JOURNAL OF RECENT ADVANCES IN APPLIED SCIENCES (JRAAS) 28:01-11, 2013
ISSN 0970 1990, ST CODE 08 ,www.jraas.org. E MAIL: mhcdr@rediff.com

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, ANTIBACTERIAL ACTIVITIES, CYTOPATHIC AND CYTOTOXIC EFFECTS OF EXTRACTS OF *Tylophora indica, Curcuma amada* AND *Urtica dioica*

DEEPIKA KANNAN[1], RAJENDRA SINGH MEHRA[2], SHUBHAM DUBEY[3], SURAJ TIWARI[4], UPASANA MAHESHWARI[5], VINOD SINGH BISHT[6]
Dept. of biochem & biotech. Annamalai university, TN[1], deeps_dk_9@yahoo.co.in
Doon paramedical college, H.N.B Garwal University, Uttarakhand[2,5,6], rajamehara@gmail.com, stiwari1911@gmail.com, indianvickybhai@gmail.com.
Alpine college of arts and science, H.N.B Garwal University, Uttarakhand[3], shubham.sparton@gmail.com.
Sri Venkateshwara College, Delhi University4, 13.upasana@gmail.com.

ABSTRACT

Medicinal properties of many Indian herbal plants are yet to be evaluated. The aim of this study was to determine the phytochemical constituents, antibacterial activity, cytopathic and cytotoxicity effects of *Tylophora indica, Curcuma amada* and *Urtica dioica.* Various parts of the plants were collected and shade dried. The solvents used for extraction of plants were water, ethanol, methanol, petroleum ether and chloroform. *In vitro* antibacterial activity was performed by agar disc diffusion method. Normal and multi drug resistant bacterial strains of *E.coli*, *P.aeruginosa* were used. *Curcuma amada* was found to exhibit highest toxicity against both the bacterial strains. But none of the plant exhibited activity over multi drug resistant bacterial strains. Further *in vitro* cytopathic and cytotoxic effects were determined for *Tylophora indica, Curcuma amada* and *Urtica dioica* upon BHK-21 fibroblast cell lines. Cytotoxicity of the plant extracts were determined by calculating the IC_{50} values. The highest cytotoxicity was found in alcoholic extracts obtained from *Tylophora indica* with IC_{50} value ~20µg/ml respectively.

KEY WORDS: *Tylophora indica, Curcuma amada, Urtica dioica, BHK*-21 cell lines, soxhlet, phytochemical analysis.

INTRODUCTION

India is a continent with wide field of diversity. This diversity includes both flora as well as fauna. This variation is due to the varied climatic condition, vegetation, topography etc. resulting in enriched heterogeneity. As a result, many such herbs are present with increased medicinal value that is left unnoticed. These herbs may possess medicinal values, domestic values and therapeutic values.

It has been proved since ages the benefits of using these natural agents for curing various diseases. This property may be due to the presence of some active compounds that are different for each plant.

In the present study three plants were selected *Curcuma amada*, *Tylophora indica* and *Urtica dioica.* The three plants belonged to different family each belonging to Indian origin. These plants are used in daily household as spices, condiments and other primitive therapies. Based on the knowledge provided by the surrounding aged people these plants were selected for evaluating their antibacterial activity and cytopathic effects against some basic bacteria and fibroblast cell line. For the following analysis aqueous extracts and alcoholic extracts were chosen. Phytochemical screening was also

carried out to determine the major photochemical present.

MATERIALS AND METHODS

Plant materials

Three Indian origin plants viz., *Curcuma amada* (rhizome), *Tylophora indica* (leaves and stem) and *Urtica dioica* (leaves and stem) were collected from the polyhouse and natural environment at the Institute of biotechnology, Patwadangar, G.B.Pant University, Nainital.

Bacterial cultures

The normal strains of two gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from throat and nasal swab of Indian rooster cock and human nasal secretion. Confirmatory tests for the respective bacteria's were performed.

The multi drug resistant strains of one gram positive bacteria *Staphylococcus aureus* and two gram negative bacteria *Escherichia coli* and *Pseudomonas*

aeruginosa were obtained from the microbiology department of the institute.
All the isolates were subcultured at regular interval and stored at 4°C.

Preparation of plant extracts
Aqueous extraction
Cold extraction-0.50g of dried plant powder was added to 50ml of distilled water (15°C) and was mixed thoroughly for 30 minutes in rotary shaker at 110rpm. It was then filtered through muslin cloth/centrifuged at 5000g for 10 min. The supernatant was collected and stored at 4°C.

Warm extraction-0.50g of dried plant powder was added to 50ml of distilled water (40°C) and was mixed thoroughly for 30 minutes in rotary shaker at 110rpm, 40°C. It was then filtered through muslin cloth/centrifuged at 5000g for 10 min. The supernatant was collected and stored at 4°C.

Hot extraction-0.50g of dried plant powder was added to 50ml of distilled water (70°C) and was mixed thoroughly for 30 minutes in rotary shaker at 110rpm, 70°C. It was then filtered through muslin cloth/centrifuged at 5000g for 10 min. The supernatant was collected and stored at 4°C.

