

Comparative Phytochemical Profiling of Wild and *Calotropis procera*-Cultivated *Cuscuta reflexa* Roxb. and Evaluation of the Antimicrobial Potential of Cultivated Biomass

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Abstract:

Cuscuta reflexa Roxb. is a medicinal parasitic plant recognized for its diverse secondary metabolites and associated pharmacological properties. However, reliance on wild-collected biomass may constrain its sustainable utilization. The present study investigated whether cultivation on *Calotropis procera* could retain the qualitative phytochemical profile of *C. reflexa* while providing biomass with antimicrobial potential. Wild and cultivated *C. reflexa* were extracted separately with methanol, ethanol and chloroform and subjected to preliminary qualitative phytochemical screening. The methanolic extract of cultivated biomass was further evaluated for antibacterial and antifungal activity by the disc diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans*. The qualitative phytochemical profiles of wild and cultivated plants were highly comparable across all three solvents. Flavonoids and phenolic compounds were strongly detected in methanolic extracts, whereas terpenoids and steroids were more prominently detected in chloroform extracts, demonstrating clear solvent-dependent recovery of phytochemical classes. The cultivated methanolic extract exhibited antibacterial activity against both bacterial strains, with the highest inhibition zone against *S. aureus* (13.00 ± 1.73 mm) at 1000 µg/disc. Antifungal activity was comparatively limited, with inhibition zones of 6.00 ± 0.00 mm against both *A. niger* and *C. albicans* across the tested extract amounts. Overall, the retention of major detectable phytochemical classes in cultivated biomass, together with its measurable antibacterial activity, provides preliminary evidence supporting cultivation of *C. reflexa* as a potential sustainable source of bioactive biomass and warrants further quantitative phytochemical and pharmacological evaluation.

Keywords: *Cuscuta reflexa*, *Calotropis procera*, Phytochemical Profiling, Antimicrobial Activity, Sustainable Cultivation

1. Introduction

Cuscuta reflexa Roxb. (Convolvulaceae), commonly known as giant dodder or Amarbel, is a holoparasitic medicinal plant widely distributed throughout the Indian subcontinent and other tropical

and subtropical regions of Asia. Unlike autotrophic plants, *C. reflexa* lacks functional leaves and possesses very little chlorophyll; therefore, it depends entirely on host plants for water, minerals, carbohydrates, and other metabolites through specialized haustorial connections [1]. For centuries, *C. reflexa* has been used in Ayurveda, Unani, and traditional folk medicine for treating liver disorders, skin diseases, diabetes, inflammation, fever, urinary ailments, wounds, and various infectious diseases [2]. Modern pharmacological investigations have further demonstrated its antioxidant, antimicrobial, hepatoprotective, anti-inflammatory, antidiabetic, anticancer, immunomodulatory, cardioprotective, and neuroprotective properties, thereby validating many of its traditional therapeutic claims [3,4]. The remarkable medicinal importance of *C. reflexa* is attributed to its rich diversity of secondary metabolites. Phytochemical investigations have identified numerous bioactive compounds, including flavonoids, phenolic acids, alkaloids, coumarins, lignans, tannins, saponins, steroids, terpenoids, glycosides, β -sitosterol, lupeol, quercetin, kaempferol, amarbelin, and cuscutin. These metabolites exhibit significant antioxidant and antimicrobial activities and are considered valuable sources for pharmaceutical and nutraceutical development [5,6].

Unlike most medicinal plants, however, the phytochemical composition of *C. reflexa* is greatly influenced by the host plant because of the direct vascular connection established between parasite and host. Through these haustorial connections, not only nutrients but also metabolites and metabolic precursors may be transferred, leading to qualitative and quantitative variation in secondary metabolite accumulation. Consequently, geographical location, environmental conditions, developmental stage, and particularly host species can substantially influence the medicinal quality of *C. reflexa* [7,8].

Growing industrial demand for herbal medicines has resulted in extensive harvesting of *C. reflexa* from natural habitats, posing a serious threat to the sustainability of wild populations. Sustainable cultivation has therefore emerged as an essential strategy for conserving natural resources while ensuring a continuous supply of standardized medicinal biomass [9]. However, cultivation of obligate parasitic plants presents unique challenges because successful growth depends entirely on the availability of compatible host plants capable of supporting vigorous biomass production without compromising phytochemical quality [10]. Among the potential hosts, *Calotropis procera* has attracted considerable attention owing to its perennial growth habit, widespread distribution, adaptability to arid and semi-arid environments, and ease of cultivation. In addition, *C. procera* itself possesses a diverse array of phytochemicals, including flavonoids, terpenoids, cardenolides, steroids, and phenolic compounds, which contribute to its well-documented antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities [11]. These characteristics make *C. procera* an attractive host candidate for sustainable cultivation of *C. reflexa*.

Although successful cultivation of *C. reflexa* on selected hosts has been reported, limited information is available regarding whether cultivation on *C. procera* alters the phytochemical composition of the parasite compared with naturally occurring wild populations. This knowledge gap is particularly important because any significant variation in bioactive metabolites may influence the therapeutic efficacy and commercial acceptability of cultivated biomass [12]. Comparative phytochemical profiling therefore represents an important quality assessment tool for determining whether cultivated material can serve as a suitable alternative to wild-harvested plants. In addition to phytochemical characterization, biological validation through antimicrobial evaluation is equally important. The rapid emergence of antimicrobial resistance has intensified the search for novel plant-derived antimicrobial agents. Medicinal plants rich in phenolics, flavonoids, alkaloids, and terpenoids have demonstrated

promising inhibitory activity against a wide range of pathogenic microorganisms, making them valuable candidates for the development of new antimicrobial formulations [13,14,15]. Several studies have already demonstrated antimicrobial activity in extracts of *C. reflexa*; however, these investigations have largely focused on wild-collected plants, and comparable information on cultivated biomass remains scarce.

