Research Article

Phytochemical Profiling, Antimicrobial Potential, and Biological Activity of *Cuscuta reflexa* from Semi-Arid Regions of Pakistan

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Abstract | *Cuscuta reflexa Roxb.* a parasitic plant with significant ethnomedicinal importance, was investigated for its phytochemical composition, physicochemical properties, and antimicrobial potential. Using Soxhlet extraction, methanol was identified as the most effective solvent for isolating diverse bioactive compounds, including flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, and phenolic compounds. GC-MS profiling revealed 12 bioactive compounds, with amino compounds (40.56%) and steroids (12.34%) predominant. Physicochemical analysis demonstrated a high foaming index and substantial solubility values, further highlighting the functional attributes of *C. reflexa*. The ethanolic extract exhibited dose-dependent antibacterial and antifungal activities, with significant inhibition zones against *Pseudomonas syringae* (25.95 mm), *Xanthomonas campestris* (24.27 mm), and *Fusarium oxysporum* (20 mm) at 100 mg/mL. Despite slightly lower activity than standard antibiotics (ciprofloxacin and fluconazole), the extract showed remarkable therapeutic potential. This study underscores the pharmacological versatility of *C. reflexa* as a rich source of bioactive compounds with strong antimicrobial, antioxidant, and therapeutic properties, positioning it as a promising candidate for developing natural alternatives to chemical pesticides.

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Introduction

Cuscuta reflexa Roxb. (Solanales: Convolvulaceae), a broad climber parasite, is commonly a parasitic weed in tropical and subtropical areas. It is an angiospermic, parasitic, leafless, epiphytic plant native to Pakistan, Nepal, India, and Bangladesh (Anjum *et al*., 2013). Therapeutic plants are mainly focused on their restorative sources (Ashraf *et al*., 2020). Almost 100-160 species belonging to Convolvulaceae are associated with parasitism on ornamental plants in Pakistan (Bobbarala *et al*., 2009).

Plants possess a minimal amount of chlorophyll content and a considerably low degree of photosynthesis making them unfit for surviving individually and leading to their reliance on the host plant. *C. reflexa* is God-gifted due to their medicinal value as it plays

a crucial role against numerous diseases including constipation, diarrhea, vomiting, and antispasmodics (Abu-Izneid *et al*., 2021). *C. reflexa* is utilized all over the world due to its ethnomedicinal value (Romeilah *et al*., 2021; Hameed *et al*., 2019). World Health Organization (WHO) also acknowledged its medicinal value and reported that up to 90% of the population uses natural medicine in agricultural countries (Muhammad *et al*., 2020). *C. reflexa* has been studied for the identification of its plant properties to be used as a medicinal plant. It possesses antiviral, anticonvulsant activities, bradycardia, antisteroidogenic, antispasmodic, and hemodynamic activities (Costa-Lotufo *et al*., 2005). Ethnobotanical literature is enriched with their phytochemical values, and it provokes their utilizations against multiple disorders (Abu-Izneid *et al*., 2021).

Multiple pharmacological studies have been conducted on this medicinal plant and revealed its antifungal, antioxidant (Perveen *et al*., 2013), low blood pressure, and antibacterial potential (Mala and Sofi, 2017). Studies revealed that *C. reflexa* have the best potential to minimize antihistamine (Ambi *et al*., 2017) anti HIV, antidiabetic, anticonvulsant (Rahmatullah *et al*., 2010), analgesic, diuretic, anthelmintic (Udavant *et al*.,2012; Chatterjee *et al*., 2011). The crude ethanolic extract of *C. reflexa* showed antimicrobial activity against *Escherichia coli* and *Shigella sonnei* (Ayesha *et al*., 2011). Previously, *C. reflexa* collected from different seasons exhibited antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, E. coli, Micrococcus luteus,* and *Psedomonas aeruginosa* (Shikha *et al*., 2013). *C. reflexa* Roxb. contains a diverse array of phytochemicals with potent antimicrobial properties, and its bioactive compounds can serve as effective natural alternatives to synthetic pesticides and antimicrobial agents. Present investigations were carried out to determine the chemical composition of *C. reflexa* cultivated under semi-arid conditions in Bahawalpur, Punjab, Pakistan. Furthermore, the antifungal and antibacterial values were also determined as an alternative to the chemical pesticides.