Boiling water extraction-0.50g of dried plant powder was added to 50ml of distilled water (100°C) and was mixed thoroughly for 30 minutes by boiling. It was then filtered through muslin cloth/centrifuged at 5000g for 10 min. The supernatant was collected and stored at 4°C.
Organic solvent extraction
0.50g of dried plant powder was extracted for 8 hrs with (140ml) organic solvent (ethanol) in Soxhlet apparatus. It was then filtered through muslin cloth.

The supernatant was collected and stored at 4°C. It was then concentrated by evaporating the solvent using vacuum concentrator. The dried extract was then dissolved in PBS/DMSO. The process was repeated with other organic solvents (methanol, petroleum ether and chloroform).

Cell line
The cell line used was baby hamster kidney fibroblast cells (BHK-21). They were obtained from animal cell culture department, IVRI, Mukhteshwar. These cells were revived at regular interval and were subcultured daily for their efficient growth.

Phytochemical screening
The powdered seeds were evaluated for qualitative determination of major phytoconstituents *i.e.* Reducing sugar, non-reducing sugar, polysaccharides (starch), proteins, amino-acids, steroids, cardiac glycosides, anthraquinone glycosides, saponins, tannins and phenolic compounds, flavonoids and alkaloids (Manas boxi et.al., 2010).

Antibacterial assay by disc diffusion method
Antibacterial method of (Bauer *et al*., 1996) was adopted with slight modification. The bacterial cultures were grown in McConkey (*E.coli*) and nutrient broth (*P.aeruginosa*) at 37 °C. Muller Hinton agar sterile petriplates were used for the test cultures. 20ml of the media was poured on each petriplate. A loopful of the cultures (nearly 50µl) was uniformly spread over the surface of a sterile Muller-

Reducing sugar

EXPERIMENT	PROCEDURE
Benedict's test	Mix equal volume of benedict's reagent and test solution in test tube.Heat for 5 minutes in boiling water bath. Solution appears green, yellow and red depending on amount of reducing sugar present.

Non-reducing sugar

EXPERIMENT	PROCEDURE
	Test solution does not give response to Fehling's and benedict's tests.

Non-reducing polysaccharides (starch)

EXPERIMENT	PROCEDURE
Iodine test	Mix 3 ml test solution and few drops of dilute iodine solution. Blue color appears. It disappears on boiling and reappears on cooling.

Proteins

EXPERIMET	PROCEDURE
Biuret test	To 3 ml. test solution add 4% NaOH and few drops of 1% CuSO4 solution. Violet or pink color appears.

Amino acid

EXPERIMENT	PROCEDURE
Ninhydrin test	Heat 3 ml. test solution and then add 3 drops of 5% Ninhydrin solution, place in boiling water bath for 10 min. Purple or bluish color appears.

Steroid

EXPERIMENT	PROCEDURE
Salkowski Reaction	To 2 ml of extract add 2 ml chloroform and 2 ml conc. H2SO4. Shake well, chloroform layer appears red and acid layer shows greenish yellow florescence.

Anthraquinone glycosides

EXPERENT	PROCEDURE
Borntrager's test	To 3 ml extract add dil. H_2SO_4, boil and filter. To cold filtrate add equal volume benzene or chloroform. Shake well and separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

Cardiac glycosides

EXPERIMENT	PROCEDURE
Keller kiliani test	To 2 ml of extract add 1 ml of acetic acid, 3 drops of $FeCl_3$ and then add 1 ml H_2SO_4. Pale green colour appears on the surface.

Saponins

EXPERIMENT	PROCEDURE
Foam test	To 1 ml extract add 2 ml distilled water and shake it. Persistent foam was observed.

Flavonoids

EXPERIMENT	PROCEDURE
Shinoda test	To 1 ml ethanolic extract add few drops of conc. HCl and 0.25g magnesium ribbon. Pink color was observed.

Tannins and phenolic compounds

EXPERIMENT	PROCEDURE
Acetic acid solution	To 1 ml test solution, add 1 ml acetic acid drop by drop. Red color solution was observed.

Alkaloids

EXPERIMENT	PROCEDURE
Wagner's test	To 5mg plant extract added dilute HCl, and then filtered. To the filtrate added few drops of Wagner's reagent. Red/brown precipitate forms
Erdmann's test	To 1ml plant extract added few drops of Erdmann's reagent. Red or violet color appears

Hilton agar with a sterile bent rod. Sterile discs (4.0mm in diameter) were dipped in solution of various extracts dissolved in 1ml of PBS and dried at 40°C for 30 minutes. The disc dipped in PBS was used as a negative control and standard antibacterial agent antibiotic (tetracycline-10µg/ disc) was used as positive control. The plates were incubated at 37°C for 24 hours and antibacterial activity was measured. After this period, it was possible to observe inhibition zone. The diameter of the Zone of Inhibition was measured in mm. Overall, cultured bacteria with halos equal to or greater than 7 mm were considered susceptible to either the tested extract or phytochemical. The antibacterial experiments were performed in triplicates.

The same antibacterial sensitivity test was performed even for MDR bacteria. Three strains of MDR bacteria was taken from the laboratory cultures of the

institute namely of *E.coli, S.aureus* and *P.aeruginosa*. Plant extracts of same concentration were considered even for MDR bacteria.

