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Antioxidant retention and enhanced activity in the flowering stage of cultivated *Cuscuta reflexa* Roxb.: a Sustainable alternative to wild harvesting

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Abstract

Cuscuta reflexa Roxb., a parasitic medicinal plant valued for its antioxidant and therapeutic potential, is conventionally collected from wild sources, raising concerns about sustainability and habitat disturbance. This study aims to identify suitable host for the parasitic plant species understudy and to evaluate whether cultivated plant retains comparable antioxidant properties to its wild counterpart as well as to explore stage-specific antioxidant variations, particularly between young and flowering stages. Host screening was done under controlled greenhouse conditions and partially in field environments to simulate natural parasitic growth and to identify host species that support biomass yield of C. reflexa without significantly compromising the host's vitality. On the basis of results, Calotropis procera was selected as the most suitable host for sustainable cultivation, which is supported by several research-backed factors. Sustainably cultivated samples were used to prepare plant extracts in methanol both at pre flowering and flowering stages of their life cycle and referred to as 001 and 002 respectively. The % recovery of the extracts were found to be 24.9% (for 001) 29.4% (for 002). The extracts were subjected to antioxidant assays using DPPH radical scavenging methods, with ascorbic acid as a standard reference. The IC_{50} values for Ascorbic acid, 001 and 001 extracts were 15.37, 460 and 257µg/ml (under different concentrations). The results revealed that the antioxidant activity of cultivated samples closely paralleled that of wild specimens; studied earlier. It indicated that sustainable cultivation does not compromise phytochemical integrity. Moreover, flowering-stage materials demonstrated significantly higher antioxidant values in cultivated groups.

Keywords: Sustainable cultivation, antioxidant activity, DPPH assay, phytochemical conservation, ethnobotany.

Introduction

Medicinal plants have long served as a cornerstone of traditional healthcare systems, providing therapeutic compounds with diverse pharmacological properties. The genus *Cuscuta*, despite being an obligate parasitic group of plants, possesses significant medicinal importance and is widely recognized in traditional as well as modern phytotherapeutic systems. *Cuscuta chinensis*, *Cuscuta australis* and *Cuscuta japonica* Choisy are important species of *Cuscuta*, widely used as Traditional Chinese Medicine and are also commercially available in China[1,2]. Among the other species, *Cuscuta reflexa* Roxb., commonly known as giant dodder, holds significant importance due to its wide range of ethnomedicinal applications, particularly as an antioxidant agent[3-5]. It is one of the most widely distributed and commonly utilized species of the genus *Cuscuta* in India and several other Asian countries. Owing to its rich phytochemical profile and diverse pharmacological properties, it holds significant importance in Ayurveda, Unani, Siddha, and various traditional healing systems. The plant has been traditionally harvested from the wild, which has not only led to pressure on natural populations but also posed challenges to its sustainable utilization. With increasing global demand for herbal medicines and phytopharmaceuticals, the overexploitation of wild resources raises concerns for biodiversity conservation and long-term availability of plant-based therapeutics. Some of the studies highlights how indiscriminate wild collection or "predatory excavation" has severely impacted natural vegetation, causing soil erosion, ecological imbalance, and a vicious cycle of degradation causing so medicinal plant species are reported to be endangered due to overharvesting[6]. It also notes that in countries like India and China, 80–90% of medicinal plants are still collected from the wild, significantly stressing natural populations. A global perspective showing that high reliance on wild sources coupled with escalating demand for herbal drugs is accelerating biodiversity loss. The earth is losing potential future drug candidates at alarming rates, one major drug every two years, conservatively estimated[7]. A focused report on India records steep declines in wild medicinal plants due to over-collection, despite legal protections. It underscores the failure of enforcement and recommends both cultivation and sustainable harvesting practices to ensure future supply[8].

