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RESEARCH ARTICLE

Impact of geographical location on the antibacterial activity of *Cuscuta reflexa*

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Abstract Medicinal herbs are extensively utilized in traditional medicine to treat a variety of infectious illnesses. Bacterial infections have a significant effect on public health. The purpose of this research was to ascertain the antibacterial activity of the medicinal plant *Cuscuta reflexa* that was collected from three distinct locations: Bilaspur (Himachal Pradesh, India), Chamba (Himachal Pradesh, India), and Jalandhar (Punjab, India). Three distinct sites were chosen for the plant sample in order to determine the effect of geographical location on its bactericidal potential. Methanol extracts of the plants were produced and evaluated for antibacterial activity against five distinct bacterial species, two of which were Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and three of which were Gram-negative *(Escherichia coli, Pseudomonas fluorescens* and *Pseudomonas aeruginosa*). Different biochemical assays were used to conduct a preliminary qualitative phytochemical screening of plant extracts. The plant's antibacterial potential was determined using the well diffusion technique. All of the plant extracts demonstrated antibacterial action, with a particular emphasis on Gram-positive bacteria. Notably, preparations of *C. reflexa* from Bilaspur had the greatest antibacterial activity when compared to those from Chamba and Jalandhar. The examination of the Bilaspur sample using Fourier-transform infrared spectroscopy reveals the presence of a variety of functional groups.

Key words: Antibacterial, Bacteria, *Cuscuta reflexa*, Fourier-transform infrared spectroscopy, Phytochemical

Introduction

There have been numerous new microbial illnesses in recent years, which has resulted in an overuse of antibiotics, which in turn has led to an increase in antibiotic resistance that has been steadily increasing over time. Furthermore, synthetic antibiotics are linked with a wide range of severe adverse effects, including hypersensitivity responses, nephrotoxicity and other complications. Many microbial illnesses are not well protected by vaccinations because of a severe lack of availability. The quest for a natural antibiotic with the least amount of side effects on the human body must thus be undertaken. Natural items with antibacterial properties may hold the key to finding a solution to this issue (Ahmad and Aqil 2007). In the past, it has been shown that not only are they efficient against the microbiological illness, but they are also capable of alleviating the side-effects associated with the medication (Iwu *et al*. 1999). One alternative is *Cuscuta reflexa*, also known as Amarbel in Hindi. This is a parasitic vine that belongs to the family Convolvulaceae (Chopra *et al.* 1992). It has been discovered to parasitize on a variety of host plants, including *Cassia fistula* and *Ficus benghalensis*, and it has a light greenish yellow colour. Traditionally, tribal plant medicine was utilised as an anticonvulsant medication to treat epileptic seizures (Gupta *et al.* 2003). Besides this, *Cuscuta reflexa* is also used to treat a variety of conditions like, skin irritation and fever (Chopra *et al.* 1992). *Cuscuta reflexa* stem, according to preliminary chemical analysis, has a high concentration of flavonoids, which may explain why it has been used for therapeutic reasons for a long period of time. (Yadav *et al.* 2000). Previous research has also shown that the phytochemical makeup of plants may change depending on the meteorological variables present, such as temperature, wind patterns and rainfall, among others (Kumar *et al.* 2017). This may also have an impact on the antibacterial activity of the medicinal plants in certain instances. This is taken into

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consideration while designing the present research, which seeks to determine the effect of a change in geographical location on the bactericidal potential of *Cuscuta reflexa.*

Material and methods

Sources of test microorganisms

Five lyophilized cultures were procured from the Microbial Type Culture Collection and Gene Bank, Chandigarh. Three of these bacterial strains were Gram negative (*Pseudomonas aeruginosa, Pseudomonas fluorescens* and *E. coli*) and two were Gram positive (*Bacillus cereus* and *Staphylococcus aureus*). They were revived by incubating them at the temperature specified in Table 1 in nutrient broth.

Sources of *Cuscuta reflexa*

The medicinal plant (*Cuscuta reflexa*) was collected from three distinct locations as shown in Figure 1 and detailed in Table 2. Dr.Inam Mohammed, Taxonomist, Department of Botany, DAV University, Jalandhar identified the plant.

Plant extract preparation

Three distinct sites were used to collect plant material, which was then shade dried for 21 days at room temperature. Using a blender, the plant material was crushed into powder. This was then weighed and diluted in methanol to achieve a 332 mg/ml concentration. Methanol was chosen as the solvent since it has been found to be ideal for extracting antibacterial components (Tekwu *et al.* 2012). These were then stored at ambient temperature for 48 hours, wrapped with aluminium foil to avoid contamination. The extracted plant material was filtered using Whatmann No. 1 filter paper and evaporated to dryness before being kept in a refrigerator at 40 ^{0}C until use (Alans *et al.* 2005).