Cell culture analysis

Sample preparation

Dilution of the plants extract in different concentration was prepared. Stock concentration of each plant was 0.20g/5ml DMSO. Serial dilution at varying concentration (20mg, 2mg, 200µg and 20µg) were carried in the ratio of 0.5:4.5ml media. Both cytopathic and cytotoxic effects of the different plants extract in varying concentration were carried out.

MTT assay (test for cytotoxicity)

96 well microtitre plates were used for MTT assay. MTT stock solution of 5mg/ml in phosphate buffer saline (PBS), pH=7.5 was prepared. It was filtered using 0.22µm syringe filter to sterilize and remove the small amount of insoluble residue. 96 well microtitre plates were prepared with 100µl of BHK-21 cell suspension in each well. After 24 hrs of growth, media was decanted and 100µl of varying concentration of different plants extracts were poured. 3hrs later 10µl of MTT stock solution was added in each well. Plates were incubated in humidified 5% CO_2 incubator at 37°c for 3 hrs. 100µl of 0.04M HCl in propan-2-ol was added to each well and mixed thoroughly to dissolve the insoluble blue formazan crystals. The absorbance was read on micro-ELISA reader at 492nm. The plates were read within ½ an hour after adding the stopping reagent (Mosmann T et.al., 1983).

Data analysis

The 50% inhibition concentration (IC_{50}) of the active substances was determined as the lowest concentration which reduced cell growth by 50% in treated. Dose-response curves between percentage of cell viability and concentrations of the extracts were constructed. The IC_{50} was determined from the plotted curve.

Result

Qualitative analysis of extracts for their phytoconstituents

Phytochemical analysis of plants extracts were carried out for both fresh plant and dry plant samples. Both showed nearly similar phytochemicals presence. However, in case of fresh plant samples the minute amount and sensitive phytochemicals were also detected. This could be because as time goes by few phytochemicals might get exhausted. The qualitative determination of these phytochemicals has been presented in table 1 & 2. The phytoconstituents detected in the plant materials could be responsible for their antimicrobial activity though their exact mode of action was not understood.

Table 1: Phytochemical analysis of fresh plant sample:

S.No.	Phytochemicals	*Curcuma amada*		*Tylophora indica*		*Urtica dioica*	
		Aq	Eth	Aq	Eth	Aq	Eth
1.	Reducing sugar	+	++	+	++	+	++
2.	Non-reducing sugar	-	-	-	-	-	-
3.	Non-reducing polysaccharides (starch)	-	-	-	-	-	-
4.	Proteins	-	-	-	-	-	-
5.	Amino acids	+	+	+	+	+	+
6.	Steroids	+	+	-	+	-	++
7.	Cardiac glycosides	++	+	++	+	-	+
8.	Anthraquinone glycosides	-	+	-	+	-	+
9.	Saponins	+	+	+	+	-	-
10.	Tannins & phenolic compounds	-	-	-	+	+	+
11.	Flavonoids	-	-	-	-	+	+
12.	Alkaloids	+	+	+	+	++	++

Aq= aqueous extract , Eth= ethanolic extract,(-) indicates absence, (+) indicates presence at good concentration, (++) indicates presence at high concentration.

The extracts were tested for their antimicrobial activity against the pathogenic micro-organisms viz., *E.coli* a most common bacterium of which virulent strains can cause gastroenteritis, urinary tract infections and *Pseudomonas aeruginosa* which infects the pulmonary tract, urinary tract, burns and wounds. Same bacterial strains along with *S. aureus* were used in case of multi drug resistant bacteria. From the different range of temperatures selected for aqueous plants extract, hot water extracts (65-75°c) of *U.dioica* was found to be effective for the normal bacterial strain of *P.aeruginosa.* However, methanol extract showed antibacterial activity against *E.coli, P.aeruginosa* in most of the plants extract. When the plant extracts (alone) of aqueous and organic solvents were subjected to MDR bacterial strains none of them showed antibacterial activity towards any bacteria. The data has been reported in table 3 & 4, fig. 1 & 2.

Table 2: Phytochemical analysis of dry plant sample:

S. No.	Phytochemicals	*Curcuma amada*					*Tylophora indica*					*Urtica dioica*				
		1	2	3	4	E	1	2	3	4	E	1	2	3	4	E
1.	Reducing sugar	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+
2	Non-reducing sugar	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-
3.	Non-reducing polysaccharides(starch)	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-
4.	Proteins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	Amino acids	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+
6.	Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.	Cardiac glycosides	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
8.	Anthraquinone glycosides	*	-	-	-	-	-	-	-	-	-	*	-	-	-	-
9.	Saponins	+	+	+	-	*	-	-	-	-	-	+	-	-	-	-
10.	Tannins & phenolic compounds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
12.	Alkaloids	-	-	-	-	*	*	-	-	-	+	+	-	-	-	+

1-cold water extract 2- warm water extract 3-hot water extract
4-boiling water extract E-ethanolic extract.
(-) indicates absence, (+) indicates presence at good concentration, (++) indicates presence at high concentration, (*) indicates presence at very low concentration.

