Research Article

Investigations on anti-bacterial effect of *Cuscuta reflexa* extract

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Abstract

Objective: The present study was carried out to provide evidence to the traditional use of *Cuscuta reflexa* in the treatment of microbial infection against various microbial species. **Material and Methods:** The plant was extracted with chloroform and ethanol. The anti-microbial activity of the extracts was screened using the disc diffusion method on in-vitro condition with different concentration of plant extracts. **Results and Conclusion:** The result examined each plate with different interval and measured the diameter of zone of complete inhibition including the diameter of disc. The study showed that 50% ethanolic and 100% chloroform extract of *Cuscuta reflexa* caused antibacterial activity against *Pseudomonas aeruginosa*, *Shigella flexneri*, *E. coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. 100% chloroform extract of *Cuscuta reflexa* was found more effective as compare to 50% ethanolic extract against different bacteria.

Keywords: Zone of Inhibition, *Cuscuta reflexa*, disc diffusion, antibacterial

Introduction

The modern era of antimicrobial chemotherapy began in 1929, with Fleming's discovery of the powerful bactericidal substance, penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity (Levy, 1994). In the early 1940's, spurred partially by the need for antibacterial agents in WW II, penicillin was isolated and purified and injected into experimental animals, where it was found not only to cure infections but also to possess incredibly low toxicity for the animals. This fact ushered into being the age of antibiotic chemotherapy, and an intense search for similar antimicrobial agents of low toxicity to animals that might prove useful in the treatment of infectious disease. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were in clinical usage.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans, as well as destroying viruses (Smith et. al., 1998). Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth

of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940's, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients—those in the hospital. Currently, bacterial resistance is combated by the discovery of new drugs.

Antibacterial is the substance that destroys bacteria or

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suppresses their growth or their ability to reproduce. Heat, chemicals such as chlorine, and antibiotic drugs all have antibacterial properties (Agarwal et al., 2009). Many antibacterial products for cleaning and hand washing are sold today. Such products do not reduce the risk for symptoms of viral infectious diseases in otherwise healthy persons. This does not preclude the potential contribution of antibacterial products to reducing symptoms of bacterial diseases in the home.

Cuscuta (Dodder) is a genus of about 100-170 species of yellow, orange or red parasitic plants. Formerly treated as the only genus in the family *cuscutaceae*, recent genetic research by the Angiosperm phylogeny group has shown that it is correctly placed in the family *convolvulaceae* (Prashanth et al., 2006). The genus is found throughout the temperate to tropical region of the world, with the greatest species diversity in subtropical and tropical regions, the genus becomes rare in cool temperate climate, with e.g. only four species native to northern Europe.

Cuscuta reflexa is leafless and rootless. (Singh & Khan 1989) Initially the starter plant would have some roots. Within a few days of germination, the plant which is touch sensitive, finds a host or dies. After establishing itself on the host body, it draws nutrition from the host as a stem parasite and the roots wither away. The twining stem develops Haustoria which are root like and penetrate the host stem to draw water and nourishment (Dawson, 1990).

The flowers are small, white, having a perfect bell shape and a fresh calyx, attached directly to the stem node. Although a few species are reported to have medicinal use, the rampant Dodder plant is a varocious and destructive vine which usually will overgrown and kill the host. It also is a cause of transmission of different viruses' diseases such as citrus mosaic and purple blotch to field crops and trees. Its seeds can remain dormant for five year and control of dodder is an important issue for crops and forests. Plants lover and conservationists enjoying this site should note the in current Science Magazine. It has been identified as an emerging threat to the plant diversity in the valley of flowers. While this flower appears like a perfect lustrous bell, the experts advise that wherever an unwanted dodder vine is growing, all its stem pieces should be removed (Preferably before seeding) and burnt. It has very low level of chlorophyll, some species such as *Cuscuta reflexa* can photosynthesis slightly, while other such as *C. europeans* nutrition (Chado and Zetsche, 1990).

