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ANTIMICROBIAL EFFECTS OF *CLEOME VISCOSA* AND *TRIGONELLA FOENUM GRAECUM* SEED EXTRACTS

UPADHYAY, R. K.[?], AHMAD, S., JAISWAL, G., DWIVEDI, P. AND TRIPATHI, R.

Department of Zoology, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur273 009, India.
Email: rkupad@yahoo.com

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Abstract: In present investigation antimicrobial activities of solvent extracts of Cleome viscosa and Trigonella foenum graecum seeds were evaluated against certain pathogenic strains of bacteria i.e. E. coli, B cereus, L. acidophilus and Pneumococcus. Initially the growth inhibitory activity was examined in agar disc and later on in suspension culture followed by biochemical estimations of DNA, RNA and protein in presence and absence of various seed extracts. The growth inhibition was calculated in form of MBC_{50} (Median effective concentration) and MIC (Minimum inhibitory concentration) values in presence of each extract. The acetone, chloroform and diethyl ether extracts of Cleome viscosa have shown pronounced growth inhibition as their MBC_{50} values have been found between i.e. 1.09-1.51µg/ml, 1.13-5.45µg/ml and 1.26-1.69 µg/ml against all the four bacterial strains while that of water extract have been found 1.66-4.10 µg/ml. In case of Trigonella foenum graecum, acetone and diethyl ether extracts have attributed significant growth inhibitory effects as their MBC_{50} values obtained were 1.39-2.22 µg/ml, 1.84-2.36 µg/ml while chloroform (3.45-4.62 µg/ml) and water (4.12-5.33 µg/ml) extracts have shown less growth inhibition in comparison to tetracycline (0.53-2.13 µg/ml). These results are also compared with one positive control of standard antimicrobial drug tetracycline a well-known antibiotic at an equivalent concentration. Based on the above findings it can be concluded that used to plant extracts from both the plants are more potent antimicrobial agents and can successfully check the microbial infection caused by these bacterial strains in comparison to standard antimicrobial drugs.

Key words: *Cleome Viscosa, Trigonella Foenum graecum,* Antimicrobial

INTRODUCTION

For treatment of many infectious pathogenic diseases, various chemical formulations such as drugs have been enormously used. Due to indiscriminate use most of the pathogenic bacterial strains have developed wide resistance against them. Another reason of resistance is that pathogens have identified immunological antigens or immunogens. Therefore, effective dose level of wide spectrum antibiotics has been increased. Most of the synthetic chemicals, used as drugs after entering inside the body, generate biochemical alterations and multiple side effects during their metabolic utilization. These synthetic chemicals or drugs raise the cross reactivity inside the body and widely inhibit bio-membrane functions. To subside efforts have been made to explore new sources of active antibacterial compounds of plant origin to combat the diseases. Normally during their life cycle, plants encounter various infectious agents viz. viruses, bacteria, fungi and other parasites and synthesize a variety of secondary metabolites which provide protection against infectious agents. So far studies have been done on a number of antibacterial extracts [1] and bioorganic compounds have been identified from so many plants [2], which have shown very good fighting potential against different drug resistant pathogens [3].

In the present investigation we have selected two

local plant spices of great medical importance i.e *Trigonella foenum graecum* and *Cleome viscosa* for exploration of anti-pathogenic effects of seed extracts. *Cleome viscosa* is one of the plants, which possess different antimicrobial activities. Traditionally this plant is well known for its medicinal importance among tribes for cure of different infections. After seeing its medicinal importance we have started to isolate new active ingredients from above plants which can be effectively used against certain pathogenic bacterial strains. The second plant is *Trigonella foenum graecum*, which is originally found in Southeastern Europe and Western India. This plant grows today in many parts of world including India, Northern Africa and the United States .The seeds of fenugreek are consumed as spices and also used as medicine. As literature reveals that various crude extracts of both the plants are quite biologically effective and can be used for the treatment so many diseases. In spite of so much work done on these plants, very little work has been done on these plant species.

