

**ANTIMICROBIAL STUDY OF *CUSCUTA REFLEXA* COLLECTED
IN DIFFERENT SEASONS****SHARMA SHIKHA¹, KAUR AMRINDER*² AND ARJUN ANANIA²**

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ABSTRACT

Antimicrobial agents are natural killer of invading micro organisms. The major advantages of these agents are their ability to cause selective toxicity on the target without hampering the natural balance. *Cuscuta reflexa* is a parasitic plant and is used as appetizer, digestive, liver stimulant, anthelmintic and reduces intestinal motility. It is also reported to have anti-microbial activity and for establishing the best variety, six samples of the plant were collected in six different seasons to evaluate the anti-microbial effect on different pathogens. Anti-microbial activity were studied using ethanolic extract of *Cuscuta reflexa* on pathogens like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*. MHA agar plates were prepared using pour plate method. The zone of inhibition was calculated and results revealed that maximum activity was shown for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and the sample of plant which was collected in the rainy season showed maximum zone of inhibition in case of *Pseudomonas aeruginosa* and *E. coli*. The sample of plant collected during the spring season showed maximum activity in case of *Staphylococcus aureus*.

KEYWORDS:- *Cuscuta reflexa*, Antimicrobial activity, Ethanolic extract, *Staphylococcus aureus*

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INTRODUCTION

Herbal plants represents rich source of phytomedicines since antiquity. Amongst various taxonomical variations in herbal plants, a substantial number of plants possess medicinal values. Herbal plants are gaining popularity owing to their polyvalent actions and lesser side effects as they are a gift of nature. While collecting or processing herbal plants geographical and seasonal variations exerts a huge impact in the percentage of active constituents that can be obtained from a particular plant. Herbal plant if collected in a specific season which can be determined by studying its morphology and taxonomy, the maximum amount of active constituent can be isolated. Medicinal plants represent a rich source of antimicrobial agent like *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* against the specific pathogens like *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*. A systematic investigation is of utmost concern to accelerate the mortality and morbidity¹. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world which demands an alarming situation from the futuristic point of view. Infectious pathogens are the major causes for dreaded diseases, like AIDS, cancer etc. Traditionally used medicinal plants produce a variety of leads to combat against infectious pathogens². The point of concern for such an alarming situation is that bacteria have the genetic ability to transmit and acquire resistance to drugs.

Consequently new infections can occur and the biggest remedy is our traditional system of healing³.

Cuscuta reflexa Roxb. is a rootless, leafless perennial parasitic twining herb of Convolvulaceae family, commonly known as Dodder⁴. The plant is distributed worldwide and in India about 6 species are found. It grows on thorny or other shrubs, sometimes completely covering the bushes and trees⁵. It is a twinning and clinging herb. It lives its entire life without attachment to the ground and grows with the help of seeds which are minute and produced in large quantities. Seeds have hard coating, and survive in the soil for 5-10 years or more. The herb grows towards the smell of nearby plants. If the host contains food which is beneficial for it, then it produces a haustorium that insert themselves into the vascular system of the host and then its original root will die. It can grow and attach itself to multiple plants⁶. The major chemical constituents that have been isolated from *Cuscuta reflexa* are cuscutin, amarbelin, stigmasterol, myricetin, quercetin, cuscutamine, luteolin, bergenin⁷ etc. *Cuscuta reflexa* stem has reported anti microbial activity against gram negative and gram positive bacteria⁸. The present study is based on collection of the plant in different seasons to validate the anti microbial property of the plant to the maximum extent. The profile of collection of plant in different seasons and clinical pathogens used in study are shown in Table-1 and Table-2.

