



PHYTOCHEMICAL ANALYSIS FOR SECONDARY METABOLITES AND *IN-VITRO* ANTIMICROBIAL ACTIVITY OF *CUSCUTA REFLEXA*

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Abstract

The present study aims to determine the secondary metabolites by phytochemical analysis and *in-vitro* antimicrobial activity of *Cuscuta reflexa* (Convolvulaceae). *Cuscuta reflexa* is commonly called as Dodder plant it is a parasitic weed plant and also an extensive climber. *Cuscuta reflexa* grows as holoparasite; it has very low level of chlorophyll and photosynthesis activity. It makes haustorial connection with the vascular tissue of the host plant and completely depends over the host plant for its survival. The shade dried and powdered parasitic plant material was extracted using ethanol by cold maceration. Antibacterial and antifungal activity of extract at different concentrations of 100mg, 75mg, 50mg, and 25mg per mL were determined by agar well diffusion method. Ciprofloxacin in concentration of 1mg/mL was used as standard reference antibiotic and Fluconazole in concentration of 10mg/mL was used as standard reference antifungal agent. The phytochemical analysis showed positive result for secondary metabolites like flavonoids, terpenoids, glycosides, tannins and phenols. The crude extract of different concentrations was effective against all the test bacteria with marked inhibitory effect against *Pseudomonas aeruginosa* [100mg/mL (23mm), 75mg/mL (21mm), 50mg/mL (19mm) and 25mg/mL (16mm)]. The crude extract of different concentration showed more effectiveness towards *Cladosporium* sp. [100mg/mL (16mm), 75mg/mL (14mm), 50mg/mL (12mm) and 25mg/mL (11mm)] compare to all other test fungal organisms. The parasitic plant *Cuscuta reflexa* appears promising to treat bacterial infectious disease and it also shows an inhibitory activity against some fungal pathogens.

Key words : *Cuscuta reflexa*, Antimicrobial activity, Agar well diffusion, Phyto-chemicals, Medicinal plants.

Introduction

Medicinal plants are the treasure of various hidden chemicals. In the traditional system of medicines, plant sources were major resource to cure diseases. Medicinal plants are getting attraction of most of the researches for the evaluation of new drugs, because of the polyvalent action and lesser side effects of plant products. Since prehistoric era of mankind, treatment and cure of the diseases was one of the main concerns of human beings. Ayurveda completely depends on the plant systems for the evaluation of new chemical entities having therapeutic potentials. Medicinal properties of the plants are due to the active phyto-constituents present in the plants; these phyto-constituents are alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, steroids etc. These phytochemicals possess potential health benefits, contributes in the prevention of cardiovascular diseases,

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cancer, osteoporosis, antioxidant activity and many more (Saini *et al.*, 2015).

Cuscuta reflexa (Dodder plant)

Botanical Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Angiospermes

Class: Eudicots

Subclass: Asterids

Order: Solanales

Family: Cuscutaceae

Fig. 1: *Cuscuta reflexa*

Genus: *Cuscuta*

Species: *reflexa*



Fig. 1: *Cuscuta reflexa*.

Cuscuta is a group of 100- 170 species of yellow, orange, red or rarely green parasitic plants. *Cuscuta* belongs to the *Cuscutaceae* family and now on the basis of angiosperm phylogeny group it is accepted as belonging to morning glory family, *Convolvulaceae*. *Cuscuta* is found at the temperate and tropical regions of the world with huge species diversity in tropical and subtropical regions. *Cuscuta reflexa* is commonly called as Dodder plant, and also known as devil's hair, witch's hair, love vine, amarbel or akashabela etc. *Cuscuta reflexa* is a parasitic weed plant and also an extensive climber. *Cuscuta* grows as holoparasite, it has very low level of chlorophyll and photosynthesis activity; completely depends over the host plant for its survival. Dodder plant sucks nutrient sap from the host plant via vascular tissue of the host plant and grows itself. This plant has no roots in the ground and it grows over the host body without touching the ground surface in its complete life span. Dodder plant has the ability not only to recognize its host plant but also to move towards its prey with significant precision and efficiency.