Despite increasing research on the phytochemistry and pharmacological properties of *C. reflexa*, comprehensive comparative studies evaluating phytochemical equivalence between wild populations and plants cultivated on a selected host are lacking. Furthermore, the antimicrobial potential of cultivated *C. reflexa* biomass has not been adequately investigated. Addressing these gaps is essential for developing sustainable cultivation protocols, conserving natural populations, and ensuring the availability of standardized medicinal raw materials for pharmaceutical applications. Therefore, the present study aimed to compare the phytochemical profiles of wild *C. reflexa* and plants cultivated on *C. procera* using qualitative and quantitative phytochemical analyses. In addition, the antimicrobial activity of the cultivated biomass was evaluated against selected microbial pathogens to determine its therapeutic potential. The findings are expected to provide scientific evidence supporting host-assisted sustainable cultivation of *C. reflexa* while contributing to quality standardization and conservation of this valuable medicinal species.

2. Materials and Methods

2.1 Plant Material

The plant material used in the present study was *Cuscuta reflexa* Roxb. (Family: Convolvulaceae), a perennial, obligate stem holoparasitic medicinal plant. For this investigation, both wild collected and experimentally cultivated plant materials were used to compare their phytochemical composition, antioxidant potential, and antimicrobial activity. Plant materials were harvested at appropriate developmental stages, processed under standardized conditions, and used for subsequent experimental analyses.

2.1.1 Wild Collection

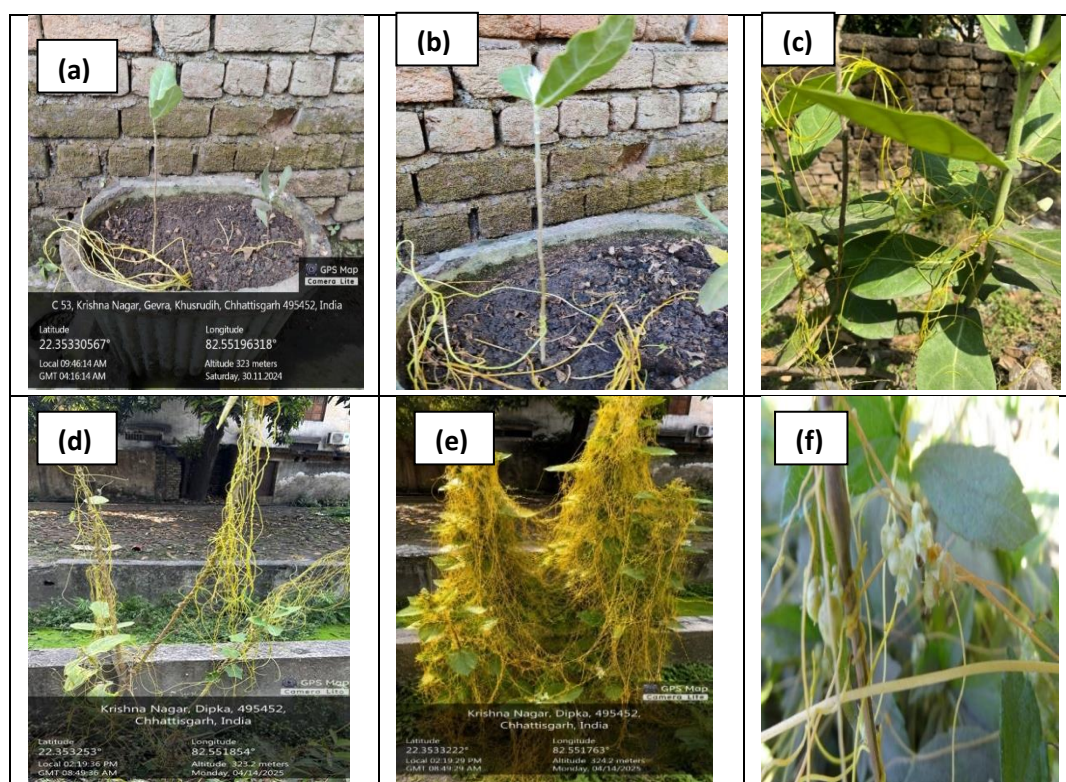
Wild specimens of plant samples were collected from Gevra (22.35° N, 82.68° E; ~300 m above mean sea level), Korba District, Chhattisgarh, India, in the month of September 2024. The area has a tropical wet-and-dry climate with an average annual rainfall of approximately 1,200–1,400 mm. Healthy parasitic vines growing naturally on their host plants were carefully harvested to ensure representative sampling. The collected plant material was thoroughly cleaned to remove dust, soil particles and adhering host plant debris before being processed for further analysis. A portion of the fresh material was preserved for taxonomic authentication, while the remaining samples were shade-dried at room temperature until a constant weight was achieved. The dried material was then pulverized into a fine powder using a laboratory grinder and stored in airtight containers until extraction.