Screening for phytochemicals

To conduct a preliminary study of the phytochemicals contained in the plant extract, several biochemical assays were conducted according to the Rao and Kaladhar's methodology (2014).

Antimicrobial activity of *Cuscuta reflexa* extracts as determined by the well diffusion test

The well diffusion analysis was used to obtain the antibacterial activity of plant extracts (Magaldi *et al.* 2004). The bacterial isolates were cultured on nutrient agar. The microbial inoculum was distributed over the agar plate in a volume of 100µl. Wells were created in the culture plate aseptically using a borer with a diameter of 6 mm. Each well

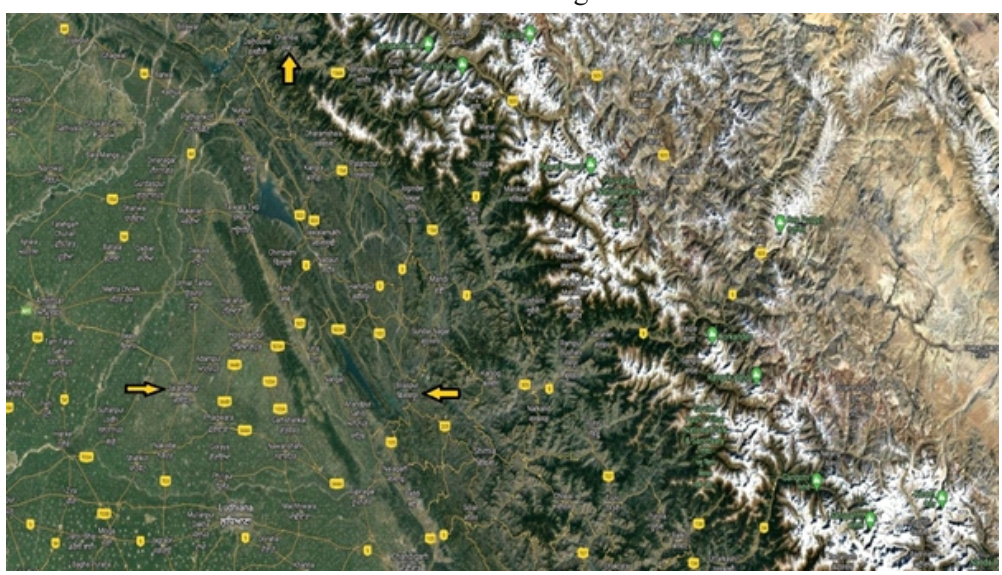

Figure-1: Google Map showing the three different geographic locations (Jalandhar, Punjab; Bilaspur, Himachal Pradesh and Chamba, Himachal Pradesh) in India from where the samples of *Cuscuta reflexa* were obtained.