Ethnomedicinal uses of *Cuscuta reflexa*

Traditional system of medicine relies on the plant sources to cure various disorders. *Cuscuta reflexa* has been studied for the identification of its plant properties to be used as medicinal plant. *C. reflexa* have anti-viral, anti-convulsant activities, bradycardia, anti-steroidogenic, anti-spasmodic and hemodynamic activities. Rural people of India used juice of *C. reflexa* for the treatment of jaundice, its warm paste is used to treat rheumatism and paste of whole plant is used for the treatment of headache. *C. reflexa* is used in the treatment of urination disorders, muscle pain and cough and also used as blood purifier.

Seeds of *C. reflexa* have carminative and anti-helminthic properties and used to treat bilious disorder.

Seeds of *C. reflexa* have neutral nature and sweet in taste; it's used in the treatment of liver and kidney disorders. This plant has the ability to control the loss of fluids from the body. *C. reflexa* is also used in the combination with other medicinal plant to cure various diseases. Juice of *C. reflexa* in the combination with the juice of *Saccharum officinarum* is used for the treatment of jaundice. *C. reflexa* is used in the treatment of constipation, flatulence, body pains, itchy skin, frequent urination, dry eyes, and white discharge from vagina, ringing in the ears, lower back pain, blurred vision and tired eyes. It is also used as hair growth promoters (Sharma and Kapoor, 2014). In the present study ethanolic extract of *Cuscuta reflexa* for phytochemicals and determined antibacterial and antifungal activity.

Materials and Methods

Collection and Identification of Plant

The plant materials were collected at Shankaraghatta, Bhadravathi(T), Shivamogga(D), Karnataka, India., during January 2018.

Extraction

The collected parasitic plant *Cuscuta reflexa* were separated from the host plant then they were washed to remove dirt and other extraneous matter and they were dried under shade for 6-8 days (complete drying) and were powdered by using mechanical grinder. For extraction, maceration process was employed in which 80g of powdered sample was transferred into conical flask containing 400mL of ethanol. The flask was left for 48 hours (during which the flask was stirred occasionally) followed by filtering the contents of flask through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness at 40°C and the obtained extract yield and colour was noted and it was stored in refrigerator at 4°C until further use (Raghavendra *et al.*, 2017).

Phytochemical Screening for secondary metabolites of Extract

Phytochemical analysis for secondary metabolites was done for Alkaloids, Flavonoids, Saponins, Terpenoids, Glycosides, Taninns, Phenols and Steroids by using standard protocol (Kalita *et al.*, 2017).

a) Test for Alkaloids

To the extract 1%Hcl was added and 6drops of Mayer's reagent and Dragendroff's reagent was also added. An organic precipitate indicated the presence of alkaloids in the sample.

b) Test for Flavonoids

5 mL of dilute ammonia solution was added to a portion of aqueous extract and concentrated H₂SO₄ was added. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing.

c) Test for Saponins

The extract with 20mL of distilled water agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam indicated the presence of saponins.

d) Test for Terpenoids

To 2mL of extract 2mL of chloroform and 3mL of concentrated H₂SO₄ was added, formation of a monolayer of reddish brown coloration at the interface indicates positive result for the terpenoids.

e) Test for Glycosides

3 mg of extract was mixed with equal quantity of anthrone and treated with 2 drops of concentrated H₂SO₄ and heated gently on water bath, development of dark green colour indicates the presence of glycosides.

f) Test for Tannins

5mL of extract was added to few drops of 1% of lead acetate. A yellow precipitate indicated the presence of tannins.

g) Test for Phenols

To 2mL of extract 3mL of ethanol and a pinch of FeCl₃ was added to form greenish yellow colour indicating the presence of phenols.

h) Test for Steroids

2 mL of acetic anhydride was added to 0.5gm of extract and then added 2mL of H₂SO₄. The colour change from violet to blue or green indicated the presence of steroids.

Antimicrobial activity of ethanolic extract

The potential of ethanolic extract of *C. reflexa* to inhibit Gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Salmonella typhi* and *Xanthomonas campestris* and also some fungi like *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium* sp., *Colletotrichum* sp., *Fusarium annulatum* and *Fusarium oxysporum* was determined by Agar well diffusion method. In this method, the test bacteria were aseptically inoculated into sterile nutrient broth tubes and incubated at 37°C for 24 h. The broth cultures were swab inoculated on sterile nutrient agar plates, test fungal spore suspensions were prepared in