Material and Methods

Collection of plant material

Fresh samples of *C. reflexa* Roxb. were collected from the host plant *Vachellia nilotica* in the semi-arid region of Bahawalpur (29.3544° N, 71.6911° E) and Muzafargarh (30.0736° N, 71.1805° E) Punjab, Pakistan, during the peak growing season in May 2021. The collected plant material was carefully separated from the host plant and washed with distilled water to remove dust and impurities to determine the morphometric characteristics (Table 1). Furthermore, the samples were air-dried in a shaded, well-ventilated environment at ambient temperature (25–30 °C) for 14 days. Once dried, the material was ground into a fine powder using a mechanical grinder, sieved through a 40-mesh sieve, and stored in an airtight container at room temperature to prevent contamination and degradation.

Table 1: *Morphometric characteristics features of C. reflexa.*

S.	Stem features	Observations
1	Shape	1 to 5 mm diameter and round shaped
2	Color	Dried stem: Light Brown
		Fresh stem: Pale greenish
3	Taste	Bitter
4	Odor	Aromatic

Preparation of extracts

Solvent selection and Soxhlet extraction: The powdered plant material was subjected to Soxhlet extraction to isolate phytochemicals using methanol, ethanol, and chloroform as solvents. The choice of solvents was based on their polarity to ensure the extraction of both polar and non-polar compounds. Fifty grams of dried powder was placed in a cellulose extraction thimble. Solvent (300 mL) was added to the Soxhlet apparatus, and the extraction process was carried out for 4 hours at a temperature corresponding to each solvent's boiling point (64.7°C for methanol, 78.4°C for ethanol, and 61.2°C for chloroform). After extraction, the solvent was evaporated using a rotary evaporator at 40°C under reduced pressure to obtain concentrated crude extracts.

Physicochemical analysis

The physicochemical parameters, including foaming index, water solubility values, alcohol solubility values, drying loss, and total ash content, were determined for the powdered plant material following the protocols outlined in the World Health Organization's guidelines on quality control methods for medicinal plant materials. The foaming Index was determined by shaking the 1% (w/v) decoction of the powdered material vigorously for 15 seconds, and the height of

the foam was recorded after standing for 15 minutes. However, the total ash, acid-insoluble ash, and water-soluble ash were determined by incinerating the sample in a muffle furnace at 450°C until a constant weight was achieved. However, phytochemical screening of secondary metabolites was determined by following the standard protocol as described by Kalita *et al*. (2017).

Phytochemical screening

Standard qualitative tests were performed to detect the presence of bioactive compounds in the methanol and chloroform extracts of *C. reflexa*. Alkaloids were determined through Dragendorff's reagent; 1% Hydrochloric acid, Dragendroffs reagent, and Mayers reagent were added to the extract and organic precipitate revealed the presence of alkaloids in the given sample. To assess the flavonoids, an alkaline reagent test was performed and a solution of dilute ammonia 5 ml was mixed with the aqueous extract and hydrosulfuric acid, leading to the formation of yellow coloration and confirming flavonoids presence. Additionally, a ferric chloride test was conducted to determine tannins, and lead acetate 1% was added to 5 ml extract and the formation of yellow precipitate expressed tannins presence. Saponins were determined by agitating plant extract along with 20 ml distilled water for 15 minutes in the graduated cylinder, and a 1cm foam layer was formed which indicates saponins formation. Steroids were assessed by adding 2ml of H_2SO_4 and acetic anhydride to the extract 0.5 gm. The color of the extract changed to green or blue from violet and revealed the presence of steroids. Terpenoids were confirmed through the addition of 3 ml of concentrated H_2SO_4 and 2 ml of chloroform to 2 ml of extract and it resulted in the formation of reddish brown monolayer coloration and represented terpenoids constituents. Tannins tests were performed by the addition of Lead acetate 1% drops to 5 ml extract and the formation of yellow precipitate expressing tannins presence. However, the Phenolic compounds were assessed by adding 3 ml of ethanol with a pinch of FeCL3 to 2 ml of extract and the formation of a greenish yellow color indicated phenol presence.