2.1.2 Cultivated Sample

Calotropis procera was selected as the most suitable host for *C. reflexa* due to its excellent host compatibility, sustained support for vigorous parasite growth, high drought tolerance, and ease of cultivation. Its ability to thrive in nutrient-poor soils with minimal inputs and without competing with food crops makes it an ideal host for the sustainable cultivation of the under study parasitic plant. Healthy host plants were established prior to inoculation, and actively growing vines of *C. reflexa* were manually introduced onto the stems of the host to facilitate haustorial attachment (Figure 1). The

cultivation experiment was maintained under natural environmental conditions and monitored for approximately 10–12 weeks across all visible seasons. Growth and establishment of the parasite were documented periodically through photographic records and field observations.

Figure 1. Morphological Development of *Cuscuta reflexa* Roxb. on the Host Plant at Different Growth Stages. **(a)** Early Establishment of Seedling; **(b)** Developing Seedling With Elongating Stem; **(c)** Initial Twining and Attachment to the Host Plant; **(d)** Progressive Vegetative Growth and Spread of the Parasite Over the Host; **(e)** Mature, Extensively Developed *C. reflexa* Exhibiting Profuse Stem Growth; and **(f)** Flowering Stage Showing Inflorescences on the Host Plant.



2.2 Authentication

The collected plant material was taxonomically authenticated by the Botanical Survey of India (BSI), Western Regional Centre, Pune, Maharashtra, India. A voucher specimen was prepared and deposited at the Herbarium of the Botanical Survey of India for future reference. The plant was authenticated as *Cuscuta reflexa* Roxb., and the authentication certificate was issued under Reference No. BSI/WRC/Tech./2025/JVD-160.

2.3 Preparation of Plant Extracts

Hydroalcoholic extracts were prepared by the maceration method. Briefly, 5 g of powdered plant material was extracted with 50 mL of 80% methanol containing 1% hydrochloric acid. The extraction mixture was placed on a rocker shaker at room temperature for 24h to facilitate efficient extraction of bioactive constituents. The extracts were subsequently filtered through Whatman No. 1 filter paper, and the filtrates were concentrated by drying in a hot-air oven maintained at 40°C until complete solvent evaporation. The dried extracts were weighed to determine extraction yield and stored at 4°C in airtight containers until further phytochemical screening and antimicrobial analysis. Separate extracts were

prepared for the wild sample and the cultivated sample to facilitate comparative phytochemical evaluation.

2.4 Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of the hydroalcoholic extracts was carried out using standard procedures described by [16,17,18]. The phytochemical classes investigated, the corresponding qualitative tests, and the criteria for positive observations are summarized in Table 1. The phytochemical constituents investigated included alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, steroids, saponins, quinones, coumarins, and anthraquinones [19]. All tests were performed in triplicate to ensure reproducibility of the results.

Table 1. Qualitative Phytochemical Tests Employed for Screening Hydroalcoholic Extracts of Wild and Cultivated *Cuscuta reflexa* Roxb.

Sl.	Phytochemical	Test Used	Positive Observation
1.	Alkaloids	Mayer's/Wagner's	Cream or reddish-brown precipitate
2.	Flavonoids	Alkaline reagent	Yellow colour disappearing on acidification
3.	Phenolic compounds	Ferric chloride	Blue-black/green colour
4.	Tannins	Ferric chloride	Blue-black/greenish-black colour
5.	Glycosides	Keller–Killiani	Brown ring at interface
6.	Terpenoids	Salkowski	Reddish-brown interface
7.	Steroids	Liebermann–Burchard	Bluish-green colour
8.	Saponins	Foam test	Persistent froth
9.	Quinones	Concentrated H ₂ SO ₄ test	Red/blue/green colour
10.	Coumarins	UV fluorescence test	Yellowish-green or blue fluorescence
11.	Anthraquinones	Bornträger's test	Pink/red/violet ammoniacal layer

2.4.1 Detection of Alkaloids

Approximately 2 mL of each extract was acidified with 1% hydrochloric acid and filtered. A few drops of Mayer's reagent were added to one portion of the filtrate, while Wagner's reagent was added to another. The formation of a creamy white precipitate with Mayer's reagent or a reddish-brown precipitate with Wagner's reagent indicated the presence of alkaloids.

2.4.2 Detection of Flavonoids

Two millilitres of the extract were treated with a few drops of 10% sodium hydroxide solution. Formation of an intense yellow colour, which disappeared upon addition of dilute hydrochloric acid, confirmed the presence of flavonoids.

2.4.3 Detection of Phenolic Compounds

Two millilitres of each extract were mixed with 2–3 drops of 5% ferric chloride solution. The appearance of a bluish-black, dark green, or violet coloration indicated the presence of phenolic compounds.

2.4.4 Detection of Tannins

About 2 mL of the extract was treated with a few drops of 1% ferric chloride solution. The development of a blue-black or greenish-black colour indicated the presence of tannins.

2.4.5 Detection of Glycosides

Two millilitres of the extract were mixed with glacial acetic acid containing one drop of ferric chloride solution, followed by careful addition of concentrated sulphuric acid along the side of the test tube. Formation of a brown ring at the interface indicated the presence of cardiac glycosides.

2.4.6 Detection of Terpenoids

Two millilitres of the extract were mixed with 2 mL of chloroform, followed by careful addition of concentrated sulphuric acid to form a separate layer. A reddish-brown coloration at the interface confirmed the presence of terpenoids.

2.4.7 Detection of Steroids

The extract was treated with acetic anhydride followed by the careful addition of concentrated sulphuric acid. Development of a bluish-green colour indicated the presence of steroidal compounds.

2.4.8 Detection of Saponins

Approximately 2 mL of the extract was vigorously shaken with 5 mL of distilled water for 2 min and allowed to stand for 15 min. The formation of a stable froth layer measuring approximately 1 cm or more indicated the presence of saponins.