Table 3: Antibacterial activity of various plants extract

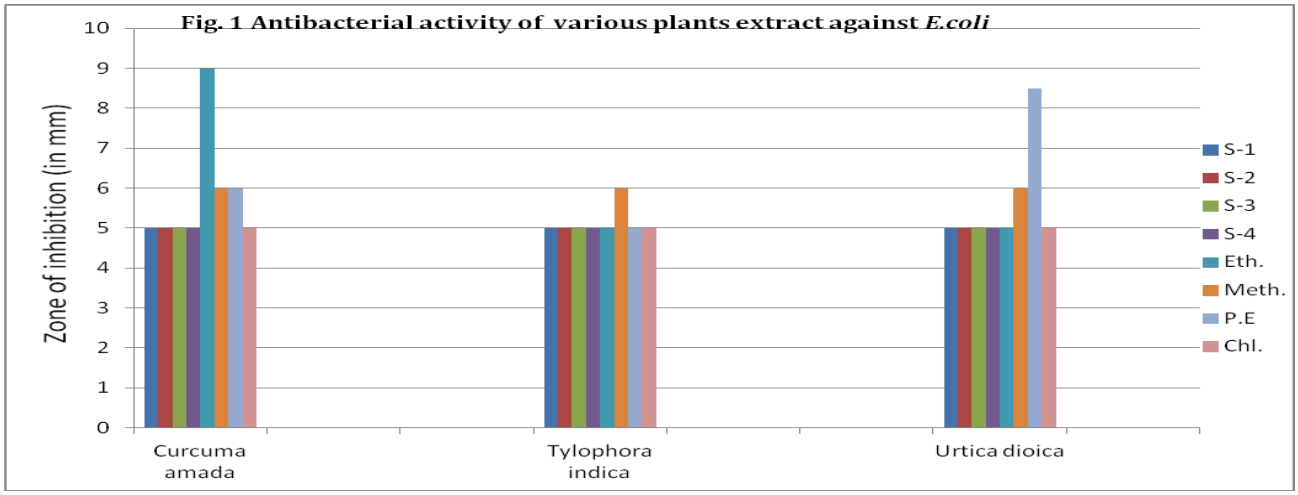
S. No.	Bacteria	*Curcuma amada*								*Tylophora indica*								*Urtica dioica*							
		Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts			
		1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C
		For normal bacterial strains																							
1.	E.C	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
2.	P.A	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-
		For MDR bacterial strains																							
3.	E.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	P.A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	S.A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-) indicates absence of antibacterial activity, (+) indicates presence of antibacterial activity
S-1 indicates cold water extract (10-15°C)
S-2 indicates warm water extract (35-45°C)
S-3 indicates hot water extract (65-75°C)
S-4 indicates boiling water extract (100°C)
(E) indicates ethanolic extract
(M) indicates methanolic extract
(P) indicates petroleum ether extract
(C) indicates chloroform extract
E.C indicates *E.coli*
P.A indicates *P.aeruginosa*

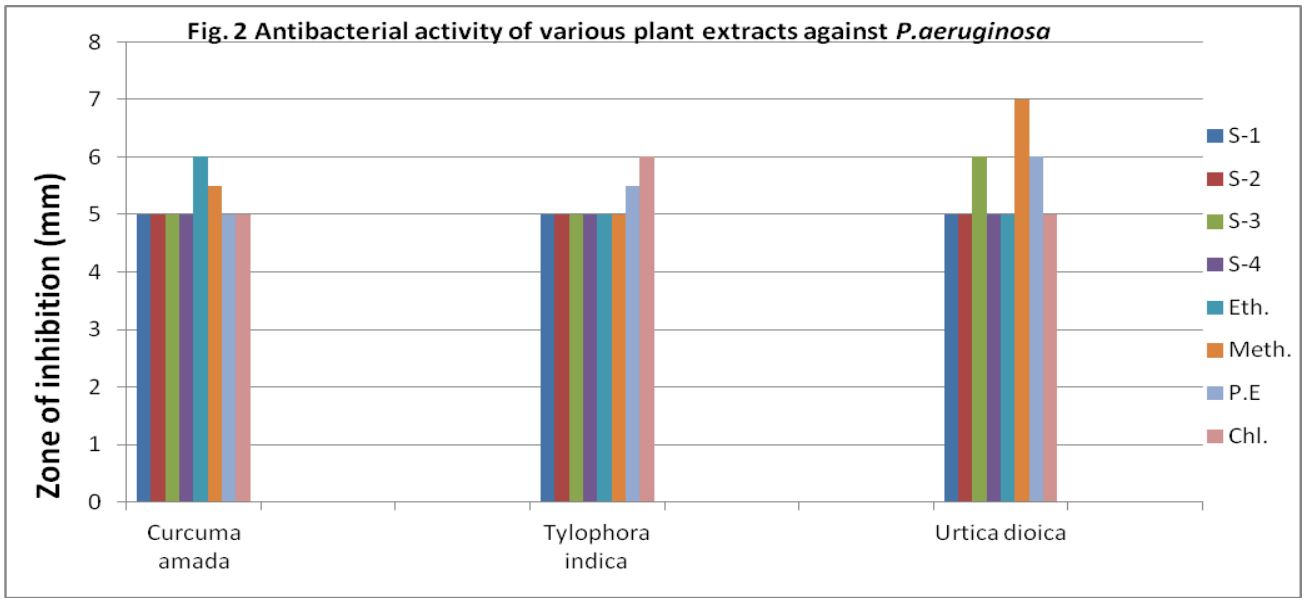
Table 4: Antibacterial activity of plants extracts in terms of zone of inhibition (in mm)

Sterile disc size=4mm. Zone of inhibition for blank (PBS) =5mm.