MATERIALS AND METHODS

Preparation of extracts: In the present study seeds of *Cleome viscosa* and *Trigonella foenum graecum* were collected from the University Campus. Before extraction seeds were dried and milled to make fine powder. The powdered seeds (500gm each) were extracted successfully with acetone, chloroform, diethyl ether and water to get different residues. The aqueous extracts were filtered using Whatman filter paper (no. 1). Solutions of known concentrations of different residues/ extracts were solibilized in known concentration of fresh solvents before testing the antimicrobial activity. Before preparation of each extract, solubility of plant products was explored and known quantity of each product was solibilized in 1ml of pure solvent. The first series of extract tested was from *Cleome viscosa* and second one from *Trigonella foenicum graecum*

Bacterial culture: Cultures of four pathogenic bacterial strains each of *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Lactobacillus acidophilus* (ATCC 53103), and *Streptococcus pneumoniae* (ATCC 12755) were maintained in the laboratory in Luria Broth (2% w/v) regularly for four days at 37^0C before use in experiments. For experiments a portion (100 µl) of the overnight culture was mixed in the tests and control for inoculation. For activity testing bacterial cultures were stored at 4 ^{0}C and sub cultured after every 8^{th} day in solid agar plates.

Determination of antimicrobial activity: For determination of antimicrobial activity bacterial growth inhibition was accessed in the presence of different increasing concentrations of crude extracts. For this dried plant extracts were dissolved in the same solvent (acetone, chloroform, diethyl ether and sterilized water) to a final concentration of 25mg/ml and sterilized through filtration by 0.45 µm millipore filters.

Micro dilution assay; For antimicrobial activity determination solvent extracts of both plant were diluted by serial micro dilution method up to a range of 32µg/ml to 0.0078 µg/ml in Luria Broth culture medium. The extracts were added to a fresh bacterial culture after following dilution up to 10^{-10}. For performing growth inhibitory assays the inoculum size of the microorganism was prepared from 12 hours broth cultures and suspensions were adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml). Each extract was assayed in triplicate. It was standardized in terms of absorbance at 600 nm in a visible spectrophotometer. The incubation of test and control cultures was performed at 37^o C for 24 hours. For comparison both negative and positive controls were set and bacterial colony number was counted in all test and control discs. Before conducting experiments all the conditions for *in vitro* anti-microbial activity were standardized to determine MIC and MBC values. Growth curve was made to observe final number of bacterial cells in presence and absence of various quantities of seed extracts prepared from both the plants.

For suspension culture sterile conical flasks of 50 ml capacity containing 15 ml of culture medium were used. For determination of MBC six different concentrations of each extract were employed in test flasks while in controls only solvent was added to the culture medium. For evaluation of inhibition two parallel controls were set up for each and every test extract. The antibacterial activity was expressed in form of growth inhibition in bacterial cultures and calculated in terms of MIC and MBC values. After 24 hours bacterial cultures were harvested to determine macromolecular biochemical inhibition.

Agar disc diffusion assay: Antimicrobial activity of various organic fractions was evaluated by agar

disc diffusion method. Molten agar was used as media for the test microorganisms. For testing the activity of various organic fractions sterile filter paper discs (Whatman No. 1) of 6 mm size were coated with six different concentrations of various organic fractions (2-32 µl) by preparing in equal volume V/V). These impregnated discs were dried under laminar flow cabinet. Before experiments inoculum size was determined and adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml) in sterile agar plates. Bacterial inoculums were spread evenly on to the surface of agar plate by using a sterile rubber pad spreader before the various organic fractions were coated on discs and positioned these discs on the inoculated agar surface. Assays were done in triplicate for each organic fraction. Sterile distilled water was used as a negative control. For comparison tetracycline, a broad-spectrum antibiotic was used as standard to compare the bacterial growth. All treated and untreated plates were incubated for 24 hrs at 37°C. The antibacterial activity was assessed in agar plates based on the size of the inhibition zone diameter surrounding the filter paper discs. Results were interpreted by using a standard table that relates to the degree of microbial resistance prescribed by NCCLS (National Committee for Clinical Laboratory Standards). A plot of MIC (on a logarithmic scale) versus zone inhibition diameters (arithmetic scale) was prepared for each organic fraction and antibiotic. These plots were used to find the zone inhibition diameters corresponding to the drug concentrations and that of organic fractions. The MIC value was considered as susceptibility of organic fraction/drug to the pathogen, while high MIC value (with a small zone inhibition diameter) was considered as resistant. The values were compared with reference bacterial strains having known MIC values and inhibition zone diameters.