Table 1
Collection of *Cuscuta reflexa* in different seasons

S.No.	Sample No.	Seasons
1	Sample-1	Vasanta (Spring)
2	Sample-2	Grishma (Summer)
3	Sample-3	Varsha (Rainy)
4	Sample-4	Sharad (Autumn)
5	Sample-5	Hemant (Winter)
6	Sample-6	Shishir (Cold & dewy)

Table 2
(Profile of pathogen used for study)

S.No.	Name of pathogen	Infection
1.	<i>Staphylococcus aureus</i>	Causes skin infections, respiratory disease and food poisoning
2.	<i>Staphylococcus epidermidis</i>	Causes infection to the patient with catheters or other surgical implants
3.	<i>Escherichia coli</i>	Causes food infection, urinary tract infection and traveler's diarrhea
4.	<i>Micrococcus luteus</i>	Causes pulmonary infections, septic arthritis and cavitating pneumonia
5.	<i>Pseudomonas aeruginosa</i>	Causes infection in Heart , CNS, Ear, Eye, Bones and joints, Urinary tract and Skin

MATERIALS AND METHODS

Plant collection and Authentication

Cuscuta reflexa was collected in different seasons from Seul, District Kangra, Himachal Pradesh, India. It was authenticated from Gurunanak Dev University, Department of Botanical and Environmental Sciences Amritsar, Punjab. The collected plants were washed thoroughly under running tap water and then shade dried for removal of moisture content and powdered using mixture grinder.

Preparation of Extracts

The air dried drug was extracted with 250 ml of ethanol using a Soxhlet extractor. The extracts were collected in sterile bottles.

Test Microorganism

The pathogenic micro-organisms were collected from Biotechnology lab in Lovely Professional University Punjab. The micro-organism are:

Staphylococcus aureus

Staphylococcus epidermidis

Escherichia coli

Micrococcus luteus

Pseudomonas aeruginosa

The pathogenic cultures were grown in Muller-Hinton agar at 37°C, maintained in Muller-Hinton agar plates, and stored at 4°C for determining the antimicrobial activity of these medicinal plants.

Antimicrobial Assay

The antimicrobial activities of different samples of *Cuscuta reflexa* were studied by agar well diffusion method. The sterilized Muller-Hinton agar media were poured into the petri-plates and allowed for solidification. After

solidification, wells were made into the petri-plate with the help of sterile cork borer (6mm). After that pathogenic cultures were swabbed on the respective agar plates using sterilized cotton swabs. The ethanolic extract were loaded into the respective wells and incubated at 37°C for 24 hrs. After incubation the diameter of inhibition zones formed around each wells were measured and expressed in millimeter (mm) to evaluate the antimicrobial activity⁹.

Phytochemical analysis

The Phytochemical analysis for major phytoconstituents of the ethanolic plant extracts was performed. Phytochemicals can exert a wide range of medicinal values. Ethanolic extracts of the plant were prepared and confirmatory tests for the presence of major phyto compounds were tested.

RESULTS AND DISCUSSION

The present study was structured to evaluate the antimicrobial property of *Cuscuta reflexa*. For this purpose ethanolic extract of the plant was taken and it investigated on various pathogens, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* by agar well diffusion method. All the samples extracts of *Cuscuta reflexa* showed varying degrees of antimicrobial activities against the clinical pathogens tested. The antimicrobial activities of ethanolic extracts of *Cuscuta reflexa* against pathogen are shown in table-3. The zone of inhibition was calculated and results revealed that maximum

activity was shown for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and the sample of plant which was collected in the rainy season showed maximum zone of inhibition in case of *Pseudomonas aeruginosa* (14mm) and *E. coli* (12mm). The sample of plant collected during the spring season showed maximum activity in case of *Staphylococcus aureus* (12mm). *Cuscuta reflexa* showed a diversified presence of

bioactive compounds that includes majorly alkaloid, tannin, saponin and carbohydrates in the preliminary phytochemical screening that were carried out in the ethanolic extract for the plant and shown in table-4. Thus the plant can be validated not only by its antimicrobial property but also various therapeutic actions which can be produced by the plant which can be validated by its presence of bioactive compounds.

