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COMPARATIVE STUDY ON ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *CUSCUTA REFLEXA* GROWN ON *CASSIA FISTULA* AND *FICUS BENGHALENSIS*

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ABSTRACT: Comparative antibacterial study of ethyl acetate extract of *Cuscuta reflexa* parasite grown on *Cassia fistula* and *Ficus benghalensis* was performed using disc diffusion method. Four different concentrations, 50, 100, 150 and 200 mg/ml of ethyl acetate extract of *Cuscuta reflexa* grown in two different host plants were prepared and tested against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcous aureus* and *Salmonella typhi* in disc diffusion method. *Cuscuta reflexa* extract from both the host trees showed effect but difference in their activity to the test organisms was found. Significantly higher activity was shown by *Cuscuta reflexa* grown on *Cassia fistula* against all the pathogenic bacteria and produced zones of inhibition ranging from 14.18-22.5 mm at 100 mg/ml concentration but extract was found inactive against *Salmonella typhi* at all tested concentration. In the same way *Cuscuta reflexa* grown on *Ficus benghalensis* was active against all the bacterial strains and produced zones of inhibition ranging from 10.4-12.98 mm at 100 mg/ml concentration but extract was found inactive against *Escherichia coli* at all the tested concentrations. This study confirms the host dependent antibacterial activity of *Cuscuta reflexa*.

INTRODUCTION: Since prehistoric times, the treatment and cure of diseases has been one of the primary concerns of mankind [1]. Medicinal plant would be the best source to obtain a variety of antibacterial drugs. Therefore they should be investigated to better understand their properties, safety and efficacy [2]. Nowadays, about 70% of the bacteria that cause infections are resistant to at least one of the antibiotic agents most commonly used for treatment [3].

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Cuscuta reflexa (Cuscutaceae), commonly known as Amarbel, is phanerogamic stem parasite. The species is rootless leafless twining annual parasites with yellowish stems.

In present work, antibacterial activity of ethyl acetate (EA) extract of *Cuscuta reflexa* grown on two different host plants separately, *Cassia fistula* and *Ficus benghalensis* against five pathogenic bacteria of different strains has been evaluated.

Cuscuta reflexa is the valuable medicinal herb. Ethanolic extract of *Cuscuta reflexa* shows antimicrobial activity against gram positive and gram negative bacteria and some fungal strains [6]. It is useful in treatment of androgen induced alopecia [7].

It also gives anti-inflammatory and anti-cancer activity. [8] The aqueous and alcoholic extract of C.*reflexa* has diuretic activity [9]. The methanol extract of the *C. reflexa* also shows the hepatoprotective activity [10]. It is the parasitic plant completely dependent on host plant for food and nutrition. The organic matter is transported from the phloem of the host to the parasite through the haustorium [11]. It is believed that the parasitic herbs extract healthy and potential sap from host plant and if their host plant is medicinal plants then these parasitic herbs show many similar properties to host plants. *Cuscuta* species feeding on commonly used medicinal herbs are given special attention by traditional healers.

Antibacterial activity of these two host plants was also confirmed by different researchers. Bhalodia and Shukla in 2011 tested the antimicrobial potential of *Cassia fistula* leaf extracts. The hydroalcoholic extract of leaf showed antibacterial effect on gram positive and gram negative bacteria.[12] Abbas et.al in 2004 had reported the activity of petroleum ether, methanolic and ethyl acetate extract of seed stem bark and leaves of *Cassia* against different pathogenic bacteria [13].

Anti-microbial activity of *Ficus benghalensis* was also confirmed by many scientists. Manimozhi *et al* performed the antibacterial activity of different extracts on *Ficus benghalensis* and found them active against gram positive and gram negative bacterial strains [14]. Ariel roots and stem bark of *Ficus* also exhibit the antibacterial activity [15].

MATERIAL AND METHOD

Collection of plant material: Stems of selected plant *C. reflexa* were collected from the trees of *Cassia fistula* (Amaltas) and *Ficus benghalensis* (bannayan tree) separately near the Jabalpur Engineering College Gokulpur, district Jabalpur during the month of September and November 2010 respectively. Collected material was carefully carried to the laboratory. Immense care was taken to avoid the mixing of host plant with that of targeted *Cuscuta* stem. Stems of *Cuscuta* were cleaned and completely separated from the stems of host plant. .

Solvent extraction: Thoroughly washed stems of *C. reflexa* from both the host trees were dried in shade for about fifteen days and then powdered with the help of blender. The shade dried powder was filled in thimble and extracted with ethyl acetate with the help of Soxhlet Apparatus. Extract was filtered in whatman filter paper. Filtrate was then concentrated under reduced pressure and preserved at 5^{o}c in air tight bottle.

