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Research Article

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ISOLATED CONSTITUENTS FROM AREAL PART OF *CUSCUTA REFLEXA* ROXB. PLANT

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ABSTRACT

Objective: The objective of the study was to investigate the pharmacological evaluation of previously isolated compounds (CR-1 to CR-5) from the areal part of *Cuscuta reflexa Roxb.* is reported.

Methods: The antimicrobial and antioxidant activity of the isolated compounds (CR-1 to CR-5) from *C. reflexa* was determined by the disc-diffusion method and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) model, respectively. The antimicrobial activity was performed against four strains *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*.

Results: The results revealed that highest zone of inhibition is measured by compound CR-5 against *E. coli.* The antioxidant activity is evaluated for *in vitro* antioxidant activity using DPPH radical scavenging activity, inhibitory concentration 50% (IC_{50}) (120.92–76.38 %), respectively. The results indicate that isolated compound CR-1 and CR-2 having IC_{50} 76.38 and 76.94 µg/ml, respectively, showed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 43.42 µg/ml).

Conclusion: This study suggests that areal part of *C. reflexa* have bioactive compounds for a new antimicrobial and antioxidant drug development.

Keywords: *Cuscuta reflexa* Roxb., Antimicrobial activity, *In vitro* antioxidant activity.

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INTRODUCTION

Cuscuta reflexa is generally called as dodder plant, otherwise called, witch's hair, devil's hair, and amarbel. *Cuscuta* belongs to the Cuscutaceae family and on the premise of Angiosperm phylogeny gather it is recognized as having a place with family, *Convolvulaceae* [1,2]. Dodder plant can similarly pick a fitting host between various plants on the preface of erratic blends release by the host plant as their ordinary technique of transpiration [3,4]. *Cuscuta* makes haustorial relationship with the vascular tissue of the host plant. This haustorium can invade the xylem and phloem of the host plant and associated with tissues of the host plant [5]. *C. reflexa* contrasts in the shade of flowers made from white to pink. Flowers generally made in the late spring and collect time also depend on the species. Seeds are made in the far-reaching sums. Seeds of *C. reflexa* can get by in the soil for quite a while in the chase of appropriate host, starting at now it depends on the sustenance spare in endosperm of the seed [6].

Distinctive parts of this plant are used as a piece of tribal medication for the disease such as antibacterial [7], antiepileptic [8], antitumor activity [9], and anti-inflammatory [10]. As our previous study, phytochemical investigation of the areal part of *C. reflexa* Roxb. yields five phytoconstituents, namely, 2,3-dihydro-3,5,7-trihydroxy-2-(3-hydroxy-4methoxyphenyl)chromen-4-one (CR-1), 2,3-dihydro-3,7dihydroxy-2-(3,4dihydroxyphenyl) chromen-4-one (CR-2) and 6-methoxy-2H-chromen-2-one (CR-3), N-(4-methoxyphenethyl)-3-(3,4dihydroxyphenyl)acrylamide (CR-4), and N-(4-butylphenethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (CR-5) [11]. The present investigation of chemical constituents of *C. reflexa* was undertaken as part of a wider study to find out the pharmacological active constituents present in this plant. Hence, in the current study, we describe the pharmacological evaluation of previously isolated compounds (CR1-CR5) from the areal part of *C. reflexa Roxb.* is reported.

METHODS

The areal part of the plant was collected from the herbal garden of A. N. D. College of Pharmacy, Babhnan, Gonda, Uttar Pradesh, India, in the month of December and identified by an expert taxonomist in the Department of Taxonomy and Pharmacognosy, National Botanical Research Institute, Lucknow. The plant specimens were authenticated (Ref. No NBRI/CIF/413/2013). The areal part of the plant was shade dried, reduced to coarse powder and stored in an airtight container till further use. The extraction and isolation were carried out our previous work [11].

Test microorganisms used in the study

The bacterial strains are identified strains and procured from Scientific and Applied Research Center, India, for antimicrobial susceptibility testing. The microorganisms are *Staphylococcus aureus* (SA/221-14), *Bacillus subtilis* (BS/222-14), *Escherichia coli* (EC/223-14), and *Pseudomonas aeruginosa* (PA/224-14). All strain were maintained preserved on Muller-Hinton agar slant throughout the antimicrobial study.

Preparation of bacterial suspension

Colonies of different strains of bacteria (S. *aureus*, B. *subtilis*, E. *coli,* and P. *aeruginosa*) were transferred to the different fresh nutrient broth in sterile conditions and were incubated at 37°C for 24 h. These suspensions were preserved in 250 ml sterile flasks for further use.