GC-MS analysis

The methanol and ethanol extracts were subjected to GC-MS analysis to identify the chemical composition. GC-MS System (Agilent Technologies 7890A GC) system equipped with a 5975C mass selective detector was used with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) and helium at a flow rate of 1 mL/min. The National Institute of Standards and Technology (NIST) database was used to identify compounds based on their mass spectra.

Antimicrobial activity

Bacterial strains and antibacterial assay: Three bacterial strains (*Pseudomonas syringae*, *Xanthomonas campestris*, and *Bacillus subtilis*) were obtained from a certified microbiological laboratory. The strains were subcultured on Mueller-Hinton agar plates and incubated at 37°C for 24 hours before use. The antibacterial activity of the ethanolic extract was evaluated using the disk diffusion method. Sterile paper disks (6 mm in diameter) were impregnated with different concentrations of the extract (25, 50, 75, and 100 mg/mL). Ciprofloxacin (10 mg/mL) was used as the positive control and evaluated against *B. subtilis*. The disks were placed on inoculated agar plates and incubated at 37°C for 24 hours. Furthermore, zones of inhibition were measured in millimeters.

Fungal strains and antifungal assay

Fungal strains (*Fusarium oxysporum*, *Aspergillus flavus*, and *Aspergillus niger*) were subcultured on Sabouraud dextrose agar plates. The well diffusion method was used to assess antifungal activity. Wells (6 mm in diameter) were created in agar plates using a sterile cork borer. Different concentrations of ethanolic extract (25–100 mg/mL) were added to the wells. Fluconazole (10 mg/mL) was used as the positive control and evaluated against A. *niger*. However, plates were incubated at 28°C for 48 hours, and inhibition zones were measured.

Antitumor and antioxidant activity

Extracts were applied orally, and Ethanol, chloroform *C. reflexa* activity was measured towards Ehrlich ascites carcinoma tumor in different mice at the concentrations of 200 and 400 mg/kg weight of their body. However, the calculation of no-enzymatic hemoglobin glycosylation under in vitro conditions was used to determine antioxidant activity.

Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard error. Statistical analysis was conducted using SPSS (v.25) with ANOVA and Tukey's post hoc test. Significance was set at $p < 0.05$.

Results

Assessment of phytochemical and physiochemical values of C. reflexa

GC-MS analysis for the phytochemical composition of *C. reflexa* revealed that fluoro and phosphorus compounds exhibited maximum percentages, indicating their predominance in the extract followed by nitrogen, aromatic compounds, alkaloids, chlorine compounds and silica (Figure 1A). Additionally, physiochemical analysis of *C. reflexa* unveiled the dominance of high foaming index followed by water-soluble values, alcohol soluble values, and drying loss and the total ash contents (Figure 1B).

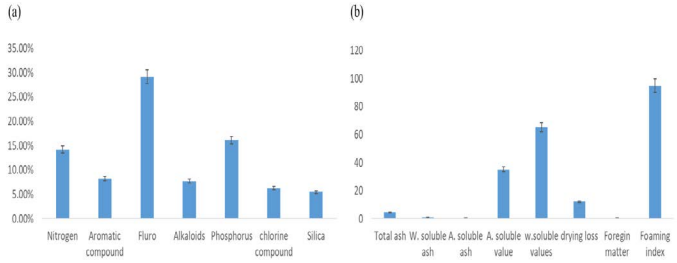

Figure 1: *(a) Screening of the phytochemical values of C. reflexa on GC-MS analysis, (b) Dominance of the physiochemical values from the C. reflexa.*

Table 2: *Phytochemical response of C. reflexa in methanol and chloroform extracts.*