Sample treatment: For different sets of treatments, various concentrations of *Cleome viscosa* and *Trigonella foenum graecum* were prepared dissolving known amount of powdered material in different solvents i.e. acetone, chloroform diethyl ether and water extract. For determination of minimum inhibitory concentration (MIC) plant extracts were serially diluted up to 10-fold dilution. A final concentration from 66 µg/ml to 0.257 µg/ml was maintained for acetone, 67.8 µg/ml to 0.266 µg/ml for chloroform, 75.6 µg/ml to 0.296 µg/ml for diethyl ether and 199.98 µg/ml to 0.78 µg/ml for water extract of *Cleome viscosa* while for *Trigonella foenum graecum* it was maintained 66 µg/ml to 0.260 µg/ml for acetone, 199.92 µg/ml to 0.78 µg/ml for chloroform, 90µg/ml to 0.35 µg/ml for diethyl ether and 199.92 µg/ml to 0.78 µg/ml for water extracts. The samples were made 12 hrs prior to experimentation and were stored in cold at 4 ^{0}C. These experiments repeated four times. After proper mixing of different extracts in the culture media in conical flasks, 200 µl of bacterial inoculants were added to each culture flask and optical density (O.D.) of each flask was noted at 490 nm at 0, 8, 16, and 24 hrs.

Determination of biochemical inhibition: Biochemical inhibition was measured in terms of macromolecules i.e. DNA, RNA and protein respectively. Biochemical estimation of proteins, DNA and RNA was made according to the methods of Lowry *et al.* [4], Schneider (Diphenylamine and Orcinol method) [5] respectively. Each assay was performed after termination of experiment at 24 hrs and for the same 10 ml of the growth medium was centrifuged. Pellet was dissolved in 5% TCA and centrifuged again at 6000 rpm for 30 minutes for washing and dissolved in 1 ml of distilled water from which 200 µl of dissolved pellet was taken for experiment. For DNA and RNA estimation supernatant was used. Especially in case of DNA estimation 1 ml of actual supernatant was taken while for RNA 0.5 ml of supernatant was used. For DNA and RNA contents absorbance was taken at 590 and 660 nm respectively.

Data Analysis: For determination of biochemical inhibition in bacterial cultures two standard parameters were considered i.e. dose and time of maximum growth according to which control and tests were compared at different time periods by taking the optical density of each and every sample. In all the tests growth inhibition was calculated based on actual concentration of extract used in sample.

RESULTS

Colony growth inhibition: The antibacterial activities of various extracts of plants are shown in table 1. The solvent extracts of both the plants screened showed various inhibitory effects against four different bacterial strains. Solvent extracts of *Cleome viscosa* and *Trigonella foenum graecum* have shown inhibitory effects, which measured in terms of inhibition zone diameter. For acetone extract of both the plants it was 22-28 mm and 23-26 mm/ 50

