A Comparative Study on Phytochemical Diversity and Bioefficacy of *Cuscuta reflexa* and *Cuscutac ampestris in* Antimicrobial and Antioxidant Assays

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Abstract - Traditionally, *Cuscuta campestris* and *Cuscuta reflexa* have been valued medically throughout different parts of Uttar Pradesh and Uttarakhand. Whole Plants extracts from both species were compared in view of their ethnobotanical relevance in order to evaluate their phytochemical composition, antibacterial efficacy, and antioxidant activity. Using the disc diffusion technique against both Gram-positive (Staphylococcus *aureus*) and Gram-negative bacteria including Pseudomonas aeruginosa, Escherichia coli and *Klebsiella pneumoniathe* antibacterial potential was assessed. The benchmark antibiotic control was ampicillin. Among the investigated extracts, ethanol-based ones showed the best antibacterial activity; *S. aureus* showed the highest sensitivity among all the bacterial strains tested. Measuring their DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity, the crude plant extracts revealed notable antioxidant potential. Several bioactive secondary metabolites were found by preliminary phytochemical screening.

Keywords:*Cuscuta reflexa, Cuscuta campestris*, phytochemical screening, Antibacterial activity, Antioxidant potential, Medicinal parasitic plants, Secondary metabolites, Natural bioactive compounds

I. INTRODUCTION

For centuries, natural sources have served as the foundation for drug discovery, with many modern pharmaceuticals being derived from bioactive compounds found in plants. These natural products continue to play a significant role in conventional therapeutic practices worldwide (Owolabi *et al*., 2017). Medicinal plants, in particular, have contributed to the development of numerous potent and widely used drugs, reflecting their critical importance in global healthcare syWholePlantss (Mahesh & Satish, 2019). Furthermore, plant-derived natural compounds and their synthetic analogs are increasingly explored for their potential in cancer prevention and treatment (Xua *et al*., 2019). *Cuscuta reflexa*, commonly known as "Amarbel," belongs to the family Cuscutaceae and is a parasitic plant native to tropical and subtropical regions. It grows as a leafless, twining vine that derives nutrients from its host plants. Phytochemical investigations have revealed the presence of various bioactive constituents in the ethanolic extract of C. *reflexa* Whole Plantss, including alkaloids, flavonoids, glycosides, carbohydrates, tannins, phenolic compounds, and steroids (Inamdar *et al*., 2011). The antimicrobial activity of this plant has been primarily attributed to its flavonoids and glycosides. Additionally, several studies have highlighted its anticancer and immunostimulatory properties (Anjum, *et al*., 2023). The Whole Plants of *C. reflexa* is traditionally used to treat fever when administered internally, and externally for relieving skin conditions such as itching (Bais & Kakka, 2024). It also demonstrates significant anti-inflammatory and anticancer properties (Pandit *et al*., 2019). Diuretic effects have been observed in both aqueous and alcoholic extracts of this species (Suresh et al., 2011), while the aqueous crude extract has shown promising anti-HIV activity (Sharma *et al*., 2019).

Cuscuta campestris, commonly referred to as "field dodder," is considered one of the most aggressive and destructive species within the genus due to its extensive host range and widespread geographical distribution (Holm *et al*., 1997). It thrives in both temperate and tropical climates and demonstrates adaptability to extreme environmental conditions. During its growth cycle, chlorophyll content in C. *campestris* increases until the flowering stage (Dinelli *et al*., 1993). This parasitic plant significantly reduces the yield of several economically important crops, underlining its agricultural significance (Farah & Alabdulsalam, 2004). Remarkably, both C. *campestris* and C. *reflexa* can grow and reproduce in vitro, producing flowers and fruits under laboratory conditions (Malik & Singh, 1980). The extracts of C. *campestris* have been found to possess antipyretic, analgesic, and anti-inflammatory activities (Agha *et al*., 1996). Given the diverse medicinal attributes of Cuscuta species, the present study was undertaken to evaluate and compare the phytochemical profiles, antibacterial efficacy, and antioxidant potential of Whole Plants extracts from *Cuscuta campestris* and *Cuscuta reflexa.*

II. MATERIALS AND METHODS

This research was carried out at Swami Vivekanand Subharti University Meerut, Uttar Pradesh, India, specifically at the Department of Biotechnology. All around the state of Uttar Pradesh and Uttarakhand, fresh plant samples of *Cuscuta campestris* and *Cuscuta reflexa* were gathered from a variety of ecological zones. At Swami Vivekanand Subharti

University, the Department of Botany was responsible for carrying out the taxonomic identification and authenticity of the specimens that were collected. These processes were validated. Whole Plants were air-dried in the shade for seven days at ambient temperature. Dried plant materials were pulverized with an electric grinder. Fifty grams of powdered Whole Plants from each plant was dissolved in 200 milliliters of methanol, distilled water, ethanol, and chloroform, and maintained in darkness with continuous shaking every 24 hours for duration of seven days. Subsequently, it was filtered into a conical flask. The residue was eliminated, and the filtrate was evaporated to provide the crude extract. Twenty milligrams of each crude extract was diluted in 2 milliliters of the corresponding solvents and utilized for biological assays. The prevalent human pathogens, including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae,* were acquired from the Microbiology section of IMTECH Chandigarh.