sterile water containing tween 20 and these spore suspensions were swab inoculated on sterile potato dextrose agar plates, with the help of a sterile gel borer, wells of 6 mm diameter were punched in the inoculated plates. Different concentrations(100mg/mL, 75mg/mL, 50mg/mL and 25mg/mL) of extract were prepared by dissolving crude extract in 10% dimethyl sulfoxide(DMSO), standard antibiotic [Ciprofloxacin, 10mg/mL of sterile distilled water(positive control)], standard antifungal [Fluconazole, 10mg/mL of sterile distilled water (positive control)] and 10% DMSO(negative control) were also prepared. 100 µL of different concentration extract along with positive and negative control, were transferred aseptically in labelled wells. The plates were left undisturbed for 30 min and then incubated in upright position for 24 hours at 37°C for bacteria and 3-4 days at 27°C for fungi. Using a zone scale or ruler, zone of inhibition formed around the wells was measured. The presence of zone of inhibition around the wells is the indication of antimicrobial activity of extract (Faiyyaz *et al.*, 2011).

Results**Colour and yield of the extract**

The colour of the obtained crude ethanolic extract of *Cuscuta reflexa* was 'Yellowish-brown' colour shown in Fig. 2.

Yield of the crude extract,

Weight of the empty petriplate (W1) = 44.34g

Weight of petriplate with extract (W2) = 51.32g and

Weight of the crude extract (W2-W1) = 6.98g

Yield (%) of crude extract (CE)

$$\frac{\text{Weight of the crude extract}}{\text{Weight of the powdered sample taken}} \times 100$$



Fig. 2: Crude extract of *C. reflexa*.

$$= 6.98/80 = 8.725$$

Therefore, the yield of the obtained ethanolic extract of *C. reflexa* was 8.725%

Secondary metabolites detected in extract of *Cuscuta reflexa*

The medicinal and pharmacological properties exhibited by plants are due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids that are distributed in the various parts of the plants. These chemicals are studied under the concept called phytochemicals. The results for presence of phyto-constituents (secondary metabolites) in *C. reflexa* were tabulated below in the table 1 and shown in Fig. 3.

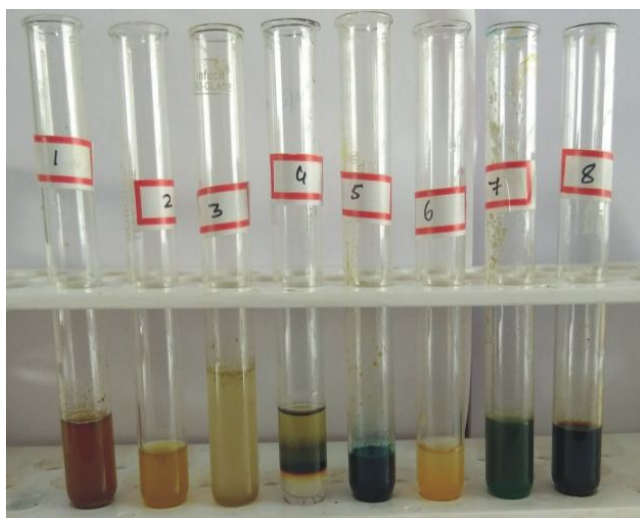


Fig. 3: Phytochemical analysis of *Cuscuta reflexa*.

Table 1: Secondary Phyto-constituents of *Cuscuta reflexa*.

| Sl. No. | Phytoconstituents | Result |
|---------|-------------------|---------|
| 01 | Alkaloids | Absent |
| 02 | Flavonoids | Present |
| 03 | Saponins | Absent |
| 04 | Terpenoids | Present |
| 05 | Glycosides | Present |
| 06 | Tannins | Present |
| 07 | Phenols | Present |
| 08 | Steroids | Absent |

Antimicrobial activity of ethanolic extract of *Cuscuta reflexa*

a) Antibacterial activity of ethanolic extract of *Cuscuta reflexa*

Screened the efficacy of ethanolic extract of *C. reflexa* by agar well diffusion assay. The result of the inhibitory activity of extracts against Gram positive and Gram negative bacteria is shown in (Table 2). The

presence of inhibition zone around the wells was considered positive for antibacterial activity. Extract of different concentrations were shown to inhibit all test bacteria, marked inhibitory effect was shown against *Pseudomonas aeruginosa* shown in Fig. 4.