2.4.9 Detection of Quinones

Approximately 2 mL of each plant extract was treated with 1 mL of concentrated sulphuric acid and allowed to stand for 2–3 min. The appearance of a red, blue, or green coloration indicated the presence of quinones.

2.4.10 Detection of Coumarins

About 2 mL of the extract was placed in a test tube and covered with filter paper moistened with 10% sodium hydroxide solution. The test tube was heated gently in a water bath for a few minutes. The filter paper was then examined under ultraviolet (UV) light (365 nm). The appearance of a yellowish-green or blue fluorescence on the filter paper indicated the presence of volatile coumarins.

2.4.11 Detection of Anthraquinones

Approximately 2 mL of the extract was shaken with an equal volume of benzene and filtered. The benzene layer was separated and treated with an equal volume of 10% ammonium hydroxide solution. The development of a pink, red, or violet colour in the ammoniacal layer indicated the presence of free anthraquinones.

2.5 Microbial Strains

The antimicrobial activity of the methanolic extract of cultivated *C. reflexa* was evaluated against two bacterial and two fungal pathogens. The bacterial strains included the Gram-positive *Staphylococcus aureus* (MTCC 96) and the Gram-negative *Pseudomonas aeruginosa* (MTCC 3541), whereas the fungal strains comprised *Candida albicans* (MTCC 854) and *Aspergillus niger* (MTCC 281). All microbial cultures were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, through a certified microbiological laboratory.

Prior to antimicrobial testing, bacterial cultures were grown in Mueller–Hinton Broth (MHB), while fungal cultures were maintained in Sabouraud Dextrose Broth (SDB). The inoculum density for all microorganisms was adjusted to 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 CFU mL⁻¹, to ensure uniform microbial growth throughout the experiment. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method following standard microbiological procedures with slight modifications [20,21,22].

2.6 Antibacterial Activity Assay

The antibacterial activity of the methanolic extract of cultivated *C. reflexa* was evaluated against *Staphylococcus aureus* (MTCC 96) and *Pseudomonas aeruginosa* (MTCC 3541) using the disc diffusion method based on the Kirby–Bauer procedure (Bauer et al., 1966). Mueller–Hinton Agar (MHA) plates were uniformly inoculated with 100 μL of bacterial suspension adjusted to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU mL^{-1}). Sterile Whatman No. 1 filter paper discs (5 mm diameter) were loaded with 5 μL of methanolic extract, corresponding to 62.5, 125, 250, 500 and 1000 μg extract per disc, prepared from the experimental dilution series.

A disc containing DMSO was used as the vehicle control, while ciprofloxacin (3 $\mu\text{g}/\text{disc}$) served as the positive control. The prepared discs were aseptically placed on the inoculated MHA plates and incubated at 37 °C for 24 h. Following incubation, the antibacterial activity was assessed by measuring the diameter of the clear zone of inhibition surrounding each disc in millimetres. The assay was performed in triplicate, and the inhibition-zone measurements were recorded for subsequent statistical analysis.

2.7 Antifungal Activity Assay

The antifungal activity of the methanolic extract of cultivated *C. reflexa* was evaluated against *Aspergillus niger* using the disc diffusion method. Sabouraud Dextrose Agar (SDA) plates were inoculated with 100 μL of the standardized fungal suspension. Sterile Whatman No. 1 filter paper discs (5 mm diameter) were loaded with 5 μL of the methanolic extract, corresponding to 62.5, 125, 250, 500 and 1000 $\mu\text{g}/\text{disc}$, prepared from a 200 mg/mL stock solution according to the experimental dilution scheme.

A disc containing the extraction solvent served as the vehicle control, while Amphotericin B (100 $\mu\text{g}/\text{disc}$) was used as the positive control. The prepared discs were aseptically placed on the inoculated SDA plates and incubated at 37 °C for 24 h. Following incubation, antifungal activity was assessed by measuring the diameter of the clear zone of inhibition surrounding each disc in millimetres. The assay was performed in triplicate, and the inhibition-zone measurements were recorded for subsequent statistical analysis.

2.8 Statistical Analysis

The antimicrobial assay data were obtained from replicate measurements of inhibition-zone diameters for each extract amount and corresponding control. The diameter of each inhibition zone was recorded in millimetres, and the results were expressed as mean $\pm$ standard deviation (SD). For the antibacterial assay, three replicate measurements were obtained for *S. aureus*, while two replicate measurements were available for *P. aeruginosa*. For the antifungal assay, three replicate measurements were obtained for each treatment against both *C. albicans* and *A. niger*. Mean inhibition-zone diameter and SD were calculated for each extract amount (62.5, 125, 250, 500 and 1000 $\mu\text{g}/\text{disc}$) and control using Microsoft Excel. The antimicrobial responses were evaluated descriptively by comparing the inhibition zones produced by the different extract amounts with those of the respective vehicle and standard antimicrobial controls. Since the antifungal replicate measurements were identical across the tested extract amounts, resulting in zero variance (SD = 0), and no inferential statistical test was applied to the datasets, the antimicrobial results were interpreted descriptively.

3. Results

3.1 Comparative Phytochemical Screening

Preliminary phytochemical screening demonstrated a broadly comparable qualitative phytochemical profile in wild and cultivated *C. reflexa* extracts (Table 3). Flavonoids and phenolic compounds were most prominently detected in methanolic extracts, whereas terpenoids and steroids were more prominent in chloroform extracts. No qualitative differences were observed between wild and cultivated samples across the solvents tested.