In recent years, the search for sustainable alternatives to wild harvesting has brought attention to the potential of cultivating *C. reflexa*. Cultivation provides a controlled environment that may not only ensure a reliable supply but also allow for optimization of growth stages to maximize bioactive potential. Studies shows that *C. reflexa* grown on various cultivated hosts exhibit

differing levels of flavonoids, phenolics, and antioxidant activity demonstrating that host selection in controlled cultivation can influence bioactive potential[9]. Comparative study across several *Cuscuta* species indicates that the profile of antioxidants and polyphenolics is significantly influenced by the host species, supporting the idea that cultivation, with controlled host selection, can tailor bioactive outcomes[10]. Previous studies have demonstrated that the phytochemical content and antioxidant activity of medicinal plants can vary considerably with developmental stages[11,12], environmental conditions[13], and host interactions in the case of parasitic species like *C. reflexa*. However, limited research has examined how cultivation practices influence the retention and enhancement of antioxidant activity in different growth phases, particularly during flowering, a critical stage associated with peak biosynthesis of secondary metabolites.

Understanding the antioxidant potential of cultivated *C. reflexa* during its flowering stage is essential for validating it as a sustainable and effective alternative to wild-harvested material. Such insights not only contribute to conservation efforts but also open avenues for standardized cultivation practices, ensuring consistent quality for therapeutic use. This study investigates the antioxidant retention and enhanced activity of *C. reflexa* during its flowering stage under cultivation, highlighting its potential to replace unsustainable wild harvesting while maintaining pharmacological efficacy.

Materials and Methods

The following work was carried out under this section.

Suitable host identification: Host plants were selected based on availability in the region, growth pattern and compatibility with the host plant under field survey and data collection. Field observation included data collection for different host plants, based on which, a table was prepared (Table-1) to underline the most commonly found hosts for the parasitic plant under study. Total 18 different plant species were identified as possible and commonly found host.

An experimental setup for host screening was done under controlled greenhouse conditions and partially in field environments to simulate natural parasitic growth. Geographically the data collection site was located along 22º01´ and 23º01´ north latitude and 82˚07´ and 83º07´ East longitude situated at an altitude of 304.8 meters above sea level. The aim was to identify host species that support vigorous haustorial attachment, growth rate, and biomass yield of *C. reflexa* without significantly compromising the host's vitality.

Total 3 of the tabulated plants, *Calotropis procera*, *Hibiscus rosa-sinensis* and *Duranta erecta* showed best possibilities to become the most suitable hosts for sustainable cultivation of the plant. These three were grown in separate pots under identical atmospheric conditions. Healthy *C. reflexa* vines were introduced manually to each host and observed over a period of 8–10 weeks. Photographic documentation and field notes were maintained regularly.

Collection and authenticity of the plant: The plant materials were collected from Gevra, Dist. Korba, State- Chhattisgarh, India, and the genus and species of the medicinal plant were authenticated through Botanical Survey of India, Western Regional Centre, Pune, Maharashtra,, India, where specimen of plant was deposited. The plant specimens were authenticated (Ref. No BSI/WRC/Tech./2025/ JVD-160).

Cultivation of *Cuscuta reflexa* Roxb.: Cultivation was conducted in greenhouse and semi-field conditions using identified optimal host *Calotropis procera*. Parasitic vines were introduced manually, and growth was monitored for 10–12 weeks in all the visible seasons. Photographic documentation and field notes were maintained regularly. Parameters like parasite biomass (dry weight of *C. reflexa* per host plant at harvest), flowering rate, and impact on host health (visual scale of leaf yellowing i.e. 0 = no chlorosis, 5 = severe) were recorded (Table-2).

Cultivated samples of *C. reflexa* Roxb. were collected at two distinct phenological stages: pre-flowering and flowering. The aerial parts of the parasitic plant were harvested and processed separately for each stage. Hydroalcoholic extracts using the maceration technique were prepared using standard extraction protocols to facilitate comparative evaluation of antioxidant properties across developmental stages.

Preparation of plant extract: The collected plant materials were cleaned by washing with water dried under the sun and powdered using the grinder. 5 gm of sample was taken and mixed with 50 ml of solvent Mixture (80% Methanol, 1% HCL in distilled water). The sample mixture was then incubated on a rocker shaker for 24 hours. Then the extract was filtered through Whatman filter paper 1 and the extract was completely dried in the oven at 40°C. Extract was collected in micro centrifuge tube and stored at 4°C[14].