Determination of antimicrobial activity

In vitro antimicrobial activity of the isolated compound of *C. reflexa* was studied by agar cup plate technique. The sterilized media (nutrient agar media for bacteria) were poured into the Petri plates after the medium was solidified; ditch was made into Petri plate with the help of sterile cork borer (6 mm). The different concentration of compounds as 50, 100, 150, and 200 (µg/ml) was made using dimethyl sulfoxide solvent, which was loaded into the respective well and incubated at 37°C for 24 h. Penicillin was used as positive control. The experiment was performed

under aseptic condition [12], inhibition of microbial growth determined by measuring the diameter of inhibition as shown in Table 1-5.

***In vitro* antioxidant activity**

1,1-diphenyl-2-picryl-hydrazyl (DPPH) method is the most excellent, easiest and commonly used method for testing preliminary free radical-scavenging activity [13-15]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. DPPH is known to abstract labile hydrogen [16-18]. DPPH-radical scavenging activity of synthesized compounds (CR-1 to CR-5) was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. Solution of DPPH was prepared and was added to all the synthesized compounds (CR-1 to CR-5) at different concentrations (1–1000 mg/ml). 30 min later, the absorbance was measured at 517 nm. All the analysis was made with the use of an ultraviolet-visible spectrophotometer (Shimadzu 1700). Absorbance of various concentrations was taken, and percentage inhibition was calculated. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as a standard antioxidant. Inhibitory concentration 50% (IC_{50}) value denotes the concentration of sample required to scavenge 50% of the DPPH free radical Table 6. IC_{50} of all synthesized compounds (CR-1 to CR-5) was determined from % inhibition v/s concentration graph Fig. 1.

The percentage discoloration was calculated as follows:

DPPH radical scavenging activity (%)=[AC_{517} – AE_{517}/AC_{517}]×100

Where; AC_{517} is absorbance of a DPPH solution without fraction; AE_{517} is the absorbance of the tested compounds with DPPH.

Table 1: Screening of antimicrobial activity of isolated compound (CR-1)

Species	Concentration (μg/ml) of isolated compound (CR-1) used with the zone of inhibition (mm)				
	50	100	150	200	Standard 100 (Penicillin)
S. aureus	7.5	7.8	8.1	9.6	14.1
B. subtilis	5.1	6.3	6.8	7.4	14.9
E. coli	6.1	7.3	8.5	9.8	16.5
P. aeruginosa	6.4	7.1	7.9	9.1	13.2

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

Table 2: Screening of antimicrobial activity of isolated compound (CR-2)

Species	Concentration (μg/ml) of isolated compound (CR-2) used with the zone of inhibition (mm)				
	50	100	150	200	Standard 100 (Penicillin)
S. aureus	+	6.7	8.9	10.5	14.1
B. subtilis	7.1	9.7	11.8	13.1	14.9
E. coli	+	+	8.7	10.9	16.5
P. aeruginosa	+	+	9.1	10.8	13.2

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

Table 3: Screening of antimicrobial activity of isolated compound (CR-3)

Species	Concentration (μg/ml) of isolated compound (CR-3) used with the zone of inhibition (mm)				
	50	100	150	200	Standard 100 (Penicillin)
S. aureus	+	9.3	10.3	12.4	14.1
B. subtilis	+	7.6	9.1	9.8	14.9
E. coli	+	8.2	9	11.9	16.5
P. aeruginosa	7.1	9.1	9.9	12.6	13.2

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

Table 4: Screening of antimicrobial activity of isolated compound (CR-4)

Species	Concentration (μg/ml) of isolated compound (CR-4) used with the zone of inhibition (mm)				
	50	100	150	200	Standard 100 (Penicillin)
S. aureus	+	12	12.9	13.5	14.1
B. subtilis	12.5	12.7	13.8	14	14.9
E. coli	11.1	13.6	14.3	15.1	16.5
P. aeruginosa	9.8	11.8	12.3	13	13.2

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

RESULT

Antibacterial activity

The *in vitro* antibacterial activities of various isolated compounds of *C. reflexa* against the tested bacteria were assessed by the presence or absence of inhibition zone. The isolated compounds exhibited antibacterial activity against various strains (Fig. 2-6). Isolated compounds (CR-4 and CR-5) from *C. reflexa* revealed great potential

Table 5: Screening of antimicrobial activity of isolated compound (CR-5)

