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COMPARATIVE *IN VITRO* BIOLOGICAL ACTIVITY ANALYSIS OF *CUSCUTA REFLEXA* ROXB. AND *C. CAMPESTRIS* YUNCKER.

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Abstract

Cuscuta reflexa Roxb. and *C. campestris* Yuncker are widely used as medicinal plants in different areas of Azad Jammu and Kashmir. Keeping in view of their local uses, antibacterial, antioxidant and phytochemical investigations were carried out in the stem extracts. The antibacterial activities were carried out by Disc diffusion method against Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*) bacteria. Ampicillin was used as positive control. It was found that ethanol extract of both the plants were most active as compared to other extracts and *S. aureus* (Gram positive) was most sensitive bacterium compared to other tested Gram-negative bacteria. The antioxidant potential of the crude extracts of these plants was examined on the source of their scavenging activity of the stable 1, 1-diphenyl-2-picrrazyl hydrazyl (DPPH) free radical and found to possess good antioxidant. Preliminary phytochemical screening has shown the presence of several phytochemicals.

Introduction

Modern drugs are isolated from nature for thousands of years. Based on these isolations, pharmaceutical agents are used in conventional treatments (Owolabi *et al.* 2007). Nature is a source of medicines and medicinal plants serve cause of several powerful and prevailing drugs in different countries (Mahesh and Satish 2008). Natural products and synthetic derivatives produced by plants play an important role in the improvement of new agents to prevent the beginning of cancer (Xua *et al*. 2009).

Cuscuta reflexa Roxb. generally identified as Amberbel, belongs to the family Cuscutaceae. It is tropical and subtropical herb originated as parasite weed on host plants. The presence of alkaloids, carbohydrates, some glycosides, flavonoids, tannins, phenolic compounds and steroids were determined in ethanolic extract of stem of *C. reflexa*. Antimicrobial activity was shown in flavonoides and glycosides (Inamdar *et al.* 2011). *Cuscuta* sp. showed anticancer and immune stimulatory activities (Anjum *et al.* 2013). Antibacterial properties have been observed in stem of *C. reflexa* which is used internally to treat fever and externally in itching (Bais and Kakka 2014). This plant also exhibits anticancer and anti-inflammatory activities (Pandit *et al*. 2008). The alcoholic and aqueous extract of *C. reflexa* indicated diuretic activity (Suresh *et al*. 2011). The crude extract of water of *C. reflexa* showed the anti-HIV activity (Sharma *et al.* 2009).

Cuscuta campestris Yunker is generally considered the most detrimental species possibly due to its broad geographic distribution and large variety of hosts (Holm *et al*. 1997). It is known as field dodder. *C. campestris* is extensively distributed in warm and temperate regions but can

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flourish in precarious hot and cold conditions. The entire chlorophyll content in *C. campestris* increased from propagation and extended a maximum at flowering (Dinelli *et al*. 1993). The commercial significance of *C. campestris* stems arises from the clue that it parasitizes a number of important crop plants and decreases their production (Farah and Alabdulsalam 2004). *C. campestris* and *C. reflexa* grow *in vitro* and are capable of fruiting and flowering (Malik and Singh 1980). It was indicated that the extract of *C. campestris* has antipyretic, analgesic and anti-inflammatory activities (Agha *et al*. 1996). Keeping in view the medicinal importance of *Cuscuta* species, present research was designed to investigate antibacterial, antioxidant and phytochemical screening of stem extracts of these species.

Materials and Methods

This Experiment was conducted in Department of Botany, University of Azad Jammu and Kashmir. Fresh plants of *Cuscuta reflexa* and *C. campestris* were collected from different areas of district Muzaffarabad, Azad Jammu and Kashmir. Identification of the plants was authenticated by Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. Stems were shade dried for seven days at room temperature. Dried plant materials were powdered by using an electrical grinder. Powder of stem of each plant (25 g) was dissolved in 100 ml ethanol, chloroform, methanol and distilled water and kept for 10 days in dark with continuous shaking after every 24 hrs. After that, it was filtered in conical flask. The residue was discarded, filtrate was evaporated to obtain crude extract. 10mg of each crude extract was dissolved in 1ml respective solvents and used for biological tests.

The common human pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Psedomonas aeruginosa* and *Klebsiella pneumoniae* were obtained from Microbiology section of Combined Military Hospital (CMH) Muzaffarabad, Azad Jammu and Kashmir.

The antibacterial activities of different samples of *C. reflexa* and *C. campestris* were studied by disc diffusion method (Mahesh and Satish 2008). The sterilized nutrient agar media (dissolving 28 g dehydrated nutrient agar in 1000 ml distilled water, warming and shaking) were poured into the Petri plates and kept for solidification. After solidification, sterilized disc dipping with extracts were poured in Petri plates and incubated for 24 hrs at 37ºC. After that pathogenic cultures were washed on the particular agar plates using sterilized cotton pads. The experiment was repeated thrice. After incubation the diameter of inhibition zones formed around each disc was measured and expressed in millimeter (mm) plus standard errors of means to evaluate the antimicrobial activity.