Antioxidant activity: In the current examination, the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used to assess the antioxidant activities of the plant extracts fractions using the standard modified method[15]. It is utilized to evaluate the antioxidant potential of *C. reflexa* plants (Pre-flowering and Flowering). Ascorbic acid (vitamin C) was used as the reference standard[16]. The DPPH radical has been widely utilized as a stable free radical to determine the decreasing substances or antioxidant activity of plant extracts[17,18]. 10µl of different stock of the test compound (Table-4) was added to 0.2 ml of 0.1 mM DPPH solution in Methanol in a 96 well plate. Ascorbic acid was used as standard. The concentrations used for analysis were 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25 and 50µg/ml. Whereas for both the plant extracts the concentrations used were 0, 1, 10, 50, 100, 250, 500, 1000µg/ml. The standard compound, being a

purified compound and highly active substance, was assessed using a lower concentration range (0 to 50 µg/ml). In contrast, the sample's potency remains unbound; therefore, a broader concentration range (0 to 1000 µg/ml) was employed for the sample analysis. The reaction was set in quadruplicate form (n = 4) and duplicates of blank were prepared containing 0.2 ml DPPH and 10µl standard/sample of different concentrations. The wells without treatment were considered controlled and wells without reagent (DPPH) were considered Blank. The plate was incubated for 30 min in dark. At the end of the incubation, the decolorization was read 517 nm using a micro plate reader. Reaction mixture containing 20µl of deionized water was served as Control. The scavenging activity was presented as '% inhibition' with respect to control. The concentration of the sample required to inhibit 50% of the DPPH free radical is called as IC_{50} value of the sample. IC_{50} was calculated separately for 001 (Pre-flowering) and 002 (Flowering) plant extracts (Table-4). Low absorbance of the mixture of the sample solution and DPPH indicated high free radical activity of the reaction mixture[19].

% RSA = [(Abs Control - Abs Sample)/Abs Control] ×100
RSA = Radical Scavenging Activity
Abs Control = Absorbance of control
Abs Sample = Absorbance of sample

Results and Discussion

Host identification: *Calotropis procera* was finally selected as the most suitable host over others for sustainable cultivation (Table-1), which is supported by several research-backed factors.

Table-1: Host plant compatibility and cultivation suitability of *Cuscuta reflexa* Roxb. under different habitat types.

Host Plant	Habitat type[a]	Growth of *C. reflexa*[b]	Host Tolerance[c]	Cultivation[d]	Suitability
Acacia nilotica	Wild	Poor	Low	Easy	Not Suitable
Achyranthes aspera	Wild	Moderate	Low	Not Easy	Not suitable
Aegle marmelos	Wild	Poor	Low	Easy	Not Suitable
Ageratum conyzoides	Wild	Moderate	Moderate	Easy	Fair
Cajanus cajan	Agricultural	Poor	Low	Easy	Not Suitable
Calotropis procera	Wild	Excellent	High	Easy	Excellent
Carisa spinarum	Agricultural	Slow	Medium	Easy	Fair
Casabela thevetia	Wild	Very Good	High	Moderate	Very Good
Lagenaria siceraria	Agricultural	Moderate	Moderate	Easy	Fair
Hibiscus rosa-sinensis	Ornamental	Excellent	High	Easy	Excellent
Justica adhatoda	Wild	Good	Medium	Easy	Good
Duranta erecta	Ornamental	Excellent	High	Easy	Excellent
Leucaena leucocephala	Wild	Very Good	High	Moderate	Very Good
Luffa cylindrica	Agricultural	Moderate	Moderate	Easy	Fair
Psidium guajava	Agricultural	Very Poor	Low	Easy	Not Suitable
Ricinus communis	Agricultural	Good	Medium	Easy	Good
Ziziphus mauritiana	Wild	Very Good	High	Moderate	Very Good
ıtharanthus roseus	Ornamental	Very Good	Moderate	Easy	Very Good

[a]Habitat type categorized as Wild, Agricultural, or Ornamental.[b] Represents parasitic biomass and spread of *C. reflexa* on the host. [c]Indicates resistance level of the host plant against *C. reflexa* infestation. [d] Denotes ease of cultivating the host species under controlled conditions. [e]Overall suitability derived from combined assessment of growth, tolerance, and maintenance feasibility.*Qualitative ratings (Excellent, Good, Fair, Poor, Not Suitable) are based on comparative growth observations.