Disc diffusion approach (Mahesh & Satish, 2019) was used to investigate antibacterial properties of several *C. campestris* and *C. reflexa* samples. Poured into the Petri plates and stored for solidification, the sterilized nutrient agar media—dissolving 28 g dried nutrient agar in 1000 ml distilled water was warmed and shook. Sterilized disc dipping with extracts was pour on Petri plates after solidification and incubated for 24 hours at 37ºC. Following that pathogenic cultures were cleaned on the specific agar plates with sterile cotton pads. Three times was the experiment carried out. To assess the antibacterial activity, the diameters of inhibition zones developed around each disc were measured and stated in millimeter (mm) plus standard deviations of means after incubation.

Major phytoconstituents of the plant extracts were investigated phytochemistically. Medically, phytocompounds can have a broad spectrum of effects. Prepared and confirmatory tests for the presence of significant chemicals were conducted on distilled water, ethanolic and methanolic extracts of the plants (Tiwari *et al*., 2011).

Crude extractive antioxidant activity was assessed using 1, 1-diphenyl-2-DPPH picrylhydrazyl free radical scavenging capability as the basis. Considered as the standard solution was ascorbic acid (Koleva et al. 2002).The optical density of each sample was measured against standard at λmax 517 nm by using UV visible spectrophotometer. The experiments were carried out in triplicate. The percentage radicals scavenging activity was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ tested\ sample}{Absorbance\ of\ controle} \times 100$$

With the use of the SSP10 software, we were able to determine the fifty percent inhibition (IC50) of each extract concentration for each graph of inhibition.

III. RESULTS AND DISCUSSION

Antibacterial activity

Several different solvent extracts (chloroform, methanol, ethanol, and aqueous) of the Whole Plants of *Cuscuta reflexa* and *Cuscuta campestris* were tested for their ability to inhibit the growth of four human pathogenic bacterial strains. These strains were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. (Table 1) showcases the findings, which are presented in the form of the diameter of the inhibitory zone, measured in millimeters.Following the chloroform extract, which showed significant zones of inhibition against *S. aureus* (16.00 ± 0.77 mm) and *K. pneumoniae* (16.00 ± 1.00 mm), the ethanol extract also demonstrated strong activity against *P. aeruginosa* (14.00 ± 0.58 mm) and *K. pneumoniae* (14.00 ± 0.58 mm). The chloroform extract was the most effective antibacterial agent among the *C. reflexa* extracts that were considered for testing. The methanol and aqueous extracts of *C. reflexa* exhibited a moderate level of inhibition, specifically against S. aureus, with the methanol extract measuring 14.00 ± 0.70 mm and the aqueous extract measuring 12.00 ± 0.70 mm. In comparison, the antibacterial potential of the extracts of *C. campestris* was shown to be comparatively lower. In comparison to the other extracts, the ethanol extract had the highest level of effectiveness, with inhibition zones measuring 12.00 ± 0.67 mm against both *S. aureus* and *P. aeruginosa*. The chloroform and methanol extracts exhibited zones of inhibition that were not particularly strong, while the aqueous extract shown the least amount of activity from all of the different bacterial strains. On the whole, it was discovered that *C. reflexa* possessed a higher level of potency compared to *C. campestris*, notably in chloroform and ethanol extracts. *S. aureus* and *K. pneumoniae* appeared to be more vulnerable to the extracts than the other bacterial strains that were tested, although *E. coli* consistently demonstrated stronger resistance to the extracts. The conventional antibiotic, ampicillin, which was employed as the positive control, provided the highest zones of inhibition against all of the organisms that were tested. The maximum effect was recorded against *S. aureus*, which measured 18.00 ± 0.50 mm. Based on these observations, it appears that the antibacterial capabilities of *Cuscuta species* could be linked to the existence of bioactive secondary metabolites. These metabolites include flavonoids, alkaloids, and phenolic chemicals, all of which are extracted more effectively in organic solvents such as ethanol and *chloroform* (Fig.1)