Antibacterial activity: Antibacterial activity tests were carried out on crude ethyl acetate extract of *C. reflexa* from both the host plants using disk diffusion method against five pathogenic bacteria including gram positive and gram negative bacteria, *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Salmonella typhi.* All the bacterial strains were obtained from Department of microbiology, Govt. Model Science College, Jabalpur.

To determine the antimicrobial activity of ethyl acetate extract of both the *Cuscuta* sample disc diffusion assay was carried out. Different concentrations of plant extracts were prepared in the order of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml. Pure ethyl acetate was used as negative control.

Standard antibiotic tetracycline, streptomycin and ampicillin at the concentration of 160 mg/ml were served as positive control. Discs of 6mm in diameter were saturated with ethyl acetate extract of different concentrations of plant extract and allowed to dry and transferred onto the inoculated nutrient agar medium plate. Plates were incubated at 37^{o}C for 24 hours. Antibacterial bacterial activity was determined by measuring the inhibition zone diameter around the disc. Zone of inhibition is indicated by the clear area around the disc which shows no bacterial growth.

RESULT: At the concentration of 50mg/ml C. *reflexa* extract in EA grown on C. *fistula* showed the inhibition zone of 11.6mm size and 11.4mm size against *E. coli* and B. *subtilis* respectively which are statically at par (P>0.05). Against *K. pneumoniae* at same concentration, 4.9mm size zone of inhibitions were produced which is significantly different from all treatments.

($F_{(p<0.001)}$ = 201.78, df = 16, $SE_{(d)\pm}$ 0.040 $LSD_{(p<0.05)}$= 0.855) (Table1). Inhibition zones produced by EA extract of *C. reflexa* grown on *F. benghalensis* at this concentration were 8.8mm, 8.86mm, 8.26mm, for *B. subtilis*, *S. aureus* and *S.*

typhi respectively which are statically at par (*P*>0.05). Least zone size, 7.7mm was produced against *K. pneumoniae* ($F_{(P<0.001)}$= 179.17), *df* = 16, $SE_{(d)}$± 0.106, $LSD_{(P<0.05)}$ = 0.225) (Table2).

On comparing the activity of EA extract of *C. reflexa* grown on C. *fistula*, 100 mg/ml concentration of extract produced 17.0mm, 16.78mm, 22.5mm and 14.36mm size zones of inhibition against *E. coli*, *B. subtilis*, *K. pneumoniae* and *S. aureus* respectively ($F_{(P<0.001)}$ = 571.19, *df* = 16, $SE_{(d)}$± = 0.030, $LSD_{(P<0.05)}$ = 0.064) (**Table 1**). At the same concentration, EA extract of *C. reflexa* grown on *F. benghalensis* produced 12.98mm and 11.84mm size zones of inhibition against *B. subtilis*, *S. aureus* respectively and are statistically different ($F_{(P<0.001)}$ = 270.90, *df* = 16, $SE_{(d)}$± = 0.033, $LSD_{(P<0.05)}$ = 0.071) (**Table 2**). Against *K. pneumoniae* and *S. typhi*, 10.6mm and 10.4mm size zones of inhibition respectively were produced and were statistically at par (P>0.05).

TABLE 1: ANTI-BACTERIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF C. *REFLEXA* GROWN ON C. *FISTULA* AGAINST DIFFERENT BACTERIAL SPECIES

Test organism	Concentrations of plant extract (mg/ml)							
	50	100	150	200	Solvent	Tetracycline	Streptomycin	Ampicillin
	Zones of Inhibition (in mm)							
E. *coli*	$11.6^{c*\#}$ (3.478)	17.0^{c} (14.181)	20.0^{c} (4.527)	22.6^{c} (4.805)	0.0^{a} (0.707)	17.78^{b} (4.275)	23.18^{a} (4.864)	12.92^{a} (3.663)
B. *subtilis*	11.4^{c} (3.449)	16.78^{c} (4.156)	19.94^{c} (4.521)	22.18^{c} (4.762)	0.0^{a} (0.707)	12.84^{a} (3.652)	23.08^{a} (4.855)	0.0^{a} (0.707)
K. *pneumoniae*	14.9^{d} (3.932)	22.5^{d} (4.769)	24.8^{d} (5.029)	27.0^{d} (5.244)	0.0^{a} (0.707)	31.06^{d} (5.617)	32.96^{d} (5.784)	25.88^{a} (5.136)
S. *aureus*	9.9^{b} (3.224)	14.36^{b} (3.854)	15.74^{b} (4.029)	18.06^{b} (4.308)	0.0^{a} (0.707)	36.84^{d} (6.11)	23.8^{a} (4.864)	37.76^{a} (6.185)
S. *typhi*	0.0^{a} (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	27.96^{c} (4.934)	30.9^{c} (5.603)	11.1^{a} (3.404)
$F_{(P<0.001)}$	201.78	571.19	573.35	557.05	NS	30.53	801.31	736.87
df	16	16	16	16	16	16	16	16
$SE_{(d)}$±	0.040	0.030	0.032	0.043	NS	0.253	0.224	0.018
$LSD_{(P<0.05)}$	0.855	0.064	0.068	0.920	NS	0.537	0.047	0.038

*values inside the parentheses are the square root transformations of original values. #values outside the parentheses are back transformed means of original values.