Species	Concentration (μg/ml) of isolated compound (CR-5) used with the zone of inhibition (mm)				
	50	100	150	200	Standard 100 (Penicillin)
S. aureus	10.7	11.8	12.4	13.6	14.1
B. subtilis	11.4	13	13.9	14	14.9
E. coli	10.1	12.6	13.1	15.9	16.5
P. aeruginosa	10	11.4	12	12.9	13.2

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

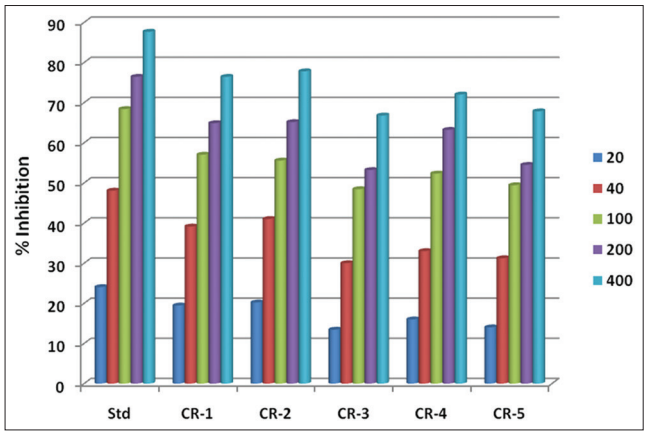

Fig. 1: 1,1-diphenyl-2-picryl-hydrazyl Scavenging assay of isolated compounds from Cuscuta reflexa plant and compared with standard ascorbic acid (% of inhibition vs. concentration) of isolated compound (CR-1 to CR-5)

Table 6: Percentage inhibition of Compounds (CR-1 to CR-5) at various concentrations

S. No.	Con. (µg/ml)	Standard (ascorbic acid)	CR-1	CR-2	CR- 3	CR-4	CR-5
1	20	24.04±0.0376	19.45±0.1624	20.22±0.2432	13.44±0.1243	16.01±0.1209	14.01±0.1056
2	40	48.08±0.1243	39.14±0.0122	41.01±0.0266	30.01±0.2167	33.01±0.1032	31.24±0.0043*
3	100	68.41±0.1821**	57.04±0.0244***	55.58±0.1623**	48.44±0.2134	52.33±0.1084*	49.44±0.0344
4	200	76.44±0.2172***	64.88±0.2442	65.15±0.0273	53.22±0.1004*	63.23±0.0234	54.49±0.1044
5	400	87.66±0.1282	76.44±0.0244***	77.81±0.1332***	66.81±0.2137	72.04±0.1034	67.85±0.1087
6	IC50	43.42±0.2201	76.38±0.1422**	76.94±0.1637**	120.92±0.1108***	92.66±0.1088*	111.08±0.1023

Data represent mean±S.E.M. Of triplicate analysis, p*<0.05 compared to control. SEM: Standard error of the mean

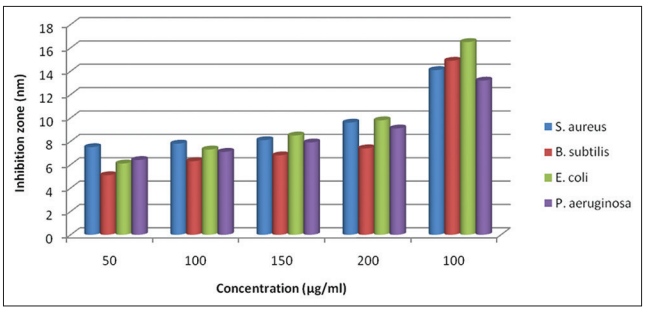

Fig. 2: Diameter of inhibition zone on the growth of different bacterial species due to the application of different concentration of isolated compound (CR-1)

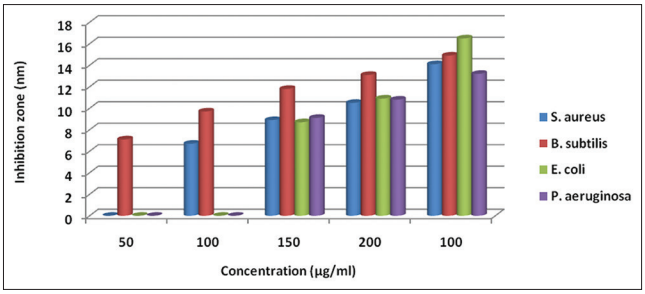

Fig. 3: Diameter of inhibition zone on the growth of different bacterial species due to the application of different concentration of isolated compound (CR-2)