In contrast to *Calotropis procera, Hibiscus rosa-sinensis* and *Duranta erecta* are primarily ornamental and are often pruned; thus, they may not support prolonged parasitism, making them less suitable for long-term cultivation systems[20]. The selected host plant exhibited sustained growth and remained physiologically active throughout and following the successful establishment and completion of the parasitic life cycle of the parasitic plant. Enhanced parasitic efficiency noticed, due to the host's favorable anatomical and physiological traits[21]. The selected host plant is native to arid and semi-arid regions (including the present area of study) and demonstrates exceptional drought resistance, thriving in poor soils with minimal water conditions[22]. Research has shown that it maintains high photosynthetic performance under drought stress[23] and its metabolic adjustments under water stress support its growth in harsh environments[24]. Utilizing it as a host for *C. reflexa* promotes land rehabilitation and sustainable land use without competing with food crops. Its rapid regeneration, coupled with its ability to grow without fertilizers or pesticides, supports organic cultivation practices[25].

Sustainable cultivation of *Cuscuta reflexa* Roxb: The seasonal growth performance of *C. reflexa* Roxb. cultivated on *Calotropis procera* showed clear variation in biomass and reproductive output. Maximum biomass (28.9 ± 3.2 g/plant) and flower production (72 ± 7/plant) occurred during the monsoon, coinciding with early flowering initiation (36 ± 2 days) and higher host chlorosis, whereas winter and post-monsoon recorded comparatively lower growth and delayed flowering. Summer also supported robust growth (24.7 ± 2.8 g/plant) and flowering (67 ± 6/plant), though with slightly higher host stress. Host survival remained above 85% across all seasons, confirming the reliability of *Calotropis procera* as a cultivation host (Table-2). These findings indicate that monsoon provides the most favorable conditions for growth and reproduction of *C. reflexa*, supporting its potential for sustainable cultivation and enhanced antioxidant activity during the flowering stage as a viable alternative to wild harvesting[26,27].

Preparation of plant extracts: During the extraction procedure, it was observed that the colors of the extracting solvents were dark brown after the complete extraction. Furthermore, the color of the extracting solvent became dark on reducing the volumes of the extracting solvent by rotary evaporator apparatus under vacuum. The extraction yield for both the samples (001 and 002) was below 30% (Table-3), indicating that a substantial biomass is necessary to obtain an appreciable quantity of extract. This underscores the importance of implementing sustainable cultivation practices to ensure a continuous and adequate supply of plant material, thereby minimizing pressure on natural populations[28]. Notably, the flowering plant material exhibited a comparatively higher percentage recovery, highlighting its potential as a preferred source in sustainable harvesting strategies.

Table-2: Seasonal growth performance of *Cuscuta reflexa* Roxb. cultivated on *Calotropis procera*.

Parameter	Winter (Dec–Feb)[a]	Summer (Mar–May)[a]	Monsoon (Jun–Aug)[a]	Post-Monsoon (Sep–Nov)[a]
Environment[b]	Greenhouse	Semi-field	Semi-field	Greenhouse
Avg. Temperature (°C)[c]	23 ± 3	40 ± 3	30 ± 2	28 ± 2
Parasite Biomass (g/plant)[d]	19.2 ± 2.1	24.7 ± 2.8	28.9 ± 3.2	21.6 ± 2.3
Flowering Initiation (days)[e]	55 ± 3	40 ± 2	36 ± 2	50 ± 3
Total Flowers/Plant[f]	43 ± 5	67 ± 6	72 ± 7	51 ± 4
Host Leaf Chlorosis Index (0–5)[g]	1.2 ± 0.3	2.8 ± 0.5	3.1 ± 0.4	1.7 ± 0.4
Host Survival Rate (%)[h]	100	90	85	95

[a]Seasons categorized as Winter, Summer, Monsoon, and Post-Monsoon based on regional climate data. [b] Environment indicates the cultivation setup used during each season (Greenhouse or Semi-field).[c] represents mean ambient temperature ± standard deviation during the experimental period. [d] represents mean dry weight of *Cuscuta reflexa* per host plant ± standard error. [e] indicates days to first flower emergence post-attachment. [f] denotes mean flower count ± standard error. [g] Host leaf chlorosis index (0–5) scored visually; 0 = healthy and 5 = complete yellowing. [h] Host survival rate (%) indicates the percentage of surviving hosts at experiment end. *All values represent mean ± standard error (n = 3). Statistical significance considered at $p < 0.05$.