The phytochemical analysis for major phytoconstituents of the plant extracts was performed. Phytocompounds can exert a wide range of medicinal values. Ethanolic, methanolic, chloroform and distilled water extracts of the plants were prepared and confirmatory tests for the presence of major compounds were tested (Tiwari *et al*. 2011).

The antioxidant activity of crude extracts was evaluated on the basis of the scavenging potential of 1, 1-diphenyl-2- DPPH picrylhydrazyl free radical scavenging potential. Ascorbic acid was considered as standard solution (Koleva *et al*. 2002). The optical density of each sample was measured against standard at λ_{max} 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:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of tested sample}}{\text{Absorbance of control}}\right] \times 100$$

Fifty per cent inhibition (IC50) of each extract concentrations against graph of inhibition was calculated by applying SSP10 software.

Results and Discussion

The antibacterial effectiveness of different solvent extracts, specifically chloroform, methanol, ethanol and distilled water of stem of *C. reflexa* and *C. campestris*, against the human pathogenic bacteria exhibited a diverse level of inhibition. The activity of different extracts of the selected plants of these species was compared with standard antibiotic ampicillin. Among all the extracts of *C. reflexa* tested, the higher activity was shown by ethanol and chloroform extracts against *S. aureus* (14.00 ± 0.67 mm) and *K. pneumoniae* (14.00 ± 1.00 mm),, as displayed in Fig. 1. *E. coli* was found to be more resistant towards stem extract while *S. aureus* and *K. pneumoniae* were more sensitive. In case of stem extract of *C. reflexa*, higher activity was shown by aqueous extract against *K. penumoniae* (10.00 ± 0.00) as shown in Fig. 2. Antibacterial studies suggested that *C. reflexa* is more active than *C. campestris*. The findings of Shikka *et al.* (2013) also showed that ethanol extract of *C. reflexa* has higher bactericidal activity against *P. aeruginosa* and *S. aureus*. Fiayyaz *et al.* (2011) also suggested that the ethanolic extract of stem has significant antibacterial activity. This activity is because of the extraction of biocompound in ethanol extract. It can be easily assumed that activity of plant extracts against microorganism is due to the presence of wide range of bioactive compounds in plants. Plant extracts are the rich source of flavonoids and polyphenols which could be the antibacterial agents. Flavonoids are well known for their antiviral (Karamoddini 2011), antimicrobial (Maria and Maria 2008) and spasmolytic (Julianeli *et al*. 2011) activity. Alkaloids extracted from plants are also known for their antimicrobial activity (Ahmed *et al.* 2010). The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and thus, inhibit the microbial growth.

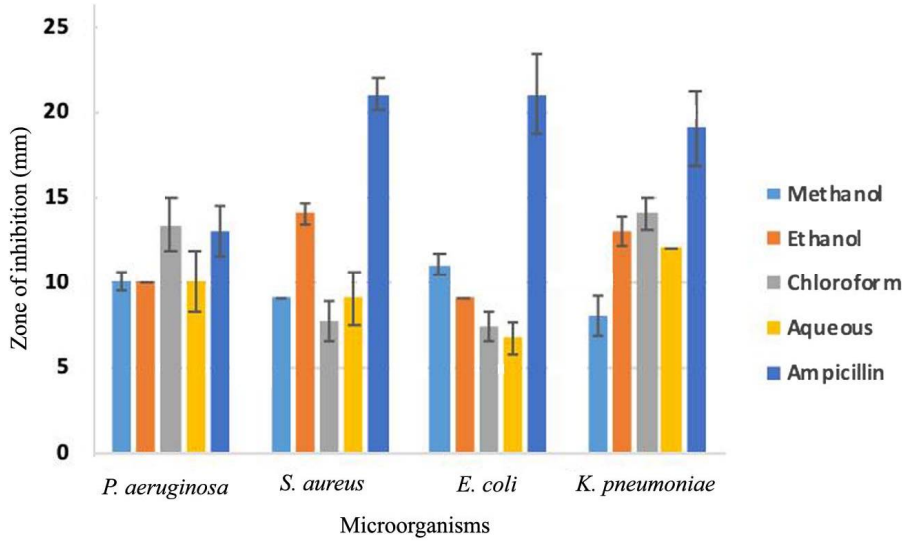

Fig. 1. Antibacterial activity of *C. reflexa.*

The preliminary phytochemical screening of *C. reflexa* and *C. campestris* shows the presence of glycosides, flavonoids, carbohydrates, tannins, proteins, alkaloids, saponins, aminoacids and phenolic compounds in different extracts (Table 1). Previous studies also reported the presence of these phytochemical in the investigated species (Thakur *et al*. 2013, Mubashr *et al*. 2015, Raza *et al*. 2015).