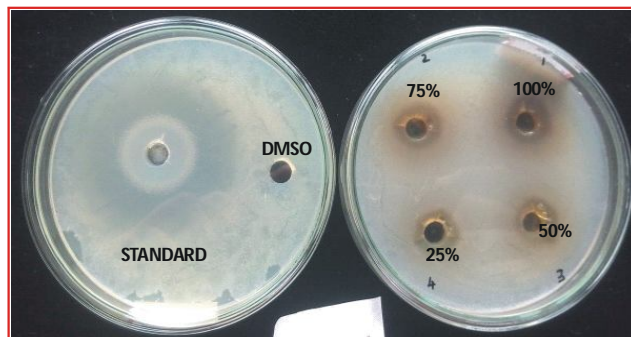


Fig. 4: *Pseudomonas aeruginosa* inhibited by crude extract of *Cuscuta reflexa*.

b) Antifungal activity of ethanolic extract of *Cuscuta reflexa*

Screened the efficacy of ethanolic extract of *C. reflexa* by agar well diffusion assay. The result of the inhibitory activity of extracts against the plant pathogenic fungi is shown in (Table 3). The presence of inhibition zone around the wells was considered positive result for antifungal activity. Extract of different concentrations were shown marked inhibitory affect against *Cladosporium* sp. shown in Fig. 5 and also inhibit some of the test fungi such as *Aspergillus niger*, *A. flavus*, *Fusarium annulatum* and *Fusarium oxysporum* respectively. The extract was not effective towards some test fungi like *Alternaria* sp. and *Colletotrichum* sp.

Discussion

Plants are known to be the sources lead compounds for the development of new drugs. Several drugs such as aspirin, digoxin, quinine, vincristine, vinblastine, reserpine, and morphine have been derived from plants. In these days, immense interest on medicinal plants and exploration of medicinal values of plants is increased because of several complications such as the development of resistance and side effects associated with the use of modern drugs such as chemically synthesized antibiotics and anticancer agents. Medicinal plants are the treasure of various hidden chemicals. In the traditional system of medicines, plant sources are major resource to cure diseases. Medicinal plants are getting research attraction of most of the researchers for the evaluation of new drugs, because of the polyvalent action and lesser side effects of plant products. Since prehistoric era of mankind, treatment and cure of the diseases was one of the main concerns of human beings.

Table 2: Antibacterial activity of ethanolic extract of *Cuscuta reflexa*.

| Test Bacteria | Ciprofloxacin (10mg/ml) Zone of inhibition (mm) | DMSO (10%) Zone of inhibition (mm) | Concentration of extract (mg/mL) | Diameter of zone of inhibition (mm) |
|-------------------------------|---|------------------------------------|----------------------------------|-------------------------------------|
| <i>Bacillus subtilis</i> | 52 | 00 | 100 | 18 |
| | | | 75 | 16 |
| | | | 50 | 13 |
| | | | 25 | 10 |
| <i>Escherichia coli</i> | 50 | 00 | 100 | 19 |
| | | | 75 | 18 |
| | | | 50 | 16 |
| | | | 25 | 15 |
| <i>Klebsiella pneumonia</i> | 44 | 00 | 100 | 22 |
| | | | 75 | 18 |
| | | | 50 | 16 |
| | | | 25 | 13 |
| <i>Pseudomonas aeruginosa</i> | 42 | 00 | 100 | 23 |
| | | | 75 | 21 |
| | | | 50 | 19 |
| | | | 25 | 16 |
| <i>Pseudomonas syringae</i> | 46 | 00 | 100 | 22 |
| | | | 75 | 20 |
| | | | 50 | 18 |
| | | | 25 | 16 |
| <i>Salmonella typhi</i> | 42 | 00 | 100 | 18 |
| | | | 75 | 17 |
| | | | 50 | 15 |
| | | | 25 | 13 |
| <i>Staphylococcus aureus</i> | 48 | 00 | 100 | 22 |
| | | | 75 | 20 |
| | | | 50 | 18 |
| | | | 25 | 12 |
| <i>Xanthomonas campestris</i> | 46 | 00 | 100 | 18 |
| | | | 75 | 16 |
| | | | 50 | 15 |
| | | | 25 | 13 |

The medicinal and pharmacological properties exhibited by plants are due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, terpenoids etc., that are distributed in the various parts of the plants. These chemicals are studied under the concept called Phytochemistry. Most of these phytochemicals have profound physiological effects on the health. Hence, it is important to detect these phytoconstituents in medicinal plants so as to correlate the possible therapeutic role played by them. There are several protocols to extract phytochemicals from plants, such as maceration, Soxhlet extraction,