TABLE 2: ANTIBACTERIAL ACTIVITY OF *C. REFLEXA* EXTRACT GROWN ON *F. BENGHALENSIS* AGAINST DIFFERENT BACTERIAL SPECIES

Test organisms	Concentrations of plant extract (mg/ml)							
	50	100	150	200	Solvent	Tetracycline	Streptomycin	Ampicillin
	Zones of inhibition (in mm)							
E.coli	$0.0^{a*\#}$ (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	0.0^{a} (0.707)	17.78^{b} (4.275)	23.18^{a} (4.864)	12.92^{c} (3.663)
B. subtilis	8.8^{c} (3.049)	12.98^{d} (3.671)	13.9^{d} (3.794)	15.98^{c} (3.993)	0.0^{a} (0.707)	12.84^{a} (3.652)	23.08^{a} (4.855)	0.0^{a} (0.707)
K. pneumoniae	7.74^{bc} (2.869)	10.6^{b} (3.331)	12.68^{c} (3.392)	14.1^{b} (3.819)	0.0^{a} (0.707)	31.06^{d} (5.617)	32.96^{d} (5.784)	25.88^{d} (5.136)
S. aureus	8.86^{c} (3.059)	11.84^{c} (3.512)	13.62^{d} (3.757)	15.1^{c} (3.949)	0.0^{a} (0.707)	36.84^{d} (6.11)	23.8^{b} (4.929)	37.76^{e} (6.185)
S. typhi	8.26^{b} (2.814)	10.4^{b} (3.3)	11.8^{b} (3.506)	14.0^{b} (3.806)	0.0^{a} (0.707)	27.96^{c} (4.934)	30.9^{c} (5.603)	11.1^{b} (3.405)
$F_{(P<0.001)}$	179.17	270.90	317.04	213.97	NS	30.53	801.31	736.87
df	16	16	16	16	16	16	16	16
$SE_{(d)}$±	0.106	0.033	0.033	0.043	NS	0.025	0.022	0.018
$LSD_{(P<0.05)}$	0.225	0.071	0.070	0.092	NS	0.537	0.047	0.038

*values inside the parentheses are the square root transformations of original values. #values outside the parentheses are back transformed means of original values

In the similar manner, antibacterial activity of *C. reflexa* from both the host plant was compared for another concentration of 150mg/ml. Extract of *C. reflexa* grown on *C. fistula* produced 24.8mm size zone of inhibition against *K. pneumoniae* and 15.74mm against *S. aureusi* which are statistically different from each other ($F_{(P<0.001)}$ = 573.35, *df* = 16, $SE_{(d)\pm}$ = 0.032, $LSD_{(P<0.05)}$ = 0.068). Against *E. coli* and *B. subtilis*, extract produced 20.0mm and 19.94mm size zone of inhibition respectively and are statistically at par (*P*>0.05) (Tale 1). Extract of *C. reflexa* grown on *F. benghalensis* produced 13.9mm and 13.62mm size zone of inhibition against *B. subtilis and S.aureus* respectively and are statically at par (*P*>0.05). Zones of inhibition were 12.68mm and 11.8mm size zone of inhibition against *K. pneumoniae* and *S. typhi* respectively and are significantly different from each other ($F_{(P<0.001)}$= 317.04), *df* = 16, $SE_{(d)\pm}$ = 0.033, $LSD_{(P<0.05)}$ = 0.070) (Table 2).

Another concentration of extract of *C. reflexa* from both the host trees was 200mg/ml. Inhibition zones given by C. *reflexa* grown on C. *fistula* were 22.6mm and 22.18mm against *E. coli* and *B. subtilis* respectively and are statically at par (*P*>0.05). Zones of inhibitions were 27.0mm against *K. pneumoniae* and 18.06mm against *S. aureus* and are significantly different from each other ($F_{(P<0.001)}$ = 557.05, *df* = 16, $SE_{(d)\pm}$ 0.043 $LSD_{(P<0.05)}$ = 0.920) (Table 1). At the same concentration, extract of *C. reflexa* grown on *F. benghalensis* were produced 15.98mm and 15.1mm size zone of inhibitions against *B. subtilis* and *S. aureus* respectively and are statically at par (*P*>0.05). Similarly 14.1mm and 14.0mm size zones of inhibition were found against *K. pneumoniae*, and *S. typhi* respectively and also are statically at par (P>0.05) ($F_{(p<0.001)}$ = 213.91, *df* = 16, $SE_{(d)\pm}$ 0.043, $LSD_{(P<0.05)}$ = 0.092) (Table 2).