Table 2. Comparative Qualitative Phytochemical Profile of Wild and Cultivated *C. reflexa* Extracts Obtained Using Different Solvents.

Phytochemical class	Wild Methanol	Wild Ethanol	Wild Chloroform	Cultivated Methanol	Cultivated Ethanol	Cultivated Chloroform
Alkaloids	++	++	+	++	++	+
Flavonoids	+++	++	–	+++	++	–
Phenolic compounds	+++	++	–	+++	++	–
Saponins	++	+	–	++	+	–
Tannins	++	+	–	++	+	–
Terpenoids	+	++	++	+	++	++
Steroids	–	+	++	–	+	++
Glycosides	++	+	–	++	+	–
Quinones	+	+	–	+	+	–
Anthraquinones	+	–	–	+	–	–

Note:+++ = strongly detected; ++ = moderately detected; + = weakly detected; – = not detected. Results represent qualitative phytochemical screening responses.

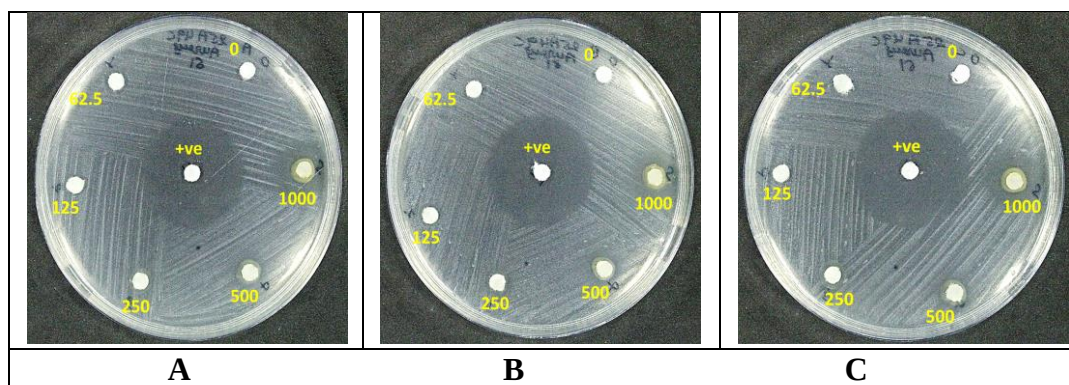
3.2 Antimicrobial Activity

The antimicrobial potential of the methanolic extract of cultivated *C. reflexa* was evaluated against two bacterial strains, *S. aureus* and *P. aeruginosa*, and two fungal strains, *C. albicans* and *A.niger*. The activity was assessed by measuring the diameter of the inhibition zone produced by different extract amounts ranging from 62.5 to 1000 µg/disc.

3.2.1 Antibacterial Activity

The methanolic extract exhibited measurable antibacterial activity against both bacterial test organisms, although the magnitude of inhibition varied between the two species (Table 4). Against *S. aureus*, the extract produced inhibition zones of 4.33 ± 3.79 , 4.33 ± 3.79 , 7.33 ± 0.58 , 8.67 ± 1.15 and 13.00 ± 1.73 mm at 62.5, 125, 250, 500 and 1000 µg/disc, respectively. Thus, the inhibition zone increased progressively from 250 to 1000 µg/disc, with the maximum inhibition observed at 1000 µg/disc. The positive control, ciprofloxacin (3 µg/disc), produced an inhibition zone of 28.33 ± 0.58 mm, which was substantially greater than that produced by the plant extract. The vehicle control did not produce a measurable antimicrobial effect.

Figure 2. Antibacterial Activity of the Methanolic Extract of Cultivated *C. reflexa* Against *S. Aureus* Using the Disc Diffusion Method. (A–C) Representative Replicate Plates Showing Extract Amounts of 62.5, 125, 250, 500 and 1000 µg/disc and Ciprofloxacin (3 µg/disc) as the Positive Control.



Against *P. aeruginosa*, the extract produced inhibition zones of 6.00 ± 0.00 , 7.00 ± 0.00 , 7.00 ± 0.00 , 7.00 ± 0.00 and 8.00 ± 0.00 mm at 62.5, 125, 250, 500 and 1000 µg/disc, respectively. The response therefore showed only a modest increase across the tested extract amounts. The positive control exhibited substantially greater inhibition.

Figure 3. Antibacterial Activity of the Methanolic Extract of Cultivated *Cuscuta reflexa* Roxb. Against *P. Aeruginosa* Determined by the Disc Diffusion Method. (A–C) Representative Replicate Plates Showing the Antibacterial Activity at Extract Concentrations of 62.5, 125, 250, 500, and 1000 µg/disc, With Ciprofloxacin as the Positive Control.

