipid-soluble antioxidants such as tocopherols or carotenoids are present (Dorman et al., 2003).

Although both plant species exhibited substantial antioxidant potential, *D. falcata* extracts generally showed marginally higher scavenging activity than *S. siamea* at comparable concentrations. This enhanced activity may be attributed to the hemiparasitic nature of *D. falcata*, which enables it to accumulate both host-derived metabolites and self-synthesized secondary compounds, thereby increasing phytochemical complexity (Pattanayak & Sunita, 2008). The parasitic association with *S. siamea* may further modulate secondary metabolite biosynthesis, potentially resulting in metabolic enrichment and improved antioxidant efficacy.

From a biomedical perspective, the demonstrated broad-spectrum scavenging activity against both ROS and RNS highlights the therapeutic relevance of these extracts in the management of oxidative stress–mediated disorders. The concentration-dependent antioxidant response observed in all active extracts indicates dose responsiveness, a critical requirement for pharmacological and nutraceutical development. Moreover, effective scavenging of hydrogen peroxide

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and superoxide radicals is particularly significant, as these species serve as precursors for the formation of highly reactive hydroxyl radicals in biological systems.

The promising antioxidant activity observed in this study provides a strong foundation for further investigations. Bioassay-guided fractionation and isolation of active constituents are warranted to identify the specific phenolic and flavonoid compounds responsible for the observed effects. Advanced analytical approaches such as HPLC-DAD, LC-MS/MS, and NMR spectroscopy would facilitate detailed structural characterization. In addition, in vivo antioxidant and oxidative stress models are necessary to validate bioavailability, metabolic stability, and safety. Future studies integrating antioxidant activity with anti-inflammatory, antidiabetic, and anticancer evaluations would further strengthen the therapeutic relevance of *D. falcata* and *S. siamea* as multifunctional medicinal plants.

## 5. CONCLUSION”

The present study demonstrates that leaf crude extracts of *Dendrophthoe falcata* and its host plant *Senna siamea* possess significant in vitro antioxidant activity against a wide range of reactive oxygen and nitrogen species. The antioxidant response of both plants was strongly influenced by extract concentration and solvent polarity, with ethyl acetate and methanol extracts consistently exhibiting superior free radical scavenging activity across all assays evaluated.

Among the two species, *D. falcata* generally showed marginally higher antioxidant efficacy than *S. siamea*, particularly in medium-to-polar solvent extracts, suggesting a richer or more diverse profile of redox-active phytochemicals. This enhanced activity may be associated with the hemiparasitic nature of *D. falcata*, which potentially enables host-mediated modulation and accumulation of bioactive secondary metabolites. The consistently poor activity observed in non-polar solvent extracts further emphasizes the dominant role of polar phenolic and flavonoid compounds in mediating antioxidant effects.

The broad-spectrum scavenging activity of the extracts against both reactive oxygen species and reactive nitrogen species underscores their therapeutic relevance in mitigating oxidative stress, a central factor in the development of chronic diseases such as inflammation, metabolic disorders, neurodegeneration, and cancer. The concentration-dependent antioxidant behavior observed in this study supports the pharmacological potential of these plants and highlights their suitability for further development as natural antioxidant sources.

The findings validate the ethnomedicinal significance of *D. falcata* and *S. siamea* and provide a strong scientific foundation for future studies focused on bioassay-guided isolation of active compounds, in vivo antioxidant validation, and mechanistic investigations. Such efforts may facilitate the development of plant-based antioxidant therapeutics or nutraceutical formulations derived from host–parasite medicinal plant systems.

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## CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest associated with this study.

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