S. No.	Bacteria	*Curcuma amada*								*Tylophora indica*								*Urtica dioica*							
		Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts			
		1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C
		For normal bacterial strains																							
1.	E. C	5	5	5	5	9	6	6	5	5	5	5	5	5	6	5	5	5	5	5	5	5	6	8.5	5
2.	P.A	5	5	5	5	6	5.5	5	5	5	5	5	5	5	5	5.5	6	5	5	6	5	5	7	6	5



.



Evaluation of the antimicrobial potential of plant extracts

Curcuma amada showed maximum antibacterial activity against E.coli with 9mm zone of inhibition for ethanol extract Urtica dioica showed maximum antibacterial activity against P.aeruginosa with 7mm zone of inhibition via methanol extract.

Synergistic effect of antibiotics and plant extracts on the bacteria samples

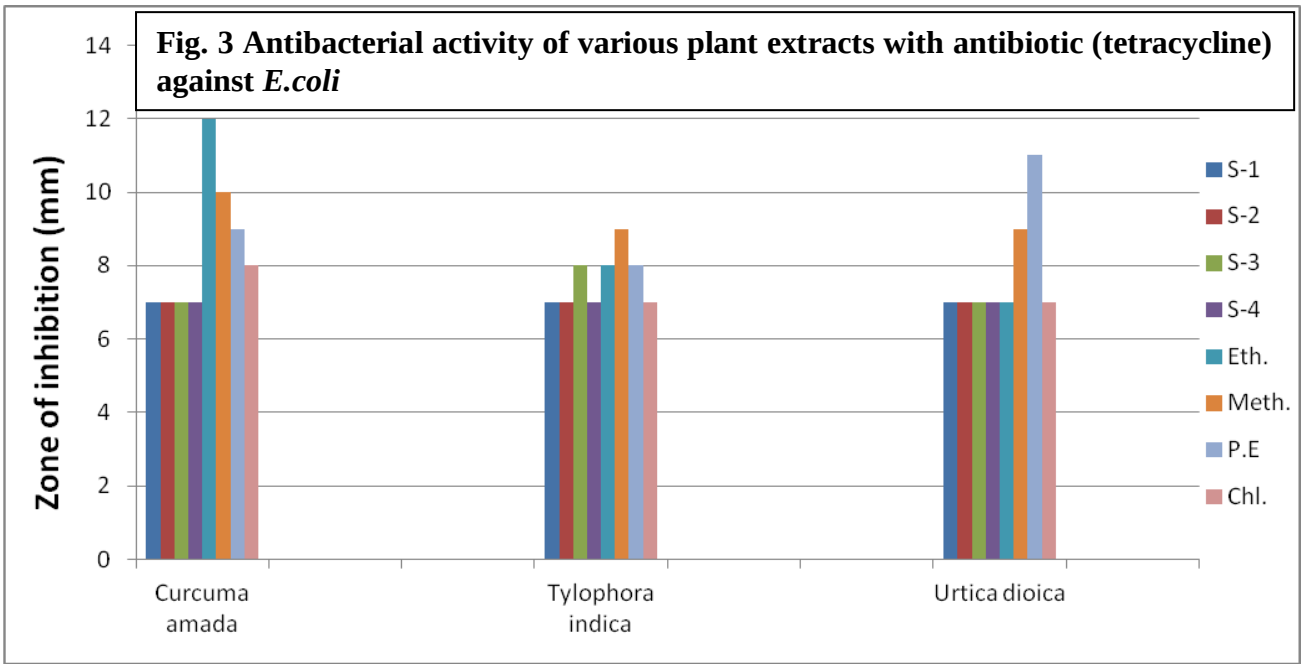
Upon the determination of the plant extracts individual effects, the combined effect of plant extract with antibiotic was determined. Tetracycline, the broad spectrum antibiotic was used. Different plants extract showed significant inhibitory zone but methanol extract in general exhibited antibacterial activity in all the plants. Hence, it can be considered that methanol is a good solvent for the extraction of various active compounds present although specificity in solvent could be exhibited. Also of all the plants extract Curcuma amada showed maximum antibacterial activity against E.coli and U.dioica against P.aeruginosa. This could be due to the phytoconstituents detected in the plant materials though their exact mode of action was not emphasized. The obtained results are presented in table 5, fig. 3 & 4.

Table 5: Antibacterial activity of plant extracts along with the antibiotic (Tetracycline) in terms of zone of inhibition (in mm)

S.No.	Bacteria	*Curcuma amada*								*Tylophora indica*								*Urtica dioica*							
		Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts				Aq. extracts				Organic solvent extracts			
		1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C	1	2	3	4	E	M	P	C
1.	E.C	7	7	7	7	12	10	9	8	7	7	8	7	8	9	8	7	7	7	7	7	7	9	11	7
2.	P.A	7	7	7	7	10	9	8	8	7	7	8	7	8	8	9	10	7	7	10	7	8	11	10	8

Antibiotic disc size=6mm.