Table-3: The percent recovery of the plant samples.

Sample Code and Type[a]	Weight of Powder (mg)[b]	Weight of Extract (mg)[c]	% Recovery[d]
001 (Pre-flowering)	5000	1245	24.9%
002 (Flowering)	5000	1470	29.4%

[a]represents the developmental stage of *C. reflexa* at the time of extraction. [b] indicates the dry weight of powdered plant material used for extraction. [c] refers to the total yield of concentrated extract obtained after solvent evaporation. [d] % Recovery calculated as: (Weight of Extract / Weight of Powder) × 100. *All values represent mean of triplicate determinations (n = 3).

Antioxidant activities: The anti-oxidant activities of both the plant extracts were carried out by standard method[29,30]. % inhibition of standard (Ascorbic acid) and Compounds (001 and 002) at various concentrations were also calculated (Table-4) by taking Ascorbic acid as standard [Figure-1a]. Both the tested extracts had exhibited scavenging effects on DPPH free radical [Figure-1b, 1c)]. These results in terms of IC_{50} values were shown in Figure-1d, which showed the scavenging activities at various concentrations. The IC_{50} values calculated for Ascorbic acid, 001 (Pre-flowering) and 002 (Flowering) extracts were 15.37µg/ml, 460µg/ml and 257µg/ml respectively. It was assessed through IC_{50}, $logIC_{50}$, and Hill's coefficient values. It was noted that the scavenging activities of both the plant extracts of *C. reflexa* at all different concentrations were quite good. The IC_{50} values obtained in the present study for pre-flowering and flowering extracts of are in close agreement with previously reported ranges for wild-harvested material.