µl, for chloroform 21-31 and 16-23 mm/50 µl , for diethyl ether 18-22 and 16-20 mm/ 50 µl, for water extract 16-20 and 16-17 mm/ 50 µl and for standard drug tetracycline it was observed between 22-27 mm/ 50 µl respectively against all four bacterial strains (Table 1). Medium effects were observed for chloroform and diethyl ether extracts of *Trigonella foenum graecum* against *S. Pneumoniae* and *L. acidophilus*. Similarly all four bacterial strains were showing medium antimicrobial susceptibility in water extract of *Trigonella foenum graecum*. On the basis of growth inhibition zones obtained in bacterial strains in presence of various solvent fractions of both plants are given in table 1. On the basis of size of growth inhibition zone pathogens activity of fractions was divided into three categories i.e. resistant (> 7), intermediate (> 12) and susceptible > 18 mm respectively The zone of inhibition above 7 mm was considered as positive result. In these experiments acetone and chloroform extracts were found to be highly susceptible against *B. cereus, E. coli and L. acidpophilous* as their growth inhibition zone diameter was in between 28 – 31 mm in size (Table 1).

The MIC of the various extracts fell in the range of 0.326-1.083 µg /ml for *Cleome viscosa* and 0.151-1.566 µg /ml for *Trigonella foenum graecum* (Table 2). The lowest MIC i.e. 0.326 µg /ml was obtained in chloroform extract against *L. acidophilous* while highest MIC 1.083 µg /ml in water extract of *Cleome viscosa* against *E coil.* Similarly highest MBC was obtained in water extract i. e. 21.74µg /ml and lowest 6.54µg /ml in chloroform extract of *Cleome viscosa.* In case of *Trigonella foenum graecum* lowest MIC 0.151µg /ml was obtained in diethyl ether extract against *L. acidophilous* and highest 1.566 µg /ml in chloroform extract of *E coli* (Table 2). However growth inhibition obtained in presence of organic fractions was considered as final bactericidal marker.

DNA Inhibition: All the different crude extracts isolated from *Cleome viscosa* and *Trigonella foenum graecum* (Fenugreek) have shown biochemical inhibition at DNA level .The IC_{50} values for acetone extract of *Cleome viscosa* and *Trigonella foenum graecum* for *E.coli, Bacillus cereus, L. acidophilous* and *S. Pneumoniae* are presented in table 3. All the four extracts of the *Cleome viscosa* were found to have low IC_{50} values in DNA level for *E. coli* and *L acidophilus* in comparison to tetracycline for which it has 5.17 and 5.14µg/ml. More exceptionally all the extracts of both plants have shown low IC_{50} value in comparison to tetracycline except *S. Pneumoniae*. It has low IC_{50}

Table.1 Antimicrobial activity (inhibition zone diameter in mm) of different seed extracts of *Cleome viscosa* and *Trigonella foenum graecum* in agar disc cultures of certain bacterial strains> a-Extract volume utilized in each case was 50 µl. l, b-Inoculum in each case contains approximately 10^8 colony forming units (CFU/ml), *Tetracyline was used as control in each set to compare growth inhibition in culture discs.

Extract[a]	Bacterial strains[b]	*Cleomeviscosa* Diameter in mm	*Trigonella foenum graecum* Diameter in mm
Acetone	*E. coli*	28	23
	B. cereus	24	20
	L. acidophilus	24	26
	Pneumococcus	22	23
Chloroform	*E. coli*	31	28
	B. cereus	25	29
	L. acidophilus	27	20
	Pneumococcus	21	16
Diethyl ether	*E. coli*	18	20
	B. cereus	19	15
	L. acidophilus	22	16
	Pneumococcus	21	17
Water	*E. coli*	18	17
	B. cereus	19	17
	L. acidophilus	20	17
	Pneumococcus	16	16
Tetracycline	*E. coli*	22*	24*
	B. cereus	22*	26*
	L. acidophilus	22*	27*
	Pneumococcus	27*	25*

Table 2. Antimicrobial activity (MEC_{50} and MIC in µg/ ml) of different seed extracts of *Cleome viscosa* and *Trigonella foenum graecum* against certain bacterial strains in Luria Broth culture*. MBC = Minimum bactericidal concentration, MIC = minimum inhibitory concentration, *Growth inhibition in bacterial cultures in presence of different seed extracts.