Zone of inhibition for antibiotic (tetracycline) for *E.coli*=7mm for *P.aeruginosa*=8mm.



Curcuma amada along with tetracycline showed maximum antibacterial activity against E.coli with 12mm zone of inhibition for ethanol extract *Urtica dioica* with tetracycline showed maximum antibacterial activity against *P.aeruginosa* with 11mm zone of inhibition for methanol extract.

Synergistic effect was observed in all the plants extracts. The plant extracts exhibited improved antibacterial activity in combination with the antibiotic

tetracycline. But the antibacterial effect of the plant extracts with antibiotic was not determined against multidrug resistant bacteria.

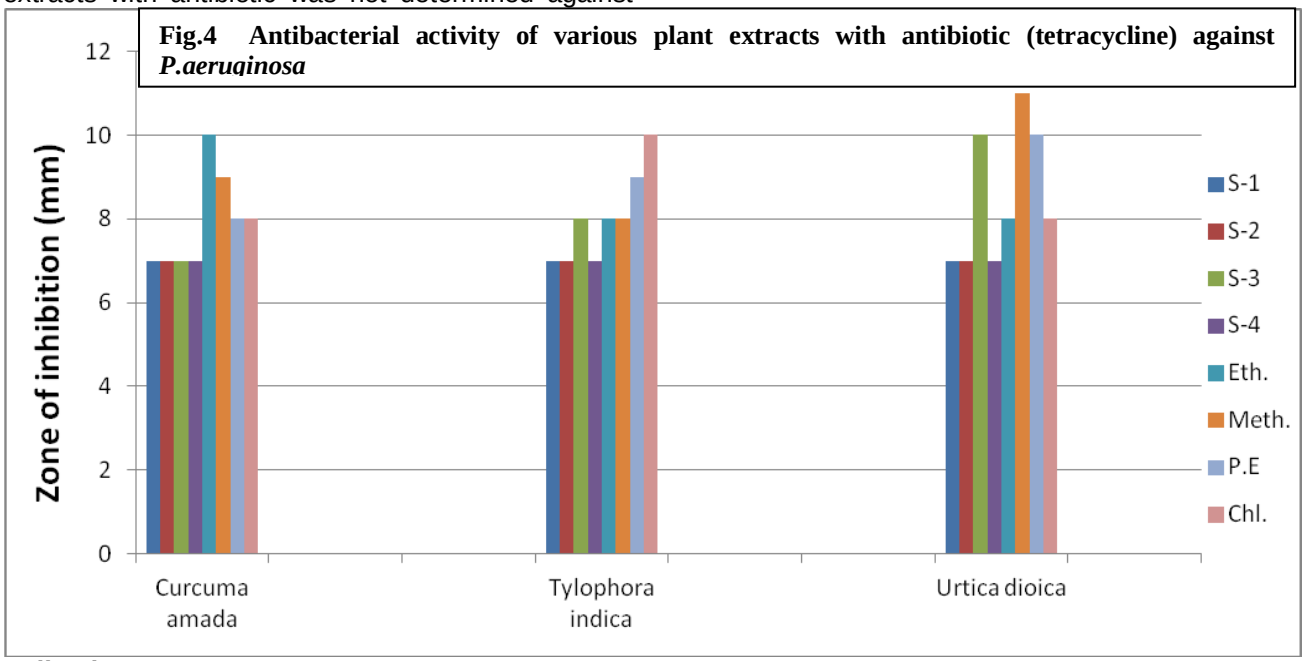


Fig.4 Antibacterial activity of various plant extracts with antibiotic (tetracycline) against *P.aeruginosa*

Cell culture

In-vitro confirmation of the toxicity of the plant extracts *Curcuma amada, Tylophora indica and Urtica dioica* on *BHK-21*cell line was determined. Percentage of viable cell was obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity was carried out by using MTT assay

Viability and characterization of cell lines Cell lines derived from Indian Vetenary Research Institute, Mukhteshwar were free from any kind of bacterial and fungal contamination. Cell count was carried out by using hemocytometer with dilution factor 2 via Trypan blue dye exclusion technique. 74% cell viability was calculated with 2.16 X 10^4 viable cells in 100µl of cell suspension. These are most suitable to perform cytoxicity studies.

Table 6: O.D values of plant samples at 492nm-

a-DMSO

S.No.	Time period	Absorbance at 492nm			
		20mg/ml	2mg/ml	200µg/ml	20µg/ml
1.	10minutes	.1950	.2040	.2197	.3085

Various plant samples

S.No.	Plant sample	Absorbance at 492nm											
		Aqueous extract				Ethanolic extract				Methanolic extract			
		20mg/ml	2mg/ml	200µg/ml	20µg/ml	20mg/ml	2mg/ml	200µg/ml	20µg/ml	20mg/ml	2mg/ml	200µg/ml	20µg/ml
1.	*C.amada*	.0945	.0995	.108	.165	.076	.092	.1435	.2250	.0965	.134	.1695	.2595
2.	*T.indica*	.0990	.1060	.1280	.1995	.0735	.0825	.0955	.1498	.0655	.070	.0760	.092
3.	*U.dioica*	.1205	.1425	.1640	.2325	.1085	.1192	.1470	.2265	.0895	.118	.151	.2135