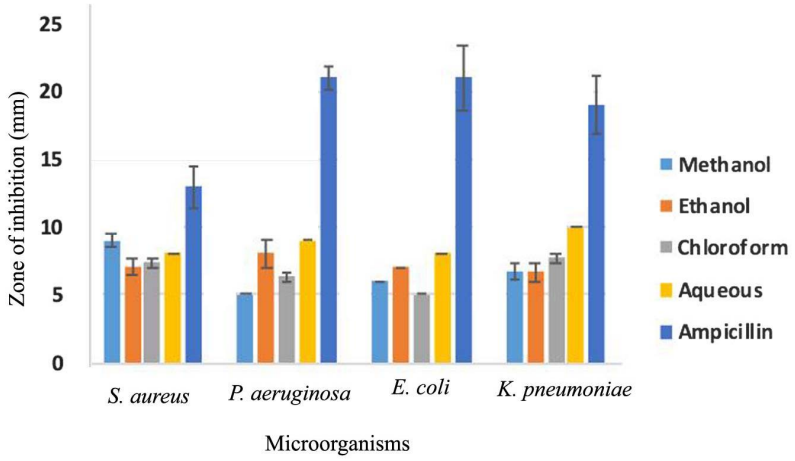

Fig. 2. Antibacterial activity of *C. campestris*.

Table 1. Screening of phytochemicals of various extracts of *C. reflexa* and *C. campestris*.

Chemical tests	*C. reflexa*				*C. campestris*			
	M	E	C	W	M	E	C	W
1. Test for glycosides								
Keller Killiani test	+	+	–	+	+	+	–	+
2. Test for saponins								
Foam test	+	–	–	+	+	–	+	+
3. Test for alkaloids								
Hager,s test	+	+	–	–	+	+	–	–
4. Test for flavonoids								
Ferric chloride test	–	+	+	+	–	+	+	+
Alkaline reagent test	–	+	–	+	–	+	–	–
Lead acetate test	+	+	–	+	–	+	–	–
5. Test for tannins								
Gelatin test	+	+	–	–	+	+	+	+
6. Test for phenols								
Ferric chloride test	+	+	–	+	+	+	+	+
7. Test for proteins								
Xanthoproteic test	+	–	+	–	–	+	–	–
8. Test for aminoacids								
Ninhydrin test	–	+	–	+	–	+	–	+
9. Test for carbohydrates								
Benedict's test	–	+	+	–	+	–	+	+

+ = Present, - = Absent.

Free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxy radicals, peroxyl radicals, and single oxygen. Many plant extracts exhibits efficient antioxidant properties due to their phyto-constituents, including phenolics

(Larson 1988). In the present study DPPH assay is used to evaluate the antioxidant potential of *Cuscuta* species. In DPPH assay, DPPH radical has been used extensively as a stable free radical to determine the reducing substances or antioxidant activities of plant extracts (Cotelle *et al*. 1996, Ozturk *et al.* 2007). Plant extracts contain polyphenols which have the ability to donate hydrogen atoms or electrons and to capture the free radicals (Wong *et al*. 2006, Stoilova *et al*. 2007). As a result, the purple coloured DPPH reduced to yellow colored complex. The DPPH assay is found most valuable method to evaluate *in vitro* antioxidant activity of plants.