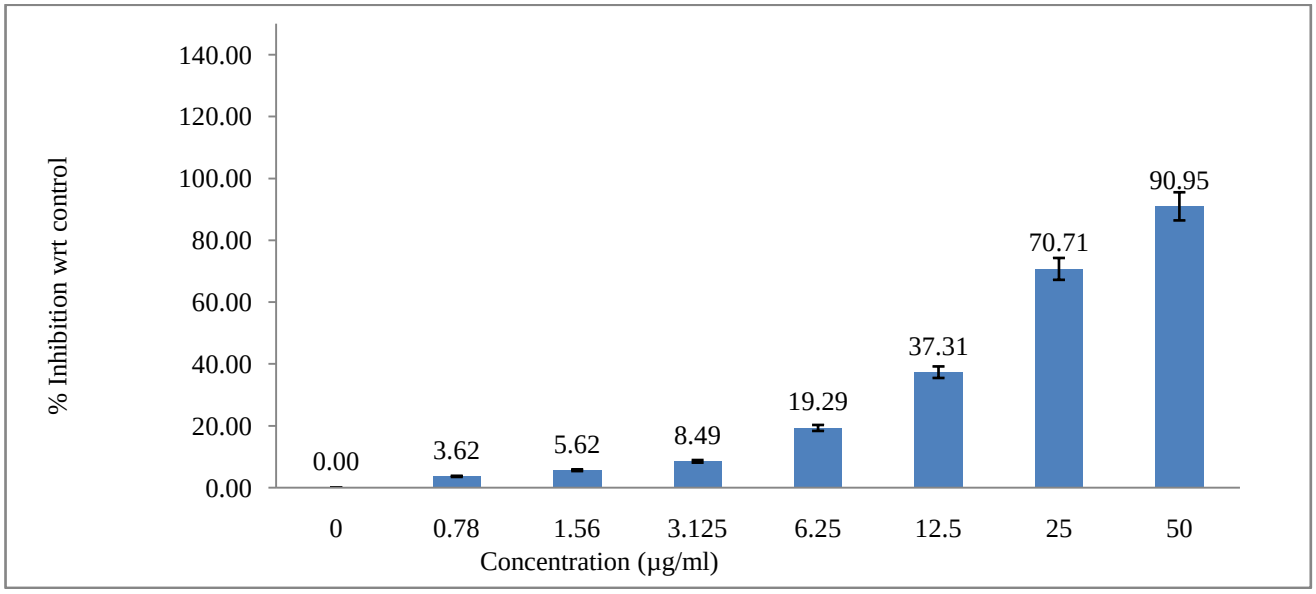

Figure-1a: DPPH Scavenging Assay - Ascorbic Acid.

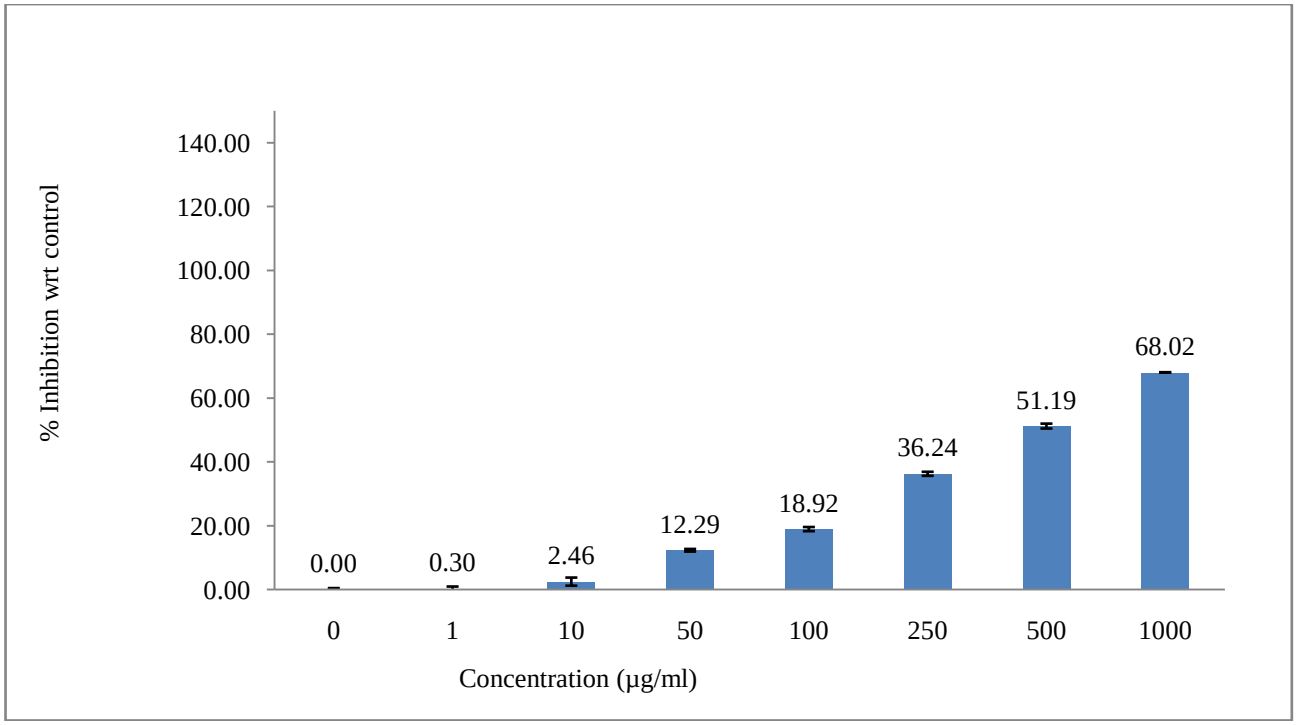

Figure-1b: DPPH Scavenging Assay – 001.