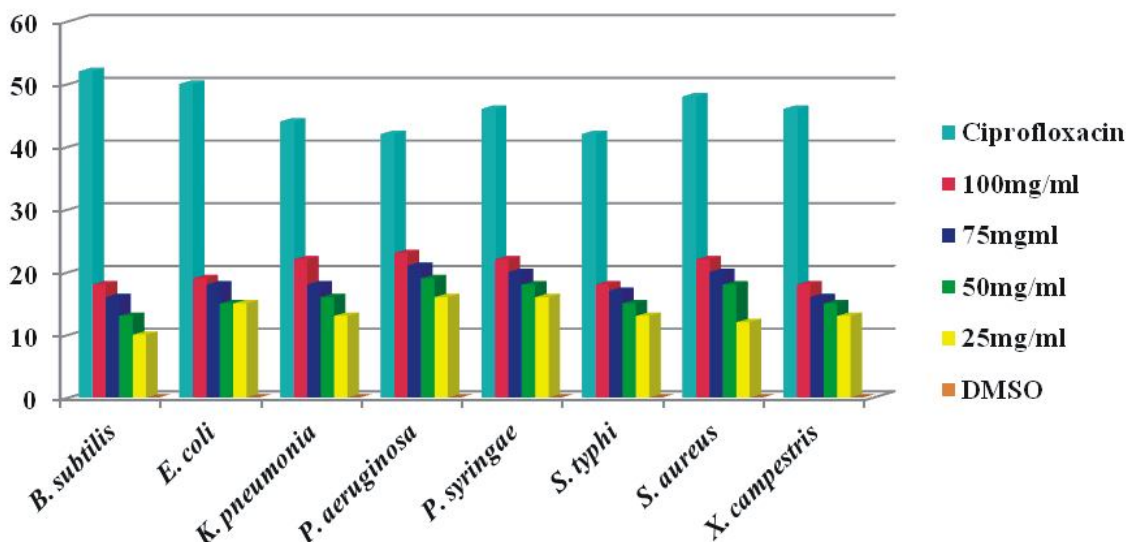
supercritical fluid extraction, and microwave-assisted extraction.

In the present study, cold maceration process was followed to get an extract from *Cuscuta reflexa* using ethanol as the extraction solvent. It has been shown that ethanol can dissolve many phytochemicals including polyphenolic compounds present in the plants. The colour of the obtained crude ethanolic extract of *Cuscuta reflexa* was 'Yellowish-brown' colour and the yield of the obtained ethanolic extract of *C. reflexa* was 8.725%.

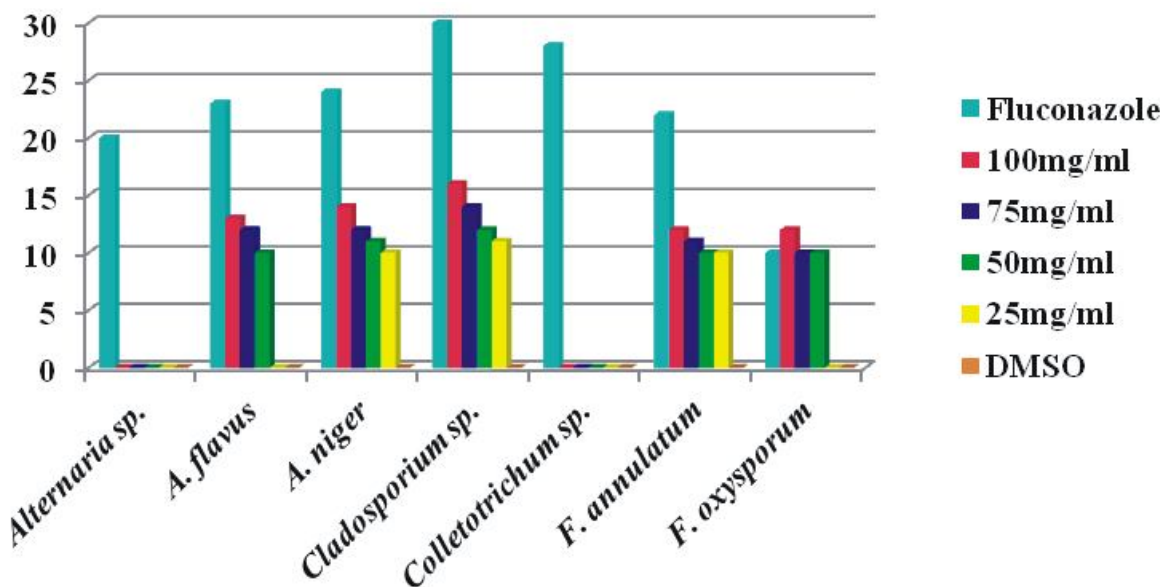
Phytochemicals which were detected in the crude extract of *Cuscuta reflexa* were flavonoids, terpenoids, glycosides, tannins and phenols. Phytoconstituents, namely, alkaloids, saponins and steroids were not detected. The study of Permesha *et al.*, (2014) on phytochemical analysis of *C. reflexa*, the methanolic extracts of stem and flower of *Cuscuta reflexa*, revealed the presence of alkaloids, flavonoids, steroids, saponins, Glycosides, phenols and tannins. The research work of Shika *et al.*, (2013) on phytochemical analysis of *C. reflexa*, the ethanolic extract of