Plant Species	Solvent Extract	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
C. reflexa	Chloroform	16.00 ± 0.77	12.00 ± 0.57	12.00 ± 0.58	16.00 ± 1.00
	Methanol	14.00 ± 0.70	9.00 ± 0.60	9.00 ± 0.00	12.00 ± 0.50
	Ethanol	14.00 ± 0.77	9.00 ± 0.48	14.00 ± 0.58	14.00 ± 0.58
	Aqueous	12.00 ± 0.70	8.00 ± 0.07	10.00 ± 0.58	12.00 ± 0.00
C. campestris	Chloroform	11.00 ± 0.48	9.00 ± 0.40	8.00 ± 0.50	10.00 ± 0.58

	Methanol	10.00 ± 0.40	8.00 ± 0.48	9.00 ± 0.58	9.00 ± 0.50
	Ethanol	12.00 ± 0.67	9.00 ± 0.68	12.00 ± 0.67	11.00 ± 0.50
	Aqueous	9.00 ± 0.58	7.00 ± 0.08	8.00 ± 0.50	9.00 ± 0.50
Control (Ampicillin)	—	18.00 ± 0.50	16.00 ± 0.78	16.00 ± 0.50	16.00 ± 0.58

Table 1. Antibacterial activity (Zone of inhibition in mm ± SD) of various solvent extracts of *Cuscuta reflexa* and *Cuscuta campestris* Whole Plants against pathogenic bacteria

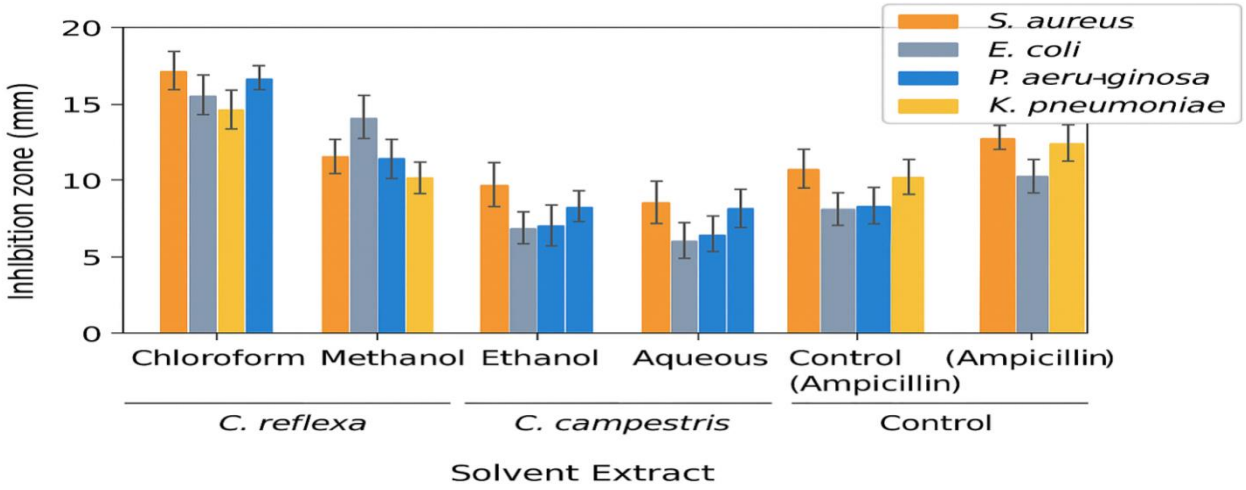

Fig.1.Anti-bacterial activity of *C. reflexa and C. campestris*

Phytochemical Analysis

The initial phytochemical analysis of *Cuscuta reflexa* and *Cuscuta campestris* revealed the presence of several bioactive constituents, including glycosides, flavonoids, carbohydrates, tannins, proteins, alkaloids, saponins, amino acids, and phenolic compounds across various solvent extracts (Table 2). These findings are consistent with earlier research that also identified similar phytochemical profiles in these species (Thakur *et al*., 2023; Mubashr *et al*., 2015; Raza *et al*., 2015).

Table2. Screening of phytochemicals of various extracts of *C. reflexa* and *C. campestris.*

S.No	Phytochemical Test	Test Method	*C. reflexa* (M)	*C. reflexa* (E)	*C. reflexa* (C)	*C. reflexa* (W)	*C. campestris* (M)	*C. campestris* (E)	*C. campestris* (C)	*C. campestris* (W)
1	Glycosides	Keller-Killiani Test	+	+	–	+	+	+	–	+
2	Saponins	Foam Test	+	–	–	+	+	–	+	+
3	Alkaloids	Hager's Test	+	+	–	–	+	+	–	–
4a	Flavonoids	Ferric Chloride Test	–	+	+	+	–	+	+	+
4b	Flavonoids	Alkaline Reagent Test	–	+	–	+	–	+	–	–
4c	Flavonoids	Lead Acetate Test	+	+	–	+	–	+	–	–
5	Tannins	Gelatin Test	+	+	–	–	+	+	+	+
6	Phenolic Compounds	Ferric Chloride Test	+	+	–	+	+	+	+	+
7	Proteins	Xanthoproteic Test	+	–	+	–	–	+	–	–
8	Amino Acids	Ninhydrin Test	–	+	–	+	–	+	–	+
9	Carbohydrates	Benedict's Test	–	+	+	–	+	–	+	+

+ = Present, -=Absent

The phytochemical screening of *Cuscuta reflexa* and *Cuscuta campestris* Whole Plants extracts, utilizing methanol (M), ethanol (E), chloroform (C), and water (W) as solvents, revealed a diverse array of bioactive compounds.