Table 3
Antimicrobial activity of ethanolic extracts of *Cuscuta reflexa*

S.No	Test Organism	Concentration of extract in 200µg/ml. Zone of inhibition in mm					
		Sample-1 (mm)	Sample-2 (mm)	Sample-3 (mm)	Sample-4 (mm)	Sample-5 (mm)	Sample-6 (mm)
1.	<i>Staphylococcus aureus</i>	12	10	9	9	11	9
2.	<i>Staphylococcus epidermidis</i>	-	-	-	-	-	-
3.	<i>Escherichia coli</i>	6	5	12	5	5	5
4.	<i>Micrococcus luteus</i>	-	-	-	-	-	-
5.	<i>Pseudomonas aeruginosa</i>	11	10	14	10	11	10

Table 4
Phytochemical analysis of ethanolic extract of *Cuscuta reflexa*

S. no	Test Name	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
1.	Alkaloid	+	+	+	+	+	+
2.	Carbohydrates	+	+	+	+	+	+
3.	Glycosides	-	-	-	-	-	-
4.	Saponins	+	+	+	+	+	+
5.	Steroids	-	-	-	-	-	-
6.	Tannins & Phenolic compounds	+	+	+	+	+	+
7.	Flavonoids	-	-	-	-	-	-
8.	Protein	+	+	+	+	+	+
9.	Aminoacid	-	-	-	-	-	-

CONCLUSION

In conclusion, the results of the study has validated scientific justification for the fact that *Cuscuta reflexa* possessing bioactive compounds showed antimicrobial action against a broad panel of bacteria which can be clinically used for very common bacterial infections. However further purifications are necessary to produce effective leads that may lead to production of good quality anti bacterial formulations and the burden of drug resistance can be minimized considering this aspect. The

presence of phytochemicals indicates a wide range of medicinal values, such as alkaloids may possess CNS stimulant activity, presence of saponin indicating anti inflammatory property, and many more. Thus from the futuristic point of view the medicinal formulation that can be prepared should be formulated by keeping all the activities in concern so as to avoid incompatibility and adverse reactions in between drugs.

REFERENCES

1. B. Mahesh and S. Satish, Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens, World J. Agric. Sci., 4: 839-843, (2008)
2. Iqbal Ahmad and Arina Z. Beg, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, Journal of Ethnopharmacology, 74: 113-123, (2001)
3. Gislene G. F. Nascimento, Juliana Locatelli, Paulo C. Freitas and Giuliana L. Silva, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant Bacteria, Brazilian Journal of Microbiology, 31: 247-256, (2000)
4. Kirtikar KR. & Basu BD, Indian Medicinal plants, vol-3, International Book Distributors: 1741, (1999)
5. Catterjee D, Sahu RK, Jha A and Dwivedi J, Evaluation of Antitumor Activity of *Cuscuta reflexa* Roxb (Cuscutaceae) Against Ehrlich Ascites Carcinoma in Swiss Albino Mice, Trop J Pharm Res, 10: 448-453, (2011)
6. Kumar A, Rani S and Sagwal S, Recent Review on Plant Molecular Biology, Phytophysiology, Phytochemistry and Ethnopharmacology of *Cuscuta reflexa* Roxb. A Wonderful Parasitic Plant, IRJP, 3: 30-38 (2012)
7. Patel S, Sharma V, Chauhan NS and Dixit VK. An updated review on the parasitic herb of *Cuscuta reflexa* Roxb.. Journal of Chinese Integrative Medicine, 10: 249-255 (2012)
8. Inamdar Faiyyaz B, Oswal Rajesh J, Chorage Trushal V and Garje Kapil, In Vitro Antimicrobial Activity of *Cuscuta reflexa* Roxb., IRJP, 2(4): 214-216, (2011)
9. Hema TA, Arya AS, Subha S, John Celestinal RK and Divya PV, Antimicrobial Activity of five South Indian Medicinal Plants against clinical pathogens, Int J Pharm Bio Sci, 4(1): 70-80, (2013)
10. Meena R, Meena AK, Mageswari S, Ramaswamy D and Ahmed SK, Evaluation of Pharmacopoeial Standards with reference to Aftimoon- whole plant (*Cuscuta reflexa* roxb.), IJPSR, 1(11): 139-144, (2010).