C. reflexa showed the presence of alkaloids, saponins, tannins and phenols. Glycosides, steroids, flavonoids were absent.

One of the major milestones in the field of medicine is the discovery of antibiotics. The use of these wonder drugs resulted in prevention and control of huge number of deaths. However, indiscriminate use of these antibiotics resulted in the emergence of resistant pathogens. Antibiotic therapy not only affects the target pathogen but also commensal inhabitants of the human host. Moreover, the ability of these pathogens to transmit the resistance to



Graph 1: Antibacterial activity of ethanolic extract of *Cuscuta reflexa*.



Graph 2: Antifungal activity of ethanolic extract of *Cuscuta reflexa*.

susceptible ones created major problem in the treatment of diseases. These antibiotic-resistant pathogens are of serious concern in the community as well as hospital settings. High cost, side effects, and the resistance problems associated with these antibiotics triggered immense interest in scientific community to search alternatives for disease control. Plants, their extracts, and the purified compounds from them are shown to be effective in inhibiting pathogenic bacteria including resistant strains.

In this study, efficacy of ethanolic extract of *C. reflexa* was screened for antibacterial activity at different concentrations of 100mg, 75mg, 50mg, and 25mg per mL by agar well diffusion assay. The result of the inhibitory

activity of extracts against Gram positive and Gram negative bacteria are shown in (Table 2). The presence of inhibition zone around the wells were considered as positive result for antibacterial activity. Extract was shown to inhibit all test bacteria with marked inhibitory effect against *Pseudomonas aeruginosa* (100mg/mL; 23mm, 75mg/mL; 21mm, 50mg/mL; 19mm, 25mg/mL; 16mm) and then followed by *P. syringae*, *S. aureus*, *K. pneumoniae*, *X. campestris*, *E. coli*, *B. subtilis* and *S. typhi* respectively. Inhibitory activity of standard reference antibiotic was higher than that of extracts. No inhibition of test bacteria was observed in case of DMSO. In the work of Faiyyaz *et al.*, (2011) on the antibacterial activity of *C. reflexa* the zone of inhibition of *B. subtilis* was 8.6mm, *S. aureus* was 7.5mm, *E. coli* was 8.4mm,

Table 3: Antifungal activity of ethanolic extract of *Cuscuta reflexa*.

| Test Fungi | Fluconazole (10mg/ml) Zone of inhibition (mm) | DMSO (10%) Zone of inhibition (mm) | Concentration of extract (mg/mL) | Diameter of zone of inhibition (mm) |
|---------------------------|---|------------------------------------|----------------------------------|-------------------------------------|
| <i>Alternaria</i> sp. | 20 | 00 | 100 | 00 |
| | | | 75 | 00 |
| | | | 50 | 00 |
| | | | 25 | 00 |
| <i>Aspergillus flavus</i> | 23 | 00 | 100 | 13 |
| | | | 75 | 12 |
| | | | 50 | 10 |
| | | | 25 | 00 |
| <i>Aspergillus niger</i> | 24 | 00 | 100 | 14 |
| | | | 75 | 12 |
| | | | 50 | 11 |
| | | | 25 | 10 |
| <i>Cladosporium</i> sp. | 30 | 00 | 100 | 16 |
| | | | 75 | 14 |
| | | | 50 | 12 |
| | | | 25 | 11 |
| <i>Colletotrichum</i> sp. | 28 | 00 | 100 | 00 |
| | | | 75 | 00 |
| | | | 50 | 00 |
| | | | 25 | 00 |
| <i>Fusarium annulatum</i> | 22 | 00 | 100 | 12 |
| | | | 75 | 11 |
| | | | 50 | 10 |
| | | | 25 | 10 |
| <i>Fusarium oxysporum</i> | 10 | 00 | 100 | 12 |
| | | | 75 | 10 |
| | | | 50 | 10 |
| | | | 25 | 00 |

13.7mm and *E. coli* was 24.6mm.

