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% (IC50) [120.92–76.38 %], respectively. The results indicate that isolated compound CR-1 and CR-2 having IC50 76.38 and 76.94 µg/ml, respectively, showed potent antioxidant activity comparable to standard ascorbic acid (IC50 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.

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-dihydroxy-3,7dihydroxy-2-(3,4dihydroxyphenyl) chromen-4-one (CR-2), 2,3-dihydro-3,5,7-trihydroxy-2-(3,4dihydroxyphenyl)chromen-4-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 (CR-1-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 strains 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) were 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
| 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*

**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)
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 mm). Compound CR-3 and CR-4 showed moderate antibacterial activity against *E. coli* and *B. subtilis*. 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 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 µg/ml 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

| S. No. | Con. (µg/ml) | Standard (ascorbic acid) | CR-1 | CR-2 | CR-3 | CR-4 | CR-5 |
|--------|--------------|--------------------------|------|------|------|------|------|
| 1      | 20           | 24.04±0.376              | 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±0.043* |
| 3      | 100          | 60.41±0.3221***          | 57.04±0.2444*** | 55.58±0.1623** | 48.44±0.2134 | 52.32±0.1094* | 49.44±0.0344 |
| 4      | 200          | 76.44±0.2172***          | 64.88±0.2422 | 65.15±0.0273 | 53.22±0.1004* | 63.23±0.0234 | 54.49±0.1044 |
| 5      | 400          | 87.66±0.1282             | 76.44±0.2444*** | 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
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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