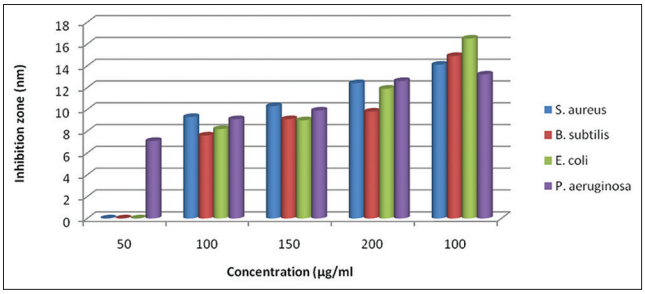

Fig. 4: Diameter of inhibition zone on the growth of different bacterial species due to the application of different concentration of isolated compound (CR-3)

of antibacterial activity against all bacteria (zone ranging from 9 to 15 mm) (Fig. 3 and 4). The highest zone of inhibition is measured by compound CR-5 against *E. coli* (15.9 nm). Compound CR-3 and CR-4 showed moderate antibacterial activity against *E. coli* and *B. sublitis*. Compound CR-3 also showed moderate activity against *P. aeruginosa*.

DPPH-scavenging activity

Fig. 6 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the isolated compounds. These

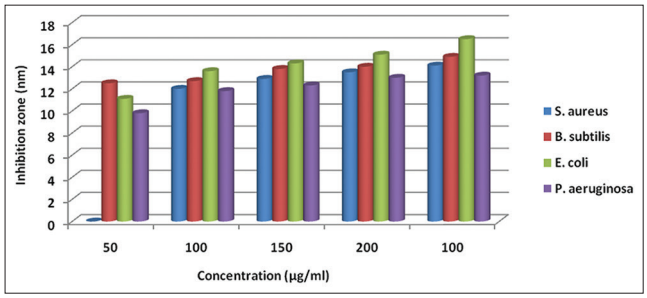

Fig. 5: Diameter of inhibition zone on the growth of different bacterial species due to the application of different concentration of isolated compound (CR-4)

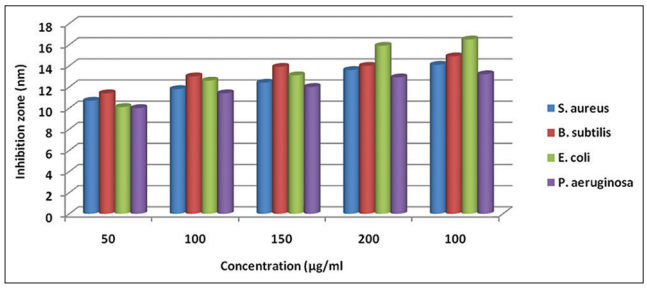

Fig. 6: Diameter of inhibition zone on the growth of different bacterial species due to the application of different concentration of isolated compound (CR-5)

results indicate that the isolated compounds from *Cuscuta reflexa* (CR-1 and CR-2) plant exhibited more antioxidant activity than the other. It may be due to the presence of OH groups, which enhance the radical scavenging activity by hydrogen donation. The results indicate that compound CR-1 and CR-2 having IC_{50} 76.38±0.14 and 76.94±0.16 µg/ml showed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 43.42±0.22 µg/ml) as shown in Table 6. The isolated compounds have shown good antioxidant effect, among CR-1 and CR-2 has shown excellent activity. Rest of the compounds (CR-3 to CR-5) showed mild-to-moderate antioxidant effect.

CONCLUSION

Due to the extremely increasing of the antibiotic unwilling pathogen, it has become predictable to find out the new drugs in pharmaceutical industries. The previous study demonstrated that extracts of C. *reflexa* showed antibacterial activity against various microorganisms. The result of the current study is relentless with previous studies [19-22]. This study showed the various compounds isolated from C. *reflexa* plant exhibited great potential inhibitory effect against the tested bacteria. Subsequently, in biological screening, the compounds (CR-1 and CR-2) showed potent antioxidant agent as compared to other isolated compounds. Further research on these plants core is needed for the discovery of a potent antioxidant agent. This study is a preliminary step which indicates the furthermore study is necessary to investigate the

toxicology and other pharmacological profile of this plant constituent for development of new antibacterial and antioxidant drugs. Thus, we observed that there is enough scope for further study in developing such compounds as a good lead molecule with the better pharmacological profile.

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