S. No	Chemicals	Methanol extract	Chloroform extract
1	Steroids	+	+
2	Flavonoids	+	-
3	Alkaloids	+	-
4	Terpinoids	+	+
5	Phenolic compounds	+	+
6	Quinones	-	-
7	Coumarins	+	+
8	Saponines	+	-
9	Tannins	+	-
10	Anthraquinone	-	-

The qualitative analysis of phytochemicals in *C. reflexa* extracts demonstrated a varied profile depending on the solvent used. The methanol extract exhibited a more comprehensive range of bioactive compounds compared to the chloroform extract, suggesting methanol is a better solvent for extracting phytochemicals. Steroids, terpenoids, phenolic compounds, and coumarins, which are known for their broad-spectrum biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties, were common in both extracts. Additionally, flavonoids, alkaloids, saponins, and tannins, known for their medicinal properties, including anticancer, antimicrobial, and hepatoprotective effects were detected only in the methanol extract. However, Quinones and anthraquinones were not detected in both extracts and it indicated that these compounds are not a characteristic feature of *C. reflexa*. The solvent-specific extraction highlighted the importance of the polarity of solvents in maximizing the yield of desired compounds (Table 2).

Assessment of the C. reflexa ethanolic extract against bacterial and fungal strains

The ethanolic extract of *C. reflexa* was further tested against multiple bacterial and fungal species to determine their inhibitory effect. Results revealed that extract caused the maximum inhibition zone of 25.95mm against *P. syringae* followed by *X. campestris* (24.27mm), and *B. subtilis* (20.68mm) at the maximum concentration of 100mg/ml on comparison to control ciprofloxacin which exhibited the maximum inhibition zone of 42.03 mm against *P. syringae* at the rate 10 mg/ml. However, the minimum inhibition zones against all tested bacterial species were recorded during the application of a minimum concentration of 25 mg/ml. *C. reflexa* exhibited an inhibition zone of 14.95 mm against *P. syringae*, and an inhibition zone of 12.01 mm against *X. campestris* (Table 3). However, the efficacy of *C. reflexa* against the bacterial species was dosage-dependent, as the maximum concentration caused maximum inhibition. Furthermore, the extract of *C. reflexa* was further tested against three fungal species and the results demonstrated that it also had a significant inhibitory effect against *F. oxysporum*, where it caused an inhibition zone of 20 mm followed by 14.97,11.92mm, 8.14mm at the concentrations of 75, 50 and 25 mg/ml, respectively. However, the inhibition zone of 17.06 mm was recorded against *A. flavus* followed by the 12.07 mm zone against *A. niger* at a maximum 100 mg/ml concentration in comparison to the application of Fluconazole which caused an inhibition zone of 25.79 mm at the concentration rate of 10mg/ml (Table 4).

The phytochemical GC-MS analysis of *C. reflexa* from Bahawalpur, Punjab, Pakistan, revealed that about 12 compounds were identified in plant samples. Among all compounds, amino compounds recorded the highest value (40.56) followed by steroids (12.34), palmitic acid (14.75), mono-unsaturated fatty acids

Table 3: *Summary of the inhibition zones caused by the C. reflexa against bacterial species at different concentrations.*

Bacterial species	Inhibition (mm) at 25 mg/ml	Inhibition (mm) at 50 mg/ml	Inhibition (mm) at 75 mg/ml	Inhibition (mm) at 100 mg/ml
P. syringae	14.95g	18.75f	20.84e	25.95c
X. campestris	12.01i	14.96g	18.017f	24.27d
B. substilis	10.38j	13.57h	15.77g	20.68e
Ciprofloxain (10mg/ml)	42.41a	42.26a	40.61 b	42.03a
SE	0.5394			
CV	1.1016			

**Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).*

Table 4: *Summary of the inhibition zones caused by the C. reflexa against bacterial species at different concentrations.*