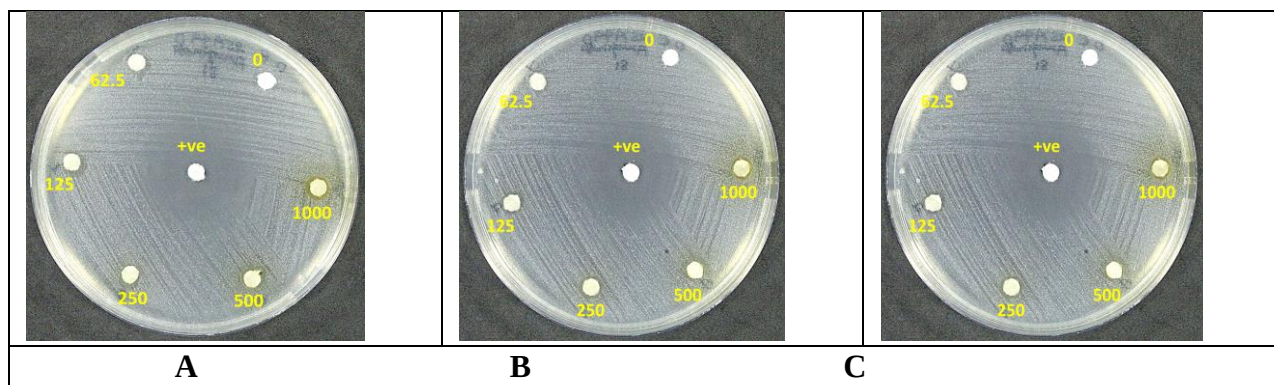


Table 3. Antibacterial Activity of Methanolic Extract of Cultivated *C. reflexa* Against Bacterial Test Strains.

Extract amount (µg/disc)	<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)
62.5	4.33 ± 3.79	6.00 ± 0.00
125	4.33 ± 3.79	7.00 ± 0.00
250	7.33 ± 0.58	7.00 ± 0.00
500	8.67 ± 1.15	7.00 ± 0.00
1000	13.00 ± 1.73	8.00 ± 0.00
Ciprofloxacin	28.33 ± 0.58	22.0 ± 0.00

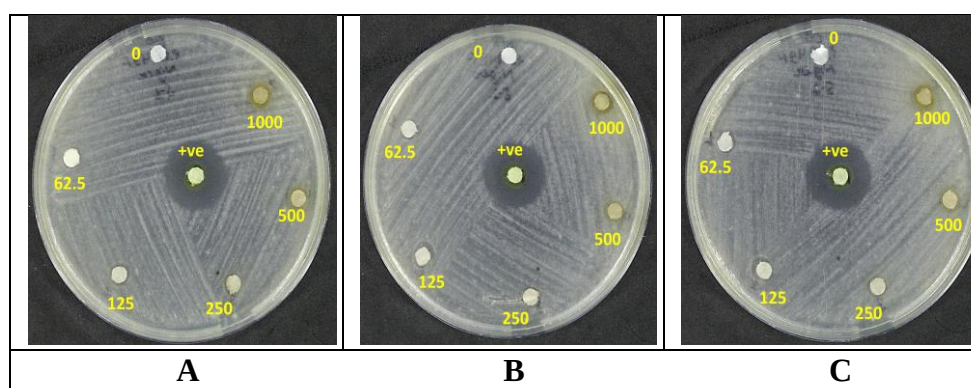
Extract amount (µg/disc)	<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)
Vehicle control	No inhibition	No inhibition

Note: Values are expressed as mean $\pm$ SD of the recorded replicate measurements. Antibacterial activity was assessed by disc diffusion.

3.2.2 Antifungal activity

The methanolic extract of cultivated *C. reflexa* also showed a limited inhibition response against the fungal test organisms. At all tested extract amounts (62.5–1000 µg/disc), the inhibition-zone diameter recorded for both *C. albicans* and *A.niger* was 6.00 ± 0.00 mm.

Figure 4. Antifungal Activity of the Methanolic Extract of Cultivated *Cuscuta reflexa* Roxb. Against *Aspergillus niger* Determined by the Disc Diffusion Method. (A–C) Representative Replicate Plates Showing the Antifungal Activity at Extract Concentrations of 62.5, 125, 250, 500, and 1000 µg/disc, With Amphotericin B (100 µg/disc) as the Positive Control.



For *A. niger*, the positive control, Amphotericin B (100 µg/disc), produced an inhibition zone of 19 mm, whereas the extract produced a 6-mm zone at all tested amounts. For *C. albicans*, the extract similarly produced a constant inhibition-zone diameter of 6.00 ± 0.00 mm across the tested range, while the corresponding positive control produced an inhibition zone of 11 mm. The absence of an increase in inhibition-zone diameter with increasing extract amount indicates that, under the conditions employed in this study, increasing the amount of methanolic extract from 62.5 to 1000 µg/disc did not produce a measurable increase in the diameter of the inhibition zone against either fungal strain.

Figure 5. Antifungal Activity of the Methanolic Extract of Cultivated *Cuscuta reflexa* Roxb. Against *Candida albicans* Determined by the Disc Diffusion Method. (A–C) Representative Replicate Plates Showing the Activity of the Extract at Concentrations of 62.5, 125, 250, 500, and 1000 µg/disc, With Amphotericin B (120 µg/disc) as the Positive Control.

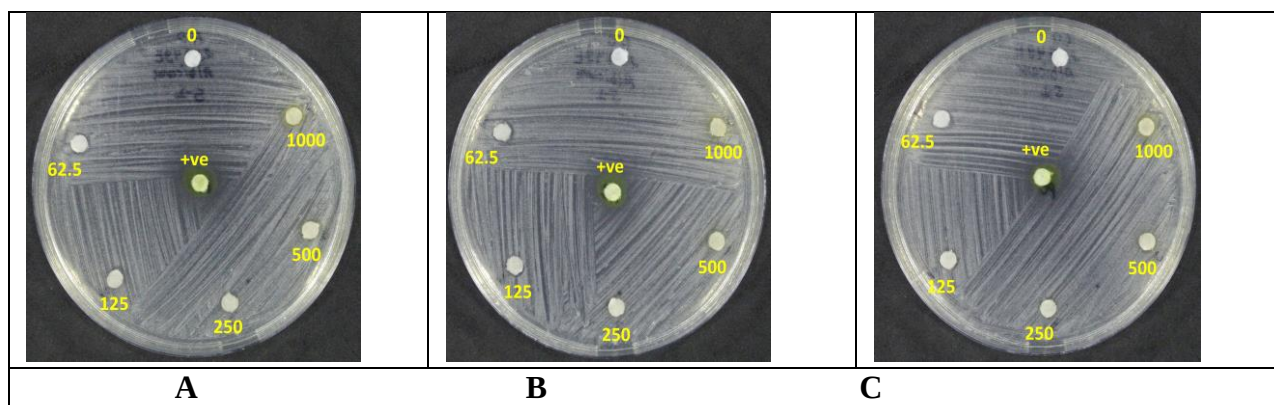


Table 4. Antifungal Activity of Methanolic Extract of Cultivated *C. reflexa* Against Fungal Test Strains.