This part provides a review of the earlier contributions made in the chemistry and pharmacology of parasitic plant *Cuscuta reflexa* and a brief discussion of the present work, with reference to the bioassay directed isolation of anti-cancer and other compounds from the ethanolic extract of *C. reflexa*. This work led to the isolation of twenty six compounds which were

characterized through the spectral studies and chemical transformations (Mhaskar et al., 2000). One (II) of them is a new constituent and the remaining twenty five (I, III-XXVI) are known compounds of which twelve are new from the plant *C. reflexa*. 21-Hydroxy odoroside H, Odoroside H, Neritaloside, Strospeptide, Gitoxigenin, Ursolic acid, B-Sitosterol glucoside, 4-O-p-Coumaroyl-O-glycoside, Methyl cinnamate, Dihydroajugapitin (Versani and Muhammad, 1997).

Materials and Methods

Collection and identification of plant material

According to opinion of the local traditional practitioner, this plant is being used for the treatment of cancer. The compiled plant therefore is subjected to identification and then literature survey to conform that the plant has not been previously investigated for the anticarcinogenic activity. So, the selection of the medicinal plant for anticarcinogenicity has been undertaken (Kokate, 2003). The herb were dried under shade, and then powered with mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further studies.

Extraction of plant material

The herb was collected from the local forest and dried for few days in shade. Then powder was made with the help of grinder, the *Cuscuta reflexa*. 50gm of powder was taken in a separating funnel and added 50% ethanol, then mixed gently. After every 24 hrs extract was collected in a beaker till the solvent appears colourless. In same way chloroform extract was prepared with 100% chloroform in place of 50% ethanol. The extract was dried into powder by water bath at 60°C and hot air oven at 45°C. The total weight of extract powder was measured and calculated % yield of each extract. On the day of experimentation, the desired amount of powder was suspended in to double distilled water (DDW) for the final administration.

Anti-bacterial activity

The test organisms (*Pseudomonas aureogenosa*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Shigella flexineri*, *Bacillus subtilis* & *E. Coli*.) were obtained from the Department of Research, JN Cancer Hospital and Research Center, Bhopal (M.P.) (Agarwal et al., 2009).

Kirby-Bauer Method (Disc diffusion method) was followed to test the antibacterial activity of different concentration of plants extract. The paper disc having the same diameter absorbed the concentration of extract. After impregnated the disc on agar plate, it diffuses their drug and in case of drug sensitive bacteria make a clear area that is known as

Inhibitory area or Zone of inhibition (Ramaswamy and Charles, 2009).

Preparation of disc

The disc of Whatmann filter paper no.1 of same diameter (0.5mm) by the help of punching machine was made. Autoclaved the disc in a Petri dish for sterilization, prepared each concentration of discs by pouring the disc in different concentration. Dried the discs and preserved in different concentration of Petri dishes at 2-8°C.

Methods: All of the required apparatus and materials were sterilized in autoclave and placed in a laminar airflow cabinet under pathogen free conditions. Test organisms were collected from department's microbial standard stock. By streaking with loop, microorganisms were inoculated in nutrient broth and incubated at 35 °C for 12 hr. Nutrient agar media was prepared and poured in Petri plates and kept for drying. Swab was dipped in broth having microbial growth and gently squeezed against the inside of the tube to remove excess fluid. Inoculated the dried surface of agar plate by streaking the swab over the entire sterile agar surface. Repeated this procedure two more times, and rotated the plate 60° each time to ensure an even distribution of inoculums. Replaced the plate top and allow 3 to 5 minutes, but

no longer than 15 minutes, for any excess surface moisture to be absorbed before applying the test and antibiotics disks (Baur, 1966). Disks were dipped in drug (*Cuscuta reflexa*) of different concentration (25%, 50%, 75% & 100%) and air dried in laminar air flow before this step. Placed the appropriate disks (Drugs and antibiotics) evenly (no closer than 24 mm from center to center) on the surface of the agar plate by using a sterile forceps. Inverted the plate and placed them in an incubator at 35°C within 15 minutes after disks were applied. After 6-8 hrs, of incubation, examined each plate at an interval of 2 hours and measured the diameters of the zones of complete inhibition, including the diameter of the disk (Khandelwal, 2005).