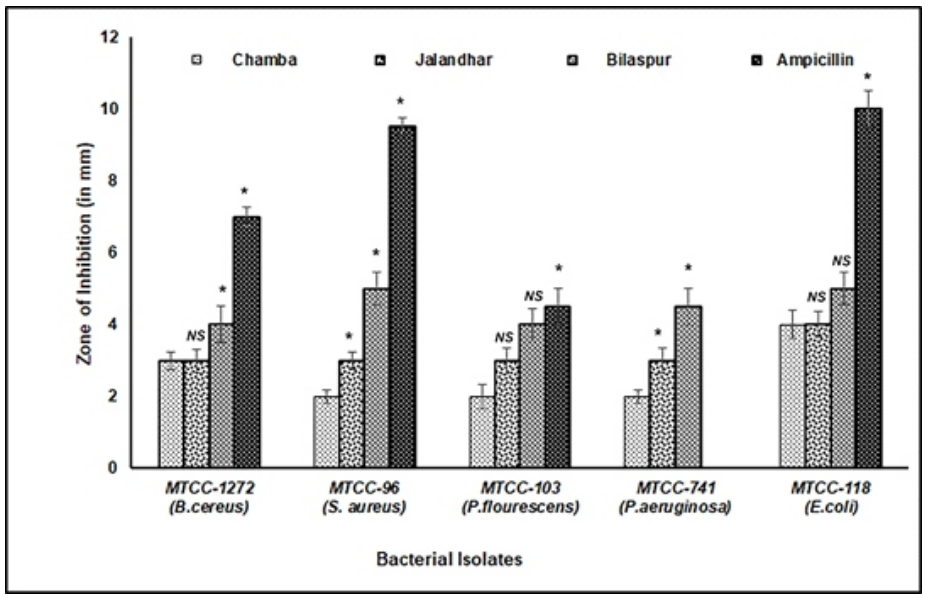

Figure-2: The graph showing the bactericidal efficacy of *Cuscuta reflexa* obtained from three different geographic locations (Jalandhar, Punjab; Bilaspur, Himachal Pradesh and Chamba, Himachal Pradesh) against five bacterial species. (NS- Not Significant, *- $p<0.05$)

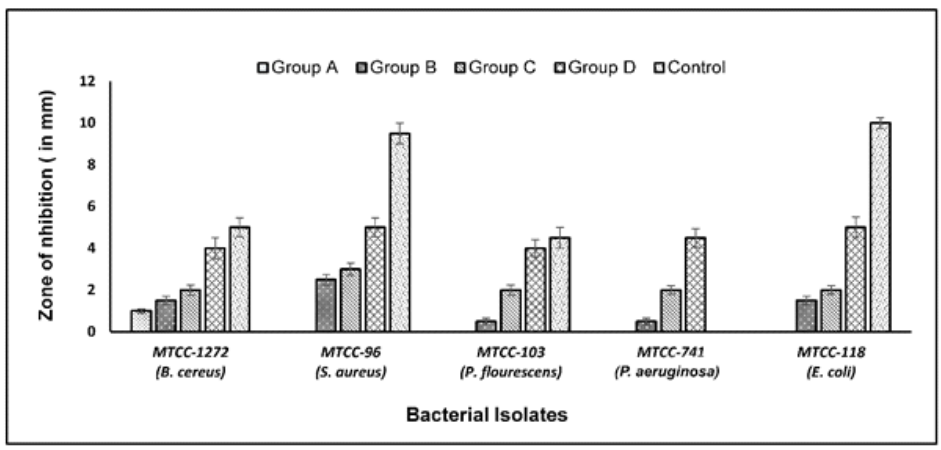

Figure-3: The graph showing the antibacterial activity of *Cuscuta reflexa* at different concentrations.
The concentration of the extract in Group A was 83 mg/ml. The concentration in Group B was 166 mg/ml and that in Group C was 221.33 mg/ml. The maximum concentration of the plant extract used was 332 mg/ml in Group D.

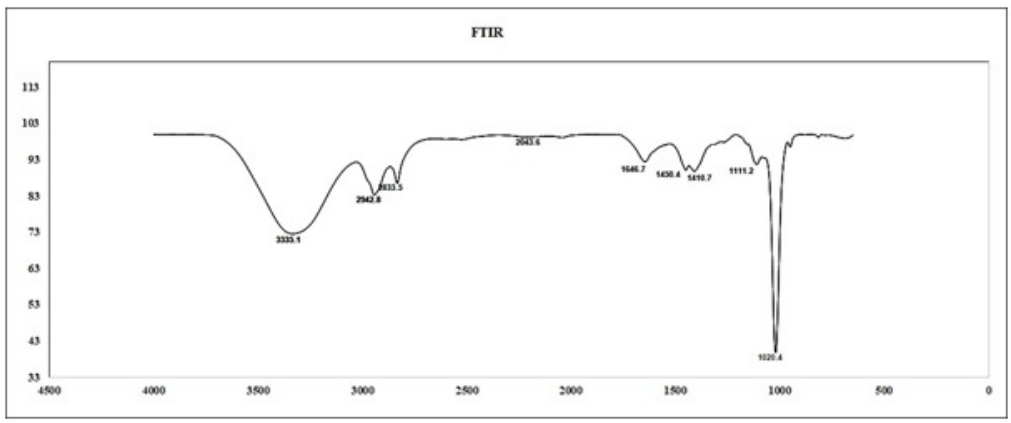

Figure-4: The FTIR spectrum wavenumbers of Bilaspur sample methanolic extract of *Cuscuta reflexa*.

Table-1: Temperature and incubation time required for the growth of bacterial cultures.