Extract amount (µg/disc)	<i>A. niger</i> (mm)	<i>C. albicans</i> (mm)
62.5	6.00 ± 0.00	6.00 ± 0.00
125	6.00 ± 0.00	6.00 ± 0.00
250	6.00 ± 0.00	6.00 ± 0.00
500	6.00 ± 0.00	6.00 ± 0.00
1000	6.00 ± 0.00	6.00 ± 0.00
Amphotericin B	19.00± 0.00	11.00± 0.00
Vehicle control	No inhibition	No inhibition

Note: Values are expressed as mean ± standard deviation (SD) of three replicate measurements. Extract amounts represent the quantity of methanolic extract loaded per disc. The inhibition-zone diameter was measured in millimetres (mm).

3.3 Comparative Phytochemical Profile of Wild and Cultivated *C. reflexa*

The comparative screening demonstrated a high degree of qualitative similarity between wild and cultivated *C. reflexa*. All phytochemical classes detected in the wild material were also detected in the corresponding cultivated material, with identical qualitative responses across methanol, ethanol and chloroform extracts. No additional phytochemical class was detected exclusively in the cultivated material. The most prominent responses were observed for flavonoids and phenolic compounds in methanolic extracts, where both wild and cultivated samples showed strong positive reactions (+++). Similarly, alkaloids, saponins, tannins and glycosides showed comparable responses in the two plant materials. In ethanol extracts, terpenoids showed a moderate response (++), whereas chloroform extracts showed pronounced detection of terpenoids and steroids (++).

The similarity between wild and cultivated samples is particularly relevant to the objective of evaluating cultivation as a potential strategy for sustainable utilization of the plant. Under the cultivation conditions

employed in the present study, cultivation on *C. procera* did not result in an apparent qualitative loss of the major phytochemical classes detected in the wild material. These findings provide preliminary evidence that cultivated *C. reflexa* can retain the phytochemical repertoire characteristic of the wild plant. However, the present qualitative screening does not establish equivalence in the concentration of individual compounds between wild and cultivated samples. The symbols +, ++ and +++ indicate the relative intensity of the qualitative test response.

4. Discussion

4.1 Solvent-dependent Extraction and Phytochemical Profile

Patel et al. (2012) reported that *C. reflexa* contains diverse bioactive constituents and has been traditionally investigated for several medicinal and pharmacological properties. The present study demonstrated that the qualitative phytochemical profile of *C. reflexa* was influenced by the extraction solvent. Methanolic extracts showed prominent detection of flavonoids and phenolic compounds in both wild and cultivated samples, whereas terpenoids and steroids were more prominently detected in the chloroform extracts. Ethanol produced an intermediate profile, with several classes of phytochemicals detected at moderate or weak levels. Such solvent-dependent differences are expected because extraction efficiency is strongly influenced by the polarity and physicochemical characteristics of both the solvent and the target metabolites. The predominance of flavonoids and phenolic compounds in the methanolic extracts is particularly relevant because *Cuscuta* species have been reported to contain diverse phenolic and flavonoid constituents, supporting the phytochemical profile observed in the present study.

The differential response observed among methanol, ethanol and chloroform extracts also indicates that the use of a single extraction solvent may provide only a partial representation of the phytochemical repertoire of the plant. Previous work on *C. rerlexa* has similarly demonstrated solvent- and fraction-dependent differences in phenolic, terpenoid and alkaloidal constituents.[23], for example, reported different phytochemical characteristics among solvent fractions of *C. reflexa*, emphasizing the importance of extraction conditions in recovering chemically diverse constituents.

However, the present screening was qualitative, further confirmation and quantification of individual compounds would require chromatographic or spectrometric approaches such as HPLC, LC-MS or related analytical techniques.

4.2 Retention of the Phytochemical Profile Following Cultivation

One of the most important findings of the present study was the remarkable qualitative similarity between wild and cultivated *C. reflexa*. The same major phytochemical classes were detected in corresponding wild and cultivated extracts across methanol, ethanol and chloroform. In particular, the strong detection of flavonoids and phenolic compounds in methanolic extracts was retained after cultivation, while the solvent-associated detection patterns of terpenoids and steroids were also maintained. This finding is important because the objective of cultivation was not merely to produce biomass but to determine whether cultivated material could retain the phytochemical characteristics associated with the wild plant. The present observations suggest that cultivation under the experimental conditions employed, using *C. procera* as the host, did not result in an apparent qualitative loss of the major phytochemical classes detected in the wild. Furthermore, chemical composition in *C. reflexa* can vary according to biological and environmental factors, including the host plant, demonstrating that cultivation conditions are potentially important determinants of phytochemical composition. [24], for

example, investigated phenolic and flavonoid constituents of *C. reflexa* grown on different hosts and demonstrated variation in bioactive compound content among host-associated samples.