Determination of Cytotoxicity by MTT assay

All the three plant samples *Curcuma amada, Tylophora indica* and *Urtica dioica* were tested for cytotoxicity by MTT assay on BHK-21 cell lines. DMSO was considered as control for MTT assay. Percentage of cell viability was estimated. It was found that methanol extract of *Tylophora indica* showed least cell viability as compared to other two plants and their extracts. In terms of cytotoxicity, lower the IC_{50} value higher the cytotoxicity. According to the table 8, *Tylophora indica* recorded the least IC_{50} value (~20 µg/ml) indicating it being the

most cytotoxic of all the three plants. Methanol and ethanol extracts of *Tylophora indica* was found to have IC_{50}value ~20 µg/ml. Other plants were found to have IC_{50} value of (2- ~20mg/ml) indicating to be less cytotoxic. Thus it can be inferred that *Tylophora indica* (mainly alcoholic extracts) is cytotoxic even at lower concentration such as 20µg/ml. Thus, dosage for *Tylophora indica* should be considered at much lower concentration towards BHK-21 fibroblast cells for further studies.**Determination of percentage of cell viability:**

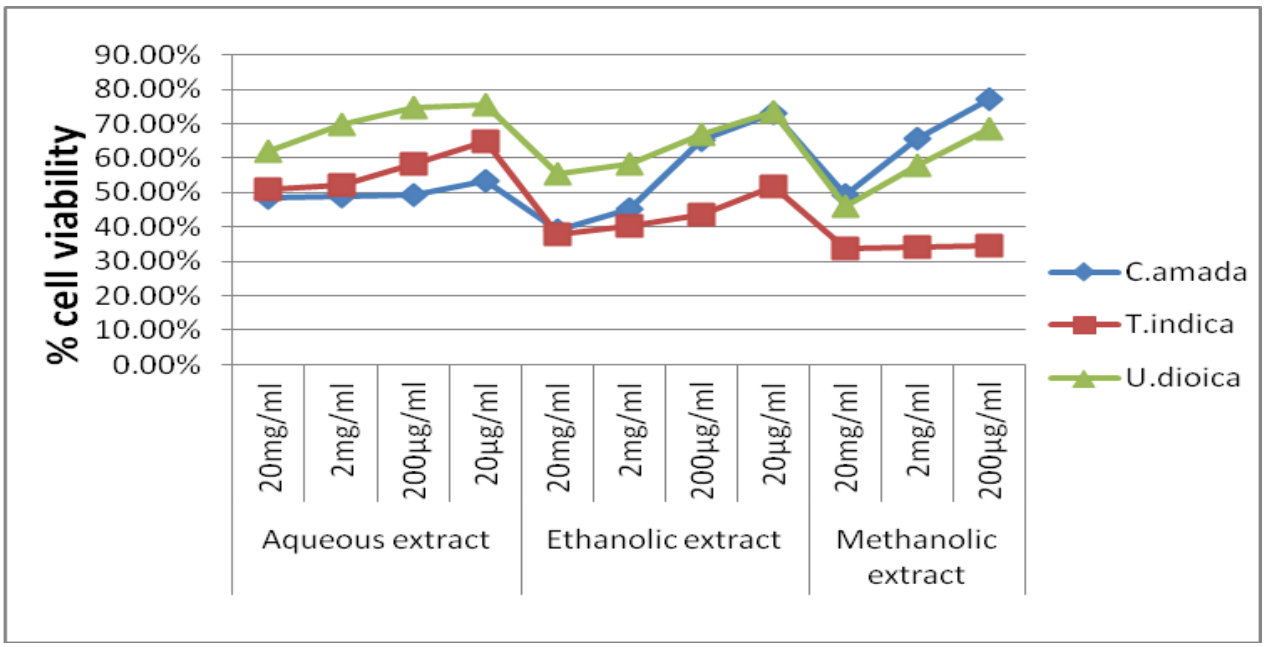
Percentage of cell viability was calculated by the following equation:

$$\% \text{ Cell survival} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

Table 7: Percentage of cell viability for various plants extracts

S.No.	Plant sample	% Cell survival											
		Aqueous extract				Ethanolic extract				Methanolic extract			
		20mg/ml	2mg/ml	200µg/ml	20µg/ml	20mg/ml	2mg/ml	200µg/ml	20µg/ml	20mg/ml	2mg/ml	200µg/ml	20µg/ml
1.	*C.amada*	48.46%	48.77%	49.16%	53.48%	38.97%	45.10%	65.35%	72.93%	49.49%	65.68%	77.15%	84.11%
2.	*T.indica*	50.77%	51.96%	58.26%	64.66%	37.69%	40.44%	43.47%	51.79%	33.58%	34.31%	34.59%	35.41%
3.	*U.dioica*	61.79%	69.85%	74.65%	75.36%	55.64%	58.43%	66.91%	73.42%	45.89%	57.84%	68.73%	69.21%

Fig. 5 Percentage of cell viability v/s concentration of different plants extracts by MTT assay



The 50% inhibition concentration (IC_{50}) of the active substances was determined as the lowest concentration which reduced cell growth by 50% in treated. Dose-response curves between percentage of cell viability and concentrations of the extracts were constructed. The IC_{50} was determined from the plotted curve.