Fungal species	Inhibition (mm) at 25 mg/ml	Inhibition (mm) at 50 mg/ml	Inhibition (mm) at 75 mg/ml	Inhibition (mm) at 100 mg/ml
F. oxysporum	8.14g	11.92 f	14.97e	20.00c
A. flavus	11.18f	12.00f	14.17e	17.06d
A. niger	6.15 h	9.18g	11.26f	12.07f
Fluconazole (10mg/ml)	26.11ab	25.13b	26.35a	25.79ab
SE	0.5753			
CV	1.1750			

**Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).*

Table 5: *Determination of plant sample constituents.*

S.	Compound	Molecular formula	Area (%)	Nature of the compound	Response
1	Dodecanoic acid	$C_{12}H_{24}O_2$	2.58	Lauric acid	Anti-inflammatory, antiseptic, antipyretic
2	Tetradecane	$C_{14}H_{30}$	0.07	Alkanes	No activity
3	Tetradecanoic acid	$C_{14}H_{28}O_2$	2.88	Myristic acid	Nematicide, antioxidant
4	Phenol, 3,5-bis (1,1- dimethylethyl)	$C_{14}H_{22}O$	0.09	Phenolic compound	Anesthetic,antiseptic, antibacterial,
5	Tris (1,3-dichloro isopropyl) phosphate	$C_9H_{15}C_{16}O_4P$	2.36	Chlorine compound	Antimicrobial
6	1,2-Benzenedi carboxylic acid, bis (2-methyl propyl)	$C_{16}H_{22}O_4$	1.93	Plasticizer compound	Antifouling
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	14.75	Palmitic acid	Pesticide, hemoytic, antioxidant,
8	Hexadecenoic acid	$C_{16}H_{30}O_2$	3.12	Palmitolic acid	Hypocholesterolemic
9	Phytol	$C_{20}H_{40}O$	2.48	Diterpene	Diuretic, antimicrobial, anti-inflammatory
10	Oleic acid	$C_{18}H_{34}O_2$	6.16	Mono unsaturated fatty	Antiandrogenic,dermatitigenic, cancer preventive,
11	Cholestan-3-one, Cyclic1,2 ethanediyl	$C_{29}H_{50}O_2$	12.34	Steroid	Antiarthritic, anti-inflammatory, antiasthma
12	Tetrazol-5-amine, N- (3,4dimethoxy-benzyl)	$C_{10}H_{13}N_5O_2$	40.56	Amino compounds	Antimicrobial

(6.16), palmitolic acid (3.12), myristic acid (2.88), diterpene (2.48), chlorine compound (2.36), plasticizer compound (1.93), and phenolic compounds (0.09) and alkanes (0.07%). Generally, identified compounds exhibited antitumor, anti-inflamatory, antimicrobial, and anticarcinogenic characteristics (Table 5).

Discussion

Medicinal plants are a treasure trove of undiscovered compounds. These plant sources are important resources in the traditional medical system due to their polyvalent action and minimum adverse effects (Mahesh and Satish, 2008). Phytochemicals

have potential health advantages against multiple disorders, including cardiovascular diseases, cancer, and osteoporosis. Plant secondary metabolites function as a plant defense system, protecting the plant against microbes, insects, and herbivores (Erb and Kliebenstein, 2020). The phytochemical and physiochemical analyses of *C. reflexa* highlight its rich bioactive composition and significant biological potential.

GC-MS profiling revealed the predominance of fluoro and phosphorus compounds, followed by nitrogen-containing, aromatic, and alkaloid compounds, underscoring the diversity of chemical constituents. Azad and Mohamed (2023) also demonstrated that *C. reflexa* stem extract, rich in phenolic and flavonoid compounds, exhibits potent antioxidant activity. These findings highlight its potential as a natural therapeutic and nutritional resource. The physiochemical analysis further demonstrated a high foaming index and substantial solubility values, providing insights into its functional attributes. Previously, Rai *et al*. (2016) and Afrin *et al*. (2019) also performed the phytochemicals analysis of *C. reflexa* extract and reported that the presence of chemical compounds mainly depends on the nature of the host plant.