S.No.	Bacterial culture	Temperature required	Incubation time
1.	MTCC-118 (*E.coli*)	37 ^{0}C	48 hours
2.	MTCC-741 (*Pseudomonas aeruginosa*)	37 ^{0}C	12 hours
3.	MTCC-103 (*Pseudomonas fluorescens*)	25 ^{0}C	48 hours
4.	MTCC-1272 (*Bacillus cereus*)	30 ^{0}C	24 hours
5.	MTCC-96 (*Staphylococcus aureus*)	37 ^{0}C	48 hours

received about 20 µl of the extracted plant samples and was incubated at 37ºC for approximately 24 hours. After the incubation time was complete, the extract diffused into the agar medium and hindered the development of the bacterial isolate, which was then measured using a clear ruler in millimetres as the zone of inhibition.

Minimum Inhibitory concentration of the plant extract

The maximal zone of inhibition as determined by a plant specimen was obtained in Bilaspur (Himachal Pradesh). As a result, this sample was examined further to determine its minimum inhibitory concentration. This sample was utilised at concentrations of 83 mg/ml, 166 mg/ml, 221.33 mg/ml and 332 mg/ml. These were designated as Groups A, B, C, and D. Concentrations of all plant extracts were prepared by dissolving them in methanol. To evaluate the produced samples, MHA (Mueller-Hinton Agar) plates were made and distributed with each bacterial solution. Each plate was punched four holes with a sterile borer about 6 mm in diameter. Then, plant extracts from each group were put into the four holes of separate plates and incubated for 24 hours at 37 ^{0}C. The inhibitory zones so generated were quantified in millimetres using a transparent ruler.

FTIR spectroscopy

Fourier Transform Infrared Spectrophotometry (FTIR) is perhaps the most effective technique for classifying chemical bonds/functional groups in phytochemicals (Dominic *et al.* 2020). The wavelength of light absorbed by the chemical bonds in the annotated spectrum is used to identify them. By analysing the infrared absorption spectra of a molecule, the chemical bonds within it may be determined. FTIR analysis was performed using liquid methanol solvent extractsof the plant material. A total of 15ml of sample was submitted to the Panjab University's Sophisticated Analytical Instrumentation Facility (SAIF) in Chandigarh.

Statistical analysis

Methanol was used as a negative control in all tests, while ampicillin was utilised as a positive control in all studies. The results were reported as the mean standard deviation of five separate experiments, and the student's t-test was used to evaluate them.

Results

Screening for phytochemicals

The results of a qualitative study on a crude methanol extract of *Cuscuta reflexa* collected from three different locations are summarised in Table 3. *Cuscuta reflexa* methanol extracts from all three locations contained amino acids, alkaloids, tannins, flavonoids, triterpinoids, steroids, cardiac glycosidases, glycoside anthraquinone, and saponins.

Zone of inhibition

The findings of the well diffusion test for extracts from three distinct Indian regions Jalandhar in

Table-2: Description of geographical locations from which plant samples were collected.

State	Place of collection	Elevation above sea level	Latitude	Longitude	Average annual rainfall	Average annual temperature
Himachal Pradesh	Chamba	996 m	32° 33' 19.12" N	76° 07' 35.29" E	2213 mm	20.7 ^{0}C
Himachal Pradesh	Bilaspur	673 m	31° 19' 48.00" N	76° 45' 0.00" E	1341 mm	22.7 ^{0}C
Punjab	Jalandhar	228 m	31° 19' 32.02" N	75° 34' 45.01" E	769 mm	23.9 ^{0}C

Table-3: Qualitative analysis of the plant sample from three different locations

S.No.	Biochemical test	Bilaspur sample	Chamba sample	Jalandhar sample
1.)	Amino acids	+	+	+
2.)	Flavonoid	+	+	+
3.)	Triterpinoids	+	+	+
4.)	Cardiac glycosides	+	+	+
5.)	Tanin	+	+	+
6.)	Steroids	+	+	+
7.)	Glycoside anthraquinone	+	+	+
8.)	Alkaloids	+	+	+
9.)	Saponins	_	_	_

Punjab; Bilaspur in Himachal Pradesh; and Chamba in Himachal Pradesh are shown in Figure 2. Table 2 summarises the geographic and climatic characteristics of these areas. The antibacterial activity of methanolic plant extracts obtained from various geographic areas was determined using five different bacterial cultures listed in Table 1. While all three samples shown antibacterial action against *Pseudomonas aeruginosa, Pseudomonas fluorescens, E. coli, Staphylococcus aureus* and *Bacillus cereus*, the medicinal plant's efficacy was observed to vary according to bacterial strain. In comparison to Chamba and Jalandhar samples, the Bilaspur sample exhibited the greatest zone of inhibition. The sample from Bilaspur had the greatest effectiveness against *E. coli* and *S. aureus*, while the samples from Jalandhar and Chamba showed the greatest antibacterial potential against *E. coli*.