Table 8: IC_{50} value determined for each plants extracts according to the concentration gradient involved.

S.No.	Plant sample	IC_{50} value		
		Aqueous extract	Ethanolic extract	Methanolic extract
1.	*Curcuma amada*	200µg/ml	20mg/ml	2mg/ml
2.	*Tylophora indica*	20mg/ml	~20 µg/ml	20 µg/ml
3.	*Urtica dioica*	~20mg/ml	20mg/ml	~20mg/ml

Tylophora indica was found to be very toxic at higher concentration. It has already been reported that this plant causes allergy to some people and sometimes may also be potentially serious. But the same allergen is reported to have anti-asthmatic effect. This plant being widely used to treat asthma contains the compound Tylophorine. Tylophorine is a Phenanthroindolizidine alkaloid which shows antitumor activity and anti-inflammatory effects. As in the present study too this plant was found to be most toxic of all for the BHK-21 fibroblast cells. It showed the maximum effect. Thus, not studied completely but it might be that this effect is too due to the higher concentration of the compound Tylophorine. As studied even in concentration as low as 20µg also the plant seemed to be toxic (Cheng-Wei Yang et,al. 2006).

CONCLUSION

In case of antibacterial effect *Curcuma amada* exhibited the maximum activity. Of the three plants the order of highest antibacterial activity was *U.dioica>C.amada>T.indica* for *P.aeruginosa* while for *E.coli* was *C.amada> U.dioica>T.indica*. All the plants showed comparable antibacterial activity which support their traditional use against infectious diseases. However in case of cytotoxicity *T.indica* showed maximum cytotoxic effects in comparison to other plants. The presence of general phytochemicals and specific active compounds might be responsible for their therapeutic effects. Further in depth study might reveal the key compounds present and their role in treating other many infectious diseases.

ACKNOWLEDGEMENT

The IBT, Patwadangar. G.B.Pant University, Uttarakhand has provided their support, lab facility for this study.

REFERENCES

1. Baur AW, Kirby WM, Sherris JC, Turck M. **Antibiotic susceptibility testing by a standard single disk method** *Am J Clin Path* 1966; 45: 493-496.
2. Bharathi Balasubramanian, Manimegalai Dhanabal, Arumugam Perumal1, Swamidoss Daniel George **Studies on the antibacterial activity and phytochemical screening of *Tylophora indica linn* on opportunistic bacterial pathogens coinfected with HIV** *Drug Invention Today* 2010, 2(9), 402-404.
3. Cheng-Wei Yang, Wei-Liang Chen, Pei-Lin Wu, Huan-Yi Tseng, and Shiow-Ju Lee **Anti-Inflammatory Mechanisms of PhenanthroindolizidineAlkaloids** *Mol. Pharmacol*. 749–758, 2006.
4. Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, et al. **Effect of tylophorine, a major alkaloid of Tylophora indica, on immunopathological and inflammatory reactions** *Indian J Med Res.* 1980;71:940–948.
5. Gurinder J Kaur and Daljit S Arora **Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*** *BMC Complementary and Alternative Medicine* 2009, doi:10.1186/1472-6882-9-30
6. Ilhami Gülçin, Irfan Küfrevioglu, Münir Oktay, Mehmet Emin Büyükokuro glu **Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.)** *Journal of Ethnopharmacology* 90 (2004) 205–215.
7. LA Betancur-Galvis, GE Morales, JE Forero, J Roldan **Cytotoxic and Antiviral Activities of Colombian Medicinal Plant Extracts of**

the Euphorbia genus *Mem Inst Oswaldo Cruz, Rio de Janeiro*, Vol. 97(4): 541-546, June 2002

8. Manas boxi, Y. Rajesh, V. Raja kumar, B. Praveen and K. Mangamma, extraction, **phytochemical screening and in-vitro evaluation of anti-oxidant properties of commicarpus chinesis (aqueous leaf extract)** International *Journal of Pharma and Bio Sciences*, Vol.1/Issue-4/Oct-Dec.2010.
9. Maridass, M. et.al., **Analysis, antifungal and anti-inflammatory activities of the rhizome essential oil of *Curcuma pseudomontana*** *International Journal of Essential Oil Therapeutics* 2009 Vol. 3 No. 2/3 pg 109-114 ISSN1961-4209)
10. Mohamed Sham Shihabudeen et.al. **Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal Plants**, *International Journal of Pharma Sciences and Research* (IJPSR) Vol.1 (10), 2010, 430-434.
11. Mosmann T **Rapid colorimetric assay for cellular grow and survival: application to proliferation and cytotoxicity assay**s *J Immunol Meth 65*, 55-63, 1983.
12. Sanjay Patel, Nirav Gheewalas, Ashok Sutha, Anand Shah ***In-vitro*** **cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and vero cell line** *International Journal of Pharmacy and Pharmaceutical Sciences,* Vol. 1, Suppl 1, Nov.-Dec. 2009.