Results and discussion

Our present study showed that 50% ethanolic and 100% chloroform extract of *Cuscuta reflexa* caused antibacterial activity against *Pseudomonas aeruginosa*, *Shigella flexneri*, *E. coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. 100% chloroform extract of *Cuscuta reflexa* was found more effective as compare to 50% ethanolic extract against different bacteria (Dubey et al., 2004).

Table 1. Antibacterial activity of chloroform extract of *C. reflexa*

S. No.	Micro-Organisms	Concentrations and Zone of inhibiton in mm			
		100%	75%	50%	25%
1.	<i>B. subtilis</i>	14	13	11	08
2.	<i>Sh. Flexinerae</i>	17	15	11	08
3.	<i>S. epidermis</i>	18	18	15	12
4.	<i>S. aureus</i>	19	17	15	11
5.	<i>E. coli</i>	17	17	16	14
6.	<i>Ps. Aurigenosa</i>	15	14	12	10

Table 2. Antibacterial activity of 50% ethanolic extract of *C. reflexa*

S.No.	Micro-Organisms	Concentrations and Zone of inhibiton in mm			
		100%	75%	50%	25%
1.	<i>B. subtilis</i>	18	16	14	13
2.	<i>Sh. Flexinerae</i>	19	16	11	08
3.	<i>S. epidermis</i>	18	18	14	12
4.	<i>S. aureus</i>	15	13	12	09
5.	<i>E. coli</i>	17	17	15	14
6.	<i>Ps. Aurigenosa</i>	15	12	12	10

Figure 1. Antibacterial activity of *C. reflexa* chloroform extract against different bacteria

Figure 2. Antibacterial activity of *C. reflexa* 50% ethanolic extract against different bacteria

Anti-bacterial activity

Table 1 shows the antibacterial activity of different concentration of chloroform extract of *C. reflexa*. The result suggest that maximum antibacterial activity in 100% concentration of *C. reflexa* extract. Table 2 shows the antibacterial activity of different concentration of ethanolic extract of *C. reflexa* against different species of micro-organisms, the result suggest that maximum antibacterial activity in 100% *C. reflexa* extract. When effect of ethanolic extract was compared with chloroform extract group it was found that ethanolic extract of *C. reflexa* was more effective against *B. subtilis*, *Sh. flexinarae* and *S. epidermis*. For *E. coli* and *P. auriginosa* both extract shows the same activity. For *S. aureus* chloroform extract was found more effective as compared to ethanolic extract. Table 3 & 4 shows the antibacterial activity of different antibiotics, which used as standard to compare the different test extract for their antibacterial activity.

The study concluded that 50% ethanolic and 100% chloroform extract of *Cuscuta reflexa* caused antibacterial activity against *Pseudomonas aeruginosa*, *Shigella flexneri*, *E. coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. 100% chloroform extract of *Cuscuta reflexa* was found

Table 3. Antibacterial activity of standard antibiotic (gram positive) against different bacteria

Name of microorganisms	Name of Standard antibiotics [zone of inhibition(mm)]			
	TE	OF	AZ	PC
<i>S. aureus</i>	15	16	16	14
<i>B. subtilis</i>	14	16	18	14
<i>S. epidermidis</i>	14	18	17	17

TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin & PC- Piperacillin

Table 4. Antibacterial activity of standard antibiotic (gram negative) against different bacteria

Name of microorganisms	Name of Standard antibiotics [zone of inhibition(mm)]			
	FU	GM	CX	NF
<i>E. coli</i>	12	16	8.0	16
<i>Sh. flexneri</i>	18	18	12	21
<i>Ps. aeruginosa</i>	14	13	18	20

FU- Nitrofurantoin, GM- Gentamicin, CX- Cefotaxime & NF- Norfloxacin

Figure 3. Antibacterial activity of standard antibiotic (gram positive) against different bacteria

Figure 4. Antibacterial activity of standard antibiotic (gram negative) against different bacteri

Figure 5. Antibacterial activity of standard antibiotic (gram positive) against bacteria

Figure 6. Antibacterial activity of standard antibiotic (gram negative) against bacteria

more effective as compare to 50% ethanolic extract against different bacteria.

Conflict of Interest

The authors declare that they have no conflict of interest.

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