Extract	Bacterial strains	*Clemoe viscose* percent growth inhibition MBC and MIC		*Trigoniella foenum graecum* percent growth inhibition MBC and MIC	
Acetone	*E. coli*	7.75	0.387	6.07	0. 398
	B. cereus	7.13	0.358	4.39	0.287
	L. acidophilus	8.67	0.437	4.29	0.281
	Pneumococcus	6.02	0.303	4.28	0.280
Chloroform	*E. coli*	7.66	0.382	23.49	1.566
	B. cereus	7.31	0.365	14.12	0.941
	L. acidophilus	6.54	0.326	12.88	0.859
	Pneumococcus	6.55	0.327	17.65	1.143
Diethyl ether	*E. coli*	7.78	0.389	7.55	0.201
	B. cereus	7.18	0.358	6.11	0.163
	L. acidophilus	7.70	0.385	5.69	0.151
	Pneumococcus	6.73	0.336	7.23	0.192
Water	*E. coli*	21.74	1.083	14.18	0.378
	B. cereus	16.93	0.846	12.58	0.335
	L. acidophilus	15.86	1.08	13.50	0.360
	Pneumococcus	17.80	0.886	14.82	0.395
Tetracycline	*E. coli*	93.48	3.61	71.98	2.87
	B. cereus	87.75	3.40	68.55	2.74
	L. acidophilus	12.75	2.14	7.974	1.78
	Pneumococcus	86.50	3.37	67.54	2.70

Table 3. Macromolecular inhibition (MEC_{50} in µg/ml) of different seed extracts of *Cleome viscosa* and *Trigonella foenum-graecum* against certain bacterial strains. [a] *Cleome viscosa*, [b] *Trigonella foenum-graecum*, * Tetracyline was used as control

Extract	Bacterial strain	DNA µg/ml	RNA µg/ml	Protein µg/ml
Acetone	*E. coli*	1.35[a] 2.22[b]	1.29[a] 1.91[b]	1.61[a] 1.89[b]
	B. cereus	1.09 1.39	1.37 1.67	1.37 1.31
	L. acidophilus	1.30 1.44	1.51 1.54	1.54 1.66
	Pneumococcous	1.51 1.42	1.24 1.48	1.58 1.09
Chloroform	*E. coli*	1.13 4.62	1.70 4.85	1.70 5.04
	B. cereus	1.13 4.46	1.18 5.00	1.55 4.96
	L. acidophilus	1.70 4.06	1.22 5.00	1.42 7.08
	Pneumococcous	5.45 3.45	1.20 3.79	1.84 3.95
Diethylether	*E. coli*	1.26 2.10	1.70 2.06	1.74 1.86
	B. cereus	1.69 2.06	1.58 2.87	1.33 2.81
	L. acidophilus	1.49 2.36	1.29 2.21	1.41 1.86
	Pneumococcous	1.66 1.84	1.26 2.10	1.90 1.42
Water	*E. coli*	1.66 4.12	4.64 4.66	3.79 4.29
	B. cereus	3.12 5.33	4.16 5.83	3.30 4.96
	L. acidophilus	4.10 4.92	4.04 6.12	3.54 4.72
	Pneumococcous	3.71 4.87	3.33 6.21	3.92 3.95
Tetracycline	*E. coli*	5.17*	2.13*	4.21*
	B. cereus	4.13*	2.87*	6.40*
	L. acidophilus	5.14*	2.81*	6.40*
	Pneumococcous	0.53*	0.29*	0.25*

values for DNA, RNA & protein against *S. Pneumoniae* in comparison to plant extracts. Similarly in case of *Trigonella foenum graecum*, various plant extracts have shown low IC_{50} value in comparison to tetracycline except water extract against *S. Pneumoniae*, which has shown very low IC_{50} value for DNA, RNA & protein (Table 3).