4.3 Relationship Between Phytochemical Composition and Antimicrobial Activity

Previous studies have documented a range of pharmacological activities associated with *Cuscuta* species, which have been attributed to their diverse phytochemical constituents [2]. The antimicrobial findings provide complementary biological evidence for the phytochemical observations. The methanolic extract of cultivated *C. reflexa* exhibited measurable antibacterial activity against both *S. aureus* and *P. aeruginosa*, although the magnitude of inhibition differed between the two organisms. The response against *S. aureus* increased with increasing extract amount, particularly at the higher amounts tested, whereas the response against *P. aeruginosa* was comparatively limited.

The antibacterial activity observed in the present study is consistent with previous reports describing antimicrobial properties of *C. reflexa*. The review by Noureen et al. (2019) summarizes antibacterial activity of the same plant extracts against several bacterial species, including *S. aureus* and *P. aeruginosa*. The comparatively strong detection of phenolic compounds and flavonoids in the methanolic extract provides a plausible phytochemical basis for the observed antibacterial response. The present study demonstrates that the cultivated extract contains several phytochemical classes and exhibits antibacterial activity.

4.4 Antifungal Response of Cultivated *C. reflexa*

In contrast to the antibacterial response, the methanolic extract showed only a limited and essentially unchanged inhibition-zone response against *A. niger* and *C. albicans* across the tested extract amounts. The measured inhibition zone remained approximately constant over the range of 62.5–1000 µg/disc, whereas the respective standard antifungal controls produced larger inhibition zones. This difference between bacterial and fungal responses may reflect differences in microbial cell structure, permeability and susceptibility to plant-derived metabolites. However, because the present study used a disc-diffusion assay, the inhibition-zone diameter is also influenced by the ability of extract constituents to diffuse through the agar medium. Consequently, differences in inhibition-zone size should not be interpreted solely as differences in intrinsic antimicrobial potency. The antifungal findings therefore provide a useful contrast to the antibacterial results: the cultivated extract demonstrated a more evident antibacterial than antifungal response under the conditions used in this study. Further investigations using quantitative susceptibility assays would be required to characterize the antimicrobial potency more precisely.

4.5 Relationship with Previously Reported Antioxidant Activity

The present findings are further supported by our earlier investigation of cultivated *C. reflexa*, in which enhanced antioxidant activity was observed at the flowering stage [25]. In that study, cultivated material grown using *C. procera* as the host retained antioxidant activity, and the flowering-stage material showed enhanced DPPH radical-scavenging activity compared with the pre-flowering material. The reported IC₅₀ values for the cultivated pre-flowering and flowering extracts were 460 and 257 µg/mL, respectively, compared with 15.37 µg/mL for ascorbic acid. Importantly, those antioxidant data were reported previously and are not presented as new results in the present manuscript. Instead, they provide complementary evidence that cultivated *C. reflexa* can retain biologically relevant properties following cultivation. The present study therefore extends the earlier antioxidant work by examining the

antimicrobial potential of cultivated biomass, thereby providing a broader assessment of its possible medicinal value [26].

4.6 Implications for Sustainable Utilization of *C. reflexa*

The most significant implication of the present findings is their relevance to the sustainable utilization of *C. reflexa*. Medicinal plants collected exclusively from natural populations may be vulnerable to repeated harvesting pressure, particularly when plant biomass is collected for commercial or medicinal purposes. Developing cultivation systems can provide a more controlled and potentially renewable source of biomass.

In the present study, *C. procera* supported the cultivation of *C. reflexa*, and the cultivated material retained the major phytochemical classes detected in the wild plant. This qualitative retention, together with the antibacterial activity observed in the cultivated extract and the previously reported antioxidant activity of cultivated material, provides preliminary support for the use of cultivated biomass as an alternative to reliance on wild-collected material. The broader literature also recognizes the biological richness of *Cuscuta* species and their potential as sources of pharmacologically relevant metabolites. However, "sustainable alternative" should be presented as a potential rather than as a conclusively demonstrated outcome. Long-term sustainability would require additional evaluation of biomass productivity, cultivation economics, host-plant effects, seasonal variation, phytochemical consistency and quantitative metabolite profiles over successive cultivation cycles.

The findings of the present study should be interpreted in light of certain limitations. Further studies involving quantitative phytochemical profiling, standardized susceptibility assays, and evaluation of different host plants, seasons and cultivation conditions would be valuable for establishing the consistency and broader applicability of cultivated *C. reflexa* biomass.

5. Conclusion

The present study demonstrated a remarkably comparable qualitative phytochemical profile between wild and cultivated *C. reflexa*, with the major phytochemical classes retained following cultivation on *C. procera*. The methanolic extract of cultivated biomass exhibited measurable antibacterial activity against *S. aureus* and *P. aeruginosa*, while comparatively limited antifungal activity was observed against *C. albicans* and *A. niger*. Together with the previously reported antioxidant activity of cultivated *C. reflexa*, the present findings support the potential of cultivated biomass as a sustainable source of bioactive material, thereby reducing reliance on wild-collected populations. The successful cultivation of *C. reflexa* on *C. procera* also indicates the potential for developing a controlled cultivation system for the production of standardized medicinal biomass. Cultivated biomass could be explored for future industrial applications in phytopharmaceutical, nutraceutical and natural-product sectors, subject to further validation of its chemical consistency, biological efficacy, safety, yield and economic feasibility. Thus, systematic cultivation and value-chain development may provide a promising approach for the sustainable utilization and potential commercialization of *C. reflexa*.

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