Solvent-specific extraction revealed methanol as a superior solvent for isolating a broader range of phytochemicals, including flavonoids, alkaloids, saponins, and tannins, known for their anticancer, antimicrobial, and hepatoprotective properties. The absence of quinones and anthraquinones suggests these are not characteristic compounds of *C. reflexa*. These findings emphasize the role of solvent polarity in optimizing the extraction of bioactive constituents.

The ethanolic extract demonstrated dose-dependent antibacterial and antifungal activities, with significant inhibitory effects against *P. syringae*, *X. campestris*, and *F. oxysporum*. Maximum inhibition zones were observed at the highest concentrations, underscoring the extract's potential as a natural antimicrobial agent. *C. reflexa* exhibits significant antibacterial properties, with its methanolic extract demonstrating substantial activity against uropathogens and potent antioxidant capabilities (Tripathy *et al*., 2024). These findings highlight its potential as a natural alternative for pharmaceutical applications.

GC-MS analysis identified 12 bioactive compounds, with amino compounds and steroids being the most abundant. These compounds exhibited antitumor, anti-inflammatory, antimicrobial, and anticarcinogenic properties, further validating the pharmacological potential of *C. reflexa*. The results of the contemporary study are in line with the agreement of Khan *et al*. (2019) and Gangarde *et al*. (2024) that *C. reflexa* Roxb. exhibits diverse pharmacological activities, including anticancer, anti-inflammatory, antibacterial, and antifungal properties. Its ability to modulate inflammation highlights its potential for managing infections. The results are also supported by the findings of Kumari *et al*. (2024), who also demonstrated that phytochemical analysis and GC-MS profiling of *C. reflexa* identified 79 bioactive compounds, including n-hexadecanoic acid, phytol, and stigmasterol, with diverse therapeutic properties. These findings underscore its potential for antioxidant, antibacterial, anti-inflammatory, and other medicinal applications. Collectively, these findings suggest that *C. reflexa* is a promising candidate for developing natural therapeutic agents targeting multiple infections.

Conclusions and Recommendations

This study establishes *C. reflexa* as a valuable source of bioactive compounds with diverse pharmacological properties, including antimicrobial and anti-inflammatory activities. The findings highlight its potential for developing natural therapeutics and eco-friendly antimicrobial agents, with methanol proving effective for extracting key phytochemicals. Further studies should explore the molecular mechanisms underlying the bioactivity of the identified compounds to validate their therapeutic efficacy. Advanced in vivo studies are essential to confirm the safety and pharmacological potential of *C. reflexa* for agricultural or human applications. Investigation of the synergistic effects of *C. reflexa* extracts with conventional antibiotics could pave the way for enhanced antimicrobial formulations. Furthermore, biotechnological approaches, such as nanoformulation and encapsulation, could be employed to enhance the bioavailability and stability of its bioactive compounds.

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In the preparation of this manuscript, advanced language processing technologies were employed

to refine its linguistic quality, ensuring clarity and coherence. The authors collectively assume full responsibility for the content, affirming that the manuscript's integrity is preserved and reflects a commitment to high standards of scholarly communication. All authors have thoroughly reviewed and endorsed the final version of the manuscript.

Novelty Statement

This study is the first to comprehensively assess the phytochemical profile, physicochemical attributes, and antimicrobial potential of *C. reflexa* from semi-arid regions of Pakistan. Utilizing Soxhlet extraction and GC-MS profiling, 12 bioactive compounds were identified, with amino compounds and steroids dominating the composition. Furthermore, the ethanolic extract displayed dose-dependent antibacterial and antifungal activities against multiple pathogens, offering an eco-friendly alternative to chemical pesticides. These findings underscore *C. reflexa* as a promising source for developing natural therapeutic and antimicrobial agents.

Author's Contribution

Shoukat Hayat: Designed the methodology, validation, writing- original draft.
Irfan Ashraf: Supervised throughout the research process, validation and project administration.

Conflict of interest
The authors have declared no conflict of interest.

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