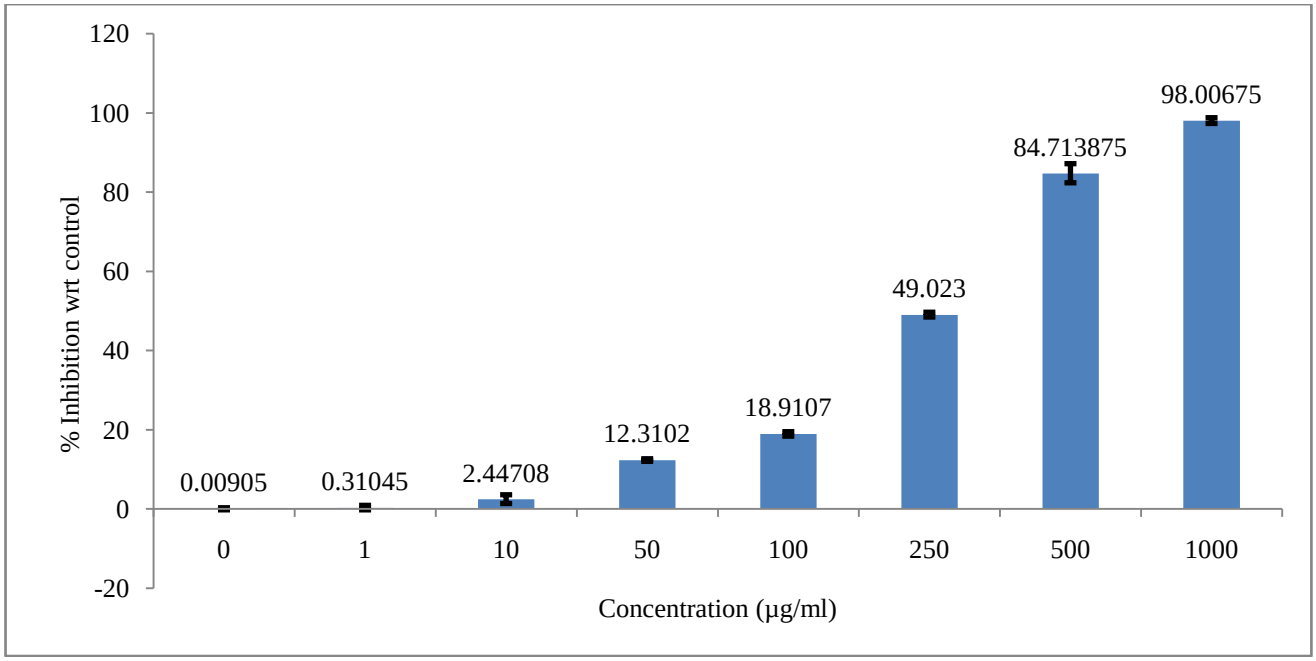

Figure-1c: DPPH Scavenging Assay – 002.

Table-4: % inhibition of standard (Ascorbic acid) and Compounds (001 and 002) at various concentrations.

Conc. (µg/ml)	Standard (Ascorbic acid)	Conc. (µg/ml)	001	002
0.78	3.62±0.5277	1	0.30±0.6315	0.31±0.6314
1.56	5.62±0.5952	10	2.46±1.2686	2.44±1.2688
3.125	8.49±0.7442	50	12.29±0.4122	12.31±0.412
6.25	19.29±0.4945	100	18.92±0.6517	18.91±0.6516
12.5	37.31±0.3494*	250	36.24±0.6355*	49.00±0.6351*
25	70.71±0.6031*	500	51.19±0.7648*	84.71±0.7647*
50	90.95±0.1192*	1000	68.02±0.0493*	98.00±0.0495*

Values represent mean ± S.E.M. of quadruplicate analysis (n = 4). *p < 0.05 indicates statistically significant difference compared to control. S.E.M.: Standard error of the mean.

Discussion: Suitability of *Calotropis procera* as host plant: The present study clearly establishes *C. procera* as the most suitable and sustainable host for cultivating *C. reflexa.* Its drought tolerance, persistent foliage, and strong physiological activity significantly enhance parasitic attachment and biomass accumulation. These observations align with earlier studies reporting that physiologically robust hosts improve parasitic establishment. In contrast, *Hibiscus rosa-sinensis* and *Duranta erecta* showed poor suitability, consistent with previous findings that ornamental plants with frequent pruning and softer tissues fail to support long-term parasitism. Given the natural abundance of *C. procera* in the semi-arid belt of Chhattisgarh, it emerges as a low-input, farmer-friendly, and ecologically safe host species for sustainable cultivation of *C. reflexa*.