Minimum inhibitory concentration

The plant extract from Bilaspur had the greatest bactericidal potential. As a result, this plant was examined further to determine its minimum inhibitory concentration against the five bacterial samples adopted in this research. The same was determined at four different concentrations (83 mg/ml, 166 mg/ml, 221.33 mg/ml, and 332 mg/ml) obtained by dissolving the plant sample in methanol. The findings of this experiment are graphed in Figure 3. The Bilaspur sample was found to have a maximum bactericidal effect at 332 mg/ml and a minimum inhibitory concentration of 166 mg/ml.

FTIR Spectroscopy

As already mentioned above the Bilaspur sample showed the greatest antibacterial activity, therefore it was selected further for FTIR. The FTIR spectrum of a *Cuscuta reflexa* methanol extract from a Bilaspur sample is shown in Figure 4. The peak values and potential functional groups contained in the methanol extracts of *Cuscuta reflexa* are listed in Table 4. (as determined by FTIR analysis). The IR radiation area, which is determined by the peak values of the FTIR spectrum, helps in the identification of the functional groups of active components contained in the extract. The absorbance bands between 400 and 4000 cm^{-1} are analysed. Alcohols, Alkanes, Alkenes, Amines, and Ester are confirmed by the FTIR findings. At 3335.1 cm^{-1}, significant peaks were seen that may be ascribed to O-H stretching vibrations. As a result of this analysis's results, alcohol seems to be the most prevalent functional category in *Cuscuta reflexa*.

Table-4: Major values of the FTIR spectral wavenumbers and functional groups obtained from the extract

S.No.	Wavenumbers	Bond	Functional group
1.)	3335.1	O–H	Alcohol
2.)	2942.8	C–H	Alkanes
3.)	2833.5	H–C=O: C–H	Aldehydes
4.)	2043.6	C=C	Alkenes
5.)	1646.7	=C–H	Alkenes
6.)	1450.4	C–H	Alkanes
7.)	1410.7	C–H	Alkanes
8.)	1111.2	C–O	Esters

Discussion

In this study, *Cuscuta reflexa* plant extracts were obtained from three different locations with three different geographical types, and the results were compared. The primary goal of this study was to examine the antibacterial characteristics of medicinal plants collected from three different locations: Jalandhar, Chamba, and Bilaspur, respectively. Five different types of pathogenic bacteria were utilised to evaluate the effectiveness of the plant from each of the three sites and to make those determinations, well diffusion test was used. In three samples collected from various sites, the antibacterial activity of the same medicinal plant was shown to be varied, with the Bilaspur sample exhibiting the greatest zone of inhibition. This may be owing to the fact that the climatic conditions in each of these three places are different. A number of previous investigations have also shown intraspecific variation in phytochemical components in response to variations in

environmental circumstances (Glassmire *et al.* 2016, Liu *et al.* 2005, and Rimkiene *et al.* 2017). Another consequence of the diversity in phytochemical composition is that secondary metabolites and antioxidant activity may differ from one medical plant to the next, which may influence the bactericidal effectiveness of the medicinal plants (Vu *et al.* 2015). The findings, on the other hand, are consistent with a previous study that demonstrated the antibacterial potential of ethanolic extracts prepared from *Cuscuta reflexa* against *E. coli* (Ayesha *et al.* 2011). The findings are also consistent with those of an earlier study, which found that extracts of *Cuscuta reflexa* had antimicrobial activity against *Bacillus subtilis, Pasteurella multocida*, and *Staphylococcus aureus*, among other bacteria (Perveen *et al.* 2013). After being collected in different seasons, *Cuscuta reflexa* was found to have antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, E. coli, Micrococcus luteus*, and *Pseudomonas aeruginosa*, among other pathogens (Sharma *et al.* 2013). According to the findings of the research conducted by Manirujjaman and colleagues, the concentration of 500 g/mL elicited the largest zone of inhibition in *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Salmonella typhi* bacteria (Manirujjaman *et al.* 2016).

Conclusion

According to the findings of the present research, the methanol extracts of *Cuscuta reflexa* have antibacterial activity that varies somewhat depending on the geographical circumstances. Based on the greatest antibacterial power shown by the Bilaspur sample, it can be concluded that the parasite vine cultivated under moderate temperature and rainfall circumstances may provide considerable protection against a wide range of bacterial infections. As a result, the research recommends that while developing antimicrobial medicines from plants, the geographic location and climatic circumstances be taken into account at all times.

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