RNA inhibition: The IC_{50} value for acetone extract of *Cleome viscosa* and *Trigonella foenum graecum* for *E.coli, Bacillus cereus, L. acidophilus* and *S. pneumoniae* are shown in table. 3. The study shows that IC_{50} values of different extracts of cleome viscosa except water extract were found to be lower than that of tetracycline. On the other hand, all the different extracts of *Trigonella foenum graecum* except water and acetone extracts have shown low IC_{50} values except *S. pneumoniae*, which showed very low IC_{50} value than that of plant extracts when treated with tetracycline.

Protein inhibition: Details of IC_{50} values of all extracts of *Cleome viscosa* and *Trigonella foenum graecum* for *E.coli, B. cereus, L. acidophilus* and *S. pneumoniae* are shown in table 3. It is evident from the data that IC_{50} values of *Cleome viscosa* water extract for *E. coli, B. cereus, L. acidophilous,* were found to be lower in comparison to that of Tetracycline. While the IC_{50} values of water extract of *Trigonella foenum graecum* were found to be lower for *B. cereus, L. acidophilus* in comparison to that of tetracycline (Table 3).

DISCUSSION

The results indicate that the crude extracts of both plants have shown good antibacterial activities towards the *E. coli, B. cereus, L. acidophilus* and *Pneumoc-occus*. In agar disc diffusion method chloroform and acetone extracts of both plants have shown larger inhibition zone diameter in comparison to other extracts; and tetracycline an antimicrobial drug. While other extracts have also shown low antibacterial activity, in comparison to other extracts. It was also observed that all bacterial strains used in this study have shown mild antibacterial activity against diethyl ether and water extracts of *Trigonella foenum-graecum* as confirmed by inhibition zone diameter. Infections caused by aforesaid bacterial strains, especially whose strains with multi drug resistance, is among the most difficult to treat and cure with conventional antibiotics. In our study the growth of these bacterial strains was remarkably inhibited by the acetone and chloroform extract of *Cleome viscosa* and *Trigonella foenum-graecum*. The micro organisms were not found as sensitive to the water extract in comparison to other extracts tested in agar disc diffusion method. The possible reason for this could be that active antibacterial components are present in acetone and chloroform.

The important chemical constituents of seeds of *Trigonella foenum graecum* are tricin, naringenin and tricin-7-O-beta-D-glucopyranoside and different alkaloids like Trigonelline, Trigocoumarin, Trimethylcoumarin and nicotinic acid which show antimicrobial activities against different pathogenic bacteria [6]. Besides this, seed extracts are also tested for their anti-diabetic effects, which can successfully improve glucose homeostasis and lipid per-oxidation in mammals [7] by regulating different

enzymes [8]. The alcoholic extract of seeds produces anti-inflammatory and neoplastic effects [9]. Similarly flavonoids isolated from fenugreek have shown antineoplastic activity [6,9,10].

In case of macromolecular inhibition in DNA, RNA and protein, according to central dogma DNA is transcribed into RNA, and RNA is responsible for translation of information in form of proteins. In some compounds DNA inhibition obtained is higher than that of RNA and protein. It means DNA synthesis is inhibited much more and synthesis of RNA and protein remains unaffected. Secondly in some cases DNA inhibition is lesser and RNA inhibition is more which indirectly inhibit protein synthesis. In third case both DNA and RNA inhibition is much lesser while protein synthesis has shown high inhibition. Ii is also possible that both DNA and RNA quantity gets enhanced from the normal level while protein synthesis gets inhibited. The differential effect of these compounds separately on DNA, RNA and protein content is probably due to the different target sites of their action in different phases of bacterial cell. Both transcription and translation get inhibited at the same time. From RNA to protein formation, if translation factors or any other factor related to the protein synthesis get inhibited, it directly shows a low protein content. It also indicates that nutrients utilized by bacteria during 24 hrs were highest in controls while in test the rate of utilization was quite low. Therefore it may be concluded that in a state of smaller nutrient deficiency inhibitory potential of compounds may increas many fold. More specifically, seeds of studied plant species have shown very good antimicrobial activity against all four different pathogenic bacterial strains and may be proved to be pharmacologically important.

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