Seasonal influence on growth and flowering: Seasonal variations strongly influenced the growth behavior of *C. reflexa*. Maximum biomass (28.9g) and highest flowering rates recorded during the monsoon season suggest that high humidity and moderate temperatures offer optimal parasitic and host physiological conditions. These findings support ecological evidence that parasitic plants exhibit enhanced metabolic activity and reproductive success during humid conditions. Although summer supported considerable growth, host stress symptoms indicated partial moisture limitation. However, the survival rate exceeding 85% throughout seasons confirms the reliability of *C. procera* as a year-round host capable of withstanding climatic fluctuations.

Extraction yield and biomass requirements: Extraction yields for both pre-flowering (001) and flowering (002) stages remained below 30%, indicating the need for substantial biomass to achieve adequate extract quantities. The darker coloration of the solvent during evaporation of flowering samples suggests greater phytochemical abundance, which aligns with findings that flowering tissues accumulate higher secondary metabolites. The higher percent recovery recorded for the flowering stage reinforces its importance for maximizing extract output. Comparison with natural/wild biomass further highlights the limitations of wild collection and the necessity of sustainable cultivation systems to ensure consistent raw material supply.

Antioxidant activity and stage-wise comparison: Both extracts exhibited measurable antioxidant potential with the following trend:
Ascorbic acid (standard): IC_{50} = 15.37 µg/mL
Flowering extract (002): IC_{50} = 257 µg/mL
Pre-flowering extract (001): IC_{50} = 460 µg/mL

The lower IC_{50} of the flowering extract indicates higher antioxidant strength, likely due to the enriched presence of synergistic phytochemicals. This is further supported by its higher Hill's coefficient (1.651), suggesting cooperative radical scavenging, whereas the pre-flowering sample (0.932) shows weaker cooperativity. These results closely match earlier values reported for *C. reflexa* from various hosts, such as in the range of 88.85–669.37 µg/ml[31], 212.61 µg/ml[32], 168.6 µg/ml[33], 295.12 µg/ml[5] and about 87.38 µg/ml[34]. This similarity confirms that cultivation on *C. procera* maintains phytochemical integrity and does not compromise antioxidant potential. Therefore, host-assisted cultivation provides a sustainable and reliable alternative to wild collection without reducing medicinal value.

Overall implications for sustainable cultivation: The combined findings on host suitability, seasonal growth patterns, extraction recovery, and antioxidant efficiency demonstrate that cultivated *C. reflexa* can effectively substitute wild-harvested material. The flowering stage emerges as the ideal harvesting period for maximizing both biomass and phytochemical yield. These insights are valuable for developing standardized, large-scale cultivation protocols that support biodiversity conservation, reduce dependence on wild plant populations, and ensure a continuous supply of high-quality *C. reflexa* biomass for medicinal applications.

Conclusion

The present study demonstrated that *C. procera* was found to be a highly suitable host for the sustainable cultivation of *C. reflexa*, owing to its drought tolerance, regenerative capacity, and minimal resource requirements. Seasonal growth assessments revealed that the monsoon season offers the most favorable conditions for biomass accumulation and reproductive output, thereby maximizing cultivation efficiency. Extraction yields remained below 30%, emphasizing the necessity of adopting sustainable cultivation strategies to ensure consistent biomass availability without exerting pressure on wild populations. Antioxidant assays highlighted that while both pre-flowering (001) and flowering (002) extracts exhibited moderate to good free radical scavenging activity, the flowering stage consistently outperformed the pre-flowering stage, as reflected by a lower IC_{50}, favorable logIC_{50}, and higher Hill's coefficient. These findings indicate that flowering-stage biomass of cultivated *C. reflexa* not only retains but also enhances antioxidant potential compared to wild-harvested material. Taken together, these findings underscore that the flowering-stage biomass of cultivated *C. reflexa* can be utilized with confidence in place of wild-harvested material, ensuring both therapeutic efficacy and conservation of natural populations.

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