In *C. reflexa*, glycosides were detected in methanol, ethanol, and water extracts. Saponins were present in methanol and water extracts. Alkaloids were identified in methanol and ethanol extracts.Flavonoids were confirmed through ferric chloride and lead acetate tests in ethanol, chloroform, and water extracts, while the alkaline reagent test indicated their presence in ethanol and water extracts.Tannins were found in methanol and ethanol extracts. Phenolic compounds were present in methanol, ethanol, and water extracts.Proteins were detected in methanol and chloroform extracts.Amino acids were identified in ethanol and water extracts. Carbohydrates were present in ethanol and chloroform extracts.

For *C. campestris*, glycosides were found in methanol, ethanol, and water extracts. Saponins were present in methanol, chloroform, and water extracts. Alkaloids were detected in methanol and ethanol extracts.Flavonoids were confirmed via ferric chloride tests in ethanol, chloroform, and water extracts, and through the alkaline reagent test in ethanol extracts.Tannins were present in methanol, ethanol, chloroform, and water extracts.Phenolic compounds were detected across all solvent extracts.Proteins were identified in ethanol extracts. Amino acids were found in ethanol and water extracts. Carbohydrates were present in methanol, chloroform, and water extracts.

Antioxidant properties

Reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, peroxyl radicals, and singlet oxygen, are known to contribute to various pathological conditions.Plant-derived compounds, particularly polyphenols, have garnered attention for their potential to neutralize these free radicals.Polyphenols possess antioxidant properties that enable them to donate hydrogen atoms or electrons, effectively scavenging free radicals and mitigating oxidative stress.

In this study, the antioxidant capacity of Cuscuta species was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a widely recognized method for evaluating free radical scavenging activity.The DPPH assay operates on the principle that antioxidants can reduce the DPPH radical, a stable free radical characterized by its deep violet color, to a non-radical form, resulting in a color change to pale yellow.This change can be quantitatively measured using spectrophotometry, providing an indication of the sample's antioxidant potential.The extent of discoloration correlates with the scavenging ability of the antioxidant compounds present in the plant extracts

The DPPH assay is favored for its simplicity, rapidity, and cost-effectiveness, making it a suitable choice for preliminary screening of antioxidant activity in plant extracts. By applying this method, the study aims to elucidate the free radical scavenging capabilities of Cuscuta species, contributing to the understanding of their potential therapeutic applications in combating oxidative stress-related diseases.

The antioxidant potential of *Cuscuta reflexa* and *Cuscuta campestris* Whole Plants was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.This method evaluates the ability of plant extracts to donate hydrogen atoms or electrons to neutralize DPPH radicals, resulting in a measurable decrease in absorbance.

For *C. reflexa*, the ethanol extract exhibited the highest scavenging activity at 81.05%, closely followed by the aqueous extract at 80.31%. In contrast, *C. campestris* demonstrated its most significant antioxidant activity with the methanol extract, achieving an 85.2% inhibition rate.

The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radical activity, was determined for each extract.This calculation involved plotting the percentage of radical scavenging activity against varying concentrations of the extracts and applying linear regression analysis. The resulting IC_{50} values are depicted in Figure 3, providing a comparative measure of the antioxidant efficacy of each plant extract.

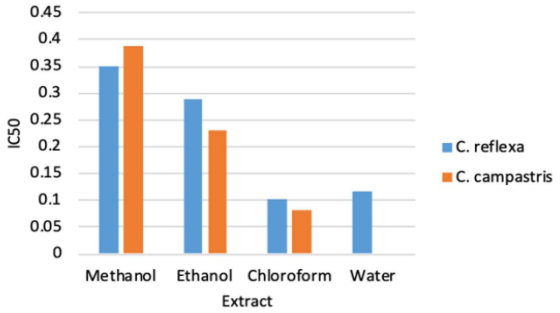

Fig.3.IC50 value of *C. reflexa and C. campestris*

These findings highlight the potent antioxidant properties of *C. reflexa* and *C. campestris*, suggesting their potential utility in mitigating oxidative stress-related conditions.

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