No effect was observed on growth of *E. coli* by extract of *C. reflexa* grown on *F. benghalensis* while no effect on growth of *S. typhi* by extract of *C. reflexa* grown on *C. fistula* is an interesting result of the study. Common positive and negative control experimental sets were prepared. No inhibition was observed by the negative control (solvent) while all the tested antibiotics could inhibit the growth of all the tested organisms significantly (Table 1 & 2, **Fig. 1 & 2**).

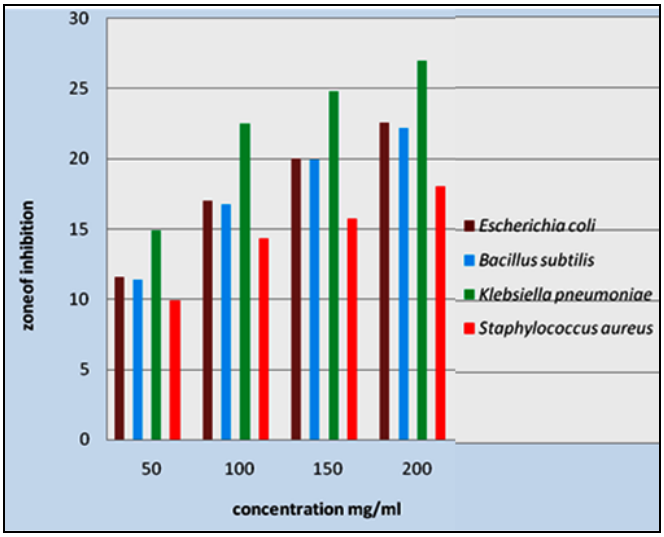

FIGURE 1: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF *CUSCUTA REFLEXA* GROWN ON *CASSIA FISTULA*

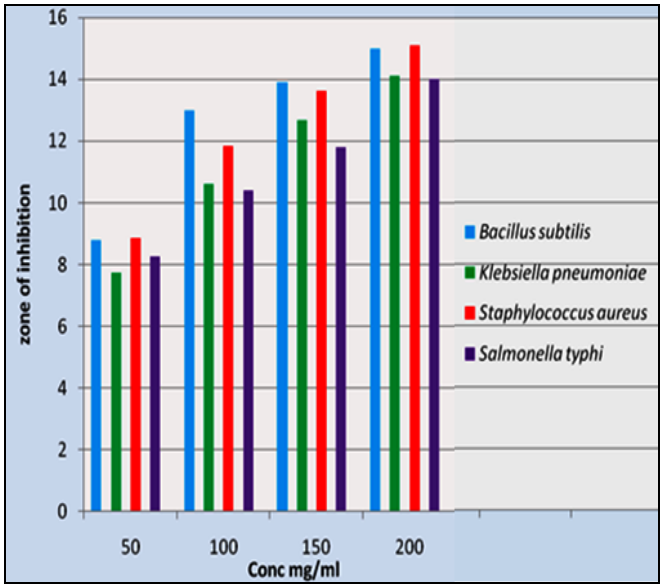

FIGURE 2: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF *CUSCUTA REFLEXA* GROWN ON *FICUS BENGHELENSIS*

DISCUSSION: Results obtained from present study revealed that ethyl acetate extract of *C. reflexa* possess potential antibacterial activity against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus and Salmonella typhi*. Monore *et al* also found significant activity of ethyl acetate extract of *C. reflexa* against *Mycobacterium tuberculosis* and *Salmonella tyhimurium* [16]. When tested by disc diffusion method the ethyl acetate extract of *Cuscuta reflexa* grown on *Cassia fistula* showed significant activity against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus* except *Salmonella typhi.*

It has been shown in table 1, that it gives maximum activity against *Klebsiella pneumonia* and least activity against *Staphylococcus aureus*. Anti-bacterial activity of ethyl acetate extract of *C. reflexa* grown on *Ficus benghalensis* is shown in table 2.

It gives antibacterial activity against *Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus and Salmonella typhi.* It was found inactive against *E. coli*. From the data it can be predicted that *C. reflexa* grown on *C. fistula* is giving larger inhibition zones thus it possess higher antibacterial activity than *C. reflexa* grown on *F. benghalensis*.

From the results it is confirmed that antibacterial activity shown by *C. reflexa* is host dependent as it absorbs the potential sap from the host plant which determines it biological activity. *C. reflexa* grown on *Cassia fistula* gives higher antibacterial activity than *C. reflexa* grown on *Ficus benghalensis.*

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