Several biological agents such as insects, bacteria, viruses, and fungi attack plants at various stages of growth and development resulting in a reduction of productivity and economic loss to farmers. As compared to other agents, the impact of fungi on crop production losses is highest. Agrochemicals such as pesticides and fungicides have been routinely used to prevent and control plant diseases and crop loss. However, many fungicides are toxic and have undesirable effects on non-target organisms present in the environment. Besides, the development of resistance that has been noticed in phyto-pathogenic fungi towards the synthetic fungicides is another great challenge. Hence, it is desirable to use alternative approaches that are eco-friendly for controlling of plant diseases. Plants appear to be promising alternatives for plant disease management. It is known that the use of natural products can reduce the population of pathogens and

control the development of diseases. Plants have been considered as potential agents in integrated pest management programs. The use of plant-based formulations is cheap, eco-friendly, and free of toxic effect on humans. A number of plants have been reported to cause inhibition of several phyto-pathogenic fungi.

Antifungal activity of the ethanolic extract of *C. reflexa* at different concentration of 100mg, 75mg, 50mg, and 25mg per mL were determined by agar well diffusion assay. The results of the inhibitory activity of extracts against some test fungi are shown in (Table 3). The presence of inhibition zone around the wells was considered as positive result for antifungal activity. Crude ethanolic extract of different concentration showed marked inhibitory effect against *Cladosporium* sp. (100mg/mL; 16mm, 75mg/mL; 14mm, 50mg/mL;

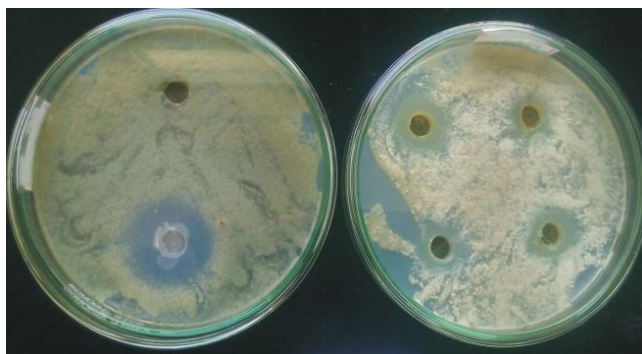


Fig. 5: *Cladosporium* sp. inhibited by crude extract of *Cuscuta reflexa*.

P. aeruginosa was 9.6mm, and *P. citrinum* was also 9.6. In the work of Manirujjaman *et al.*, (2016) on the antibacterial activity of *C. reflexa* extract showed zone of inhibition of *B. subtilis* was 18.8mm, *S. aureus* was

12mm, 25mg/ml ; 11mm) and also effective towards *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum* and *Fusarium annulatum* respectively. Inhibitory activity of standard reference antifungal agent was higher than that of extracts. The extract was not effective towards some test fungi like *Alternaria* sp. and *Colletotrichum* sp. Faiyyaz *et al.*, (2011) worked on the antifungal activity of *C. reflexa*, the zone of inhibition of *A. niger* was 9.9mm and *P. citrinum* was 10.2mm.

Conclusion

Plants have been used traditionally for the treatment of various diseases throughout the world. Extracts and purified metabolites from plants exhibit a range of bioactivities which can be exploited for drug development. The parasitic plant *Cuscuta reflexa* appears promising to treat bacterial infectious disease and it also has an inhibitory activity against some fungal pathogens. The observed antimicrobial activity against pathogenic microorganisms could be ascribed to the presence of secondary metabolites which are detected in the ethanolic crude extract of *Cuscuta reflexa*. The findings of the present study showed the potential of extract of *C. reflexa* for developing novel antimicrobial drug which can be used for treating infections caused by pathogenic microorganisms. In suitable form, the plant can be used to control phyto-pathogenic microorganisms. Further studies on isolation of active principles from the *C. reflexa* and their toxicological and pharmacological studies are to be carried out.

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