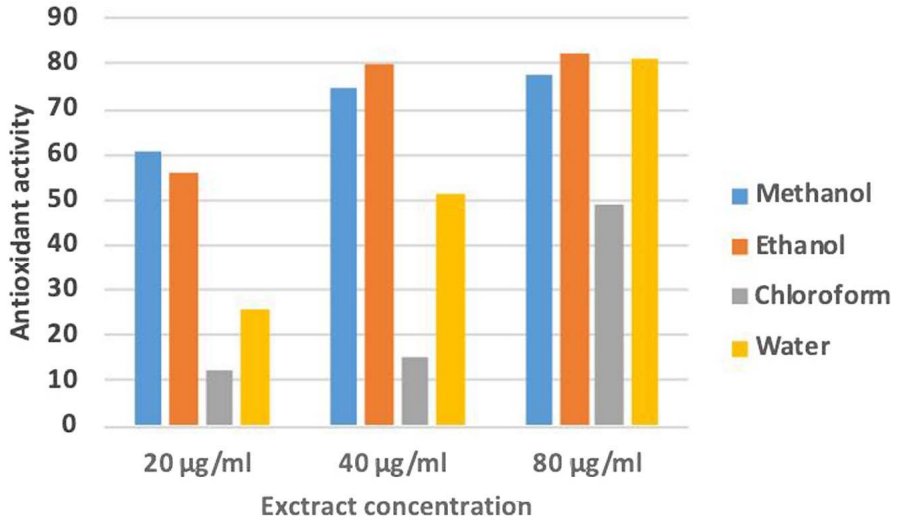

Fig. 3. Antioxidant activity of *C. reflexa.*

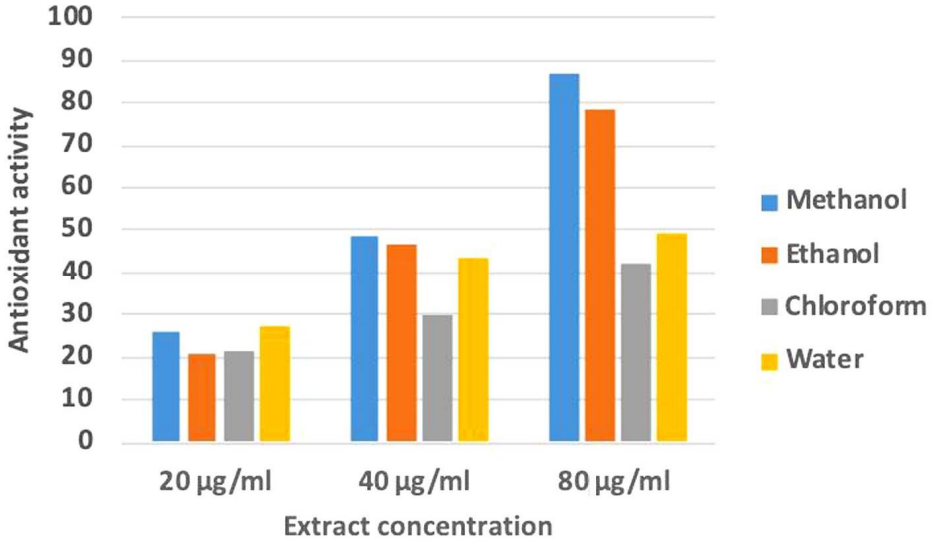

Fig. 4. Antioxidant activity of *C. campestris*.

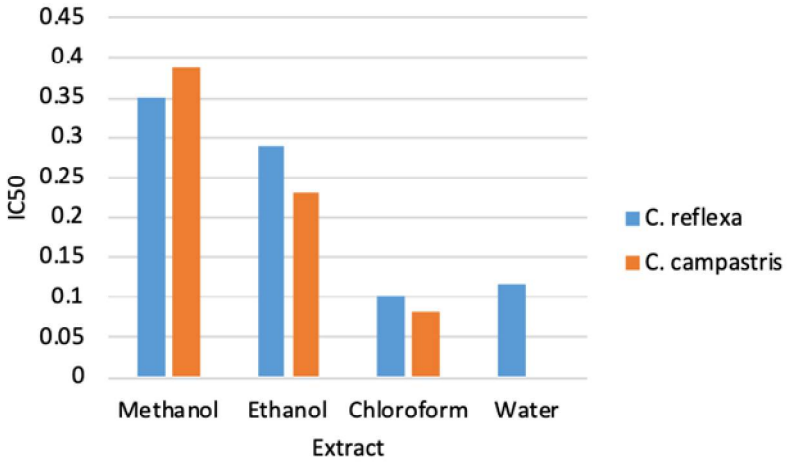

Fig. 5. IC 50 value of *C. reflexa* and *C. campestris*.

The results of free radical (DPPH) scavenging activity in percentage inhibition of stem of *C. refelxa* and *C. campestris* are shown in Figs 3 and 4, respectively. It was found that the both the plants have exerted high antioxidant power at different concentrations. In case of *C. reflexa*, the higher scavenging activity was shown by ethanol extract (82.05), followed by aqueous extract (81.31). While for *C. campestris*, methanol extract demonstrated highest antioxidant activity (87.2). Fig. 5 shows the IC50 value of both the plants. IC50 value is defined as the concentration of substrate that cause 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of anti-radical activity against the concentration of the tested compounds.

The percentage of inhibition increases due to more rapidly the absorbance decreases and the more effective the antioxidant activity of the extract. The present results were similar with the results reported by Sati *et al.* (2013). According to their results methanolic and ethanol extracts showed higher percentage DPPH scavenging activity. The methanol and ethanol extracts of investigated plants scavenged free radicals in a dose dependent manner analogous with the studies of Chandran *et al.* (2013) and Guntupalli *et al.* (2012) representing that plants secondary metabolites hold a strong antioxidative property. Flavonoids, tannins, catechins and other phenolics are the examples of common plant metabolites having prominent antioxidant activity. In the present study, *C. reflexa* and *C. campestris* have considerable antibacterial and antioxidant activity and thus could be useful as therapeutic agents in preventing and reducing the progress of aging that are related to degenerative diseases.

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