EVALUATION OF SYNERGISTIC EFFECT OF THUJAORIENTALIS WITH CIPROFLOXACIN AGAINST ESCHERICHIA COLI

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ABSTRACT

Objectives: To determine the antimicrobial effect of Thujaorientalis (TO) and ciprofloxacin alone against *Escherichia Coli* (*E.Coli*). To compare the combined antimicrobial effect of TO and ciprofloxacin against *E. Coli*.
Study Design: Laboratory experimental study.
Place and Duration of Study: This in vitro study was performed at Khyber Girls Medical College, Peshawar, from Jan to Jul 2018.
Methodology: Antibacterial activity of TO extracts alone and in combination with ciprofloxacin against E.Coli was evaluated by using disc-diffusion and minimum inhibitory concentration method. *E. coli* clinical strains were collected from Rehman Medical Institute and Northwest General Hospital Peshawar, and American Type Culture Collection [ATCC] strains (number 23922) of these bacteria were collected from Agriculture University Peshawar. The organisms were tested six times with crude extract and fractionation with different solvents such as n-hexane, chloroform, ethyl acetate and butanol at concentrations of 1, 4, 8, 12, 16, 20, 24, 30 and 36 mg/ml. The mean minimum inhibitory concentration and fractional inhibitory concentration index were obtained to report the synergism. The data was analysed using SPSS version 21.
Results: Antibacterial activity of different fractions of plant was interpreted as synergy, indifference and antagonism against *E.Coli*. Fractional inhibitory concentration index for *E.Coli* ranged from 3 to 1.05, which showed antagonism and indifferent effect of combination of plant extract with ciprofloxacin.
Conclusion: Synergism between TO and ciprofloxacin was shown against *E.Coli* by disc diffusion method while no synergistic effects were found through minimum inhibitory concentration.

Keywords: Ciprofloxacin, Drug antagonism, Drug synergism, Escherichia Coli, Fractional inhibitory concentration index, Minimum inhibitory concentration, Thuja orientalis.

INTRODUCTION

One of the major causes of death in under developed countries is infectious diseases. Allopathic medicines basically play an important role in reducing burden of infectious diseases by their ability to treat diseases efficiently. However, resistance is produced with prolong usage of these drugs due to which their effectiveness is decreased[1,2]. The bacterial resistance is a great threat to the public health; and different types of antibiotics, including the major last resort drugs are now becoming ineffective[3]. Antimicrobial drug resistance in bacteria is frequently reported from all over the world[4]. Drug resistance associated with prolong use of antimicrobial agents is very alarming situation for both underdeveloped and developed countries. Therefore, alternative strategies are thought to combat this problem. This situation makes the alternate way of therapeutic use of plants extracts as an ancient remedy[5]. Plant extracts being new may not have the problem of microbial resistance. The usage of plant extract as medicine for different diseases has become wide spread because it is realized that the effectiveness of traditional medicine has more efficacy compared to synthetic drugs[6]. The rationalization of using medicinal plants combined with the existing antimicrobial agents may help in overcoming the bacterial resistance. Furthermore, recently in year 2014 it has been reported by Brighenti and Lairungruang in their separate studies that drug synergism can be achieved by mixing

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antimicrobial agents with plant extracts to yield the desirable results[6,7].

Prehistorically medicinal plants were used as traditional medicine that have been playing significant role to heal human diseases and disorders. Thujaorientalis (More Pankh) is one such plants which is being used since long because of its medicinal value. In traditional medicine Thujaorientalis (TO) is used for treatment of different diseases like bronchial catarrh, enuresis, uterine carcinomas, and rheumatism[8]. It is also effective in the treatment of psoriasis, amenorrhea, cystitis and worm infestation. In addition, it has molluscicidal and nematicidal activity[9].

Escherichia coli (E.coli) is a gram negative organism which is responsible for various bacterial infections. They are developing resistance against flouroquinolones and other antibiotics which is becoming a problem. Research studies show that TO has great activity against *Staphylococcus aureus, E.Coli, Bacillus subtilis* and *Agrobacterium tumefaiens*[10]. Therefore, looking at the multiple uses of TO and the importance of synergism we aimed to evaluate the synergistic effects of this compound in combination with ciprofloxacin against in vitro samples of *E. coli.*

METHODOLOGY

Plant Material

Fresh plant leaves of Thujaoriental is weighting 13kg were collected from Bagh-e-Naran, Hayatabad, Peshawar. The plant specimen was identified by taxonomist in Botany department, University of Peshawar and a specimen was placed there in the herbarium with voucher number Bot-224/2016. The sample was dried for 7 days and soaked in methanol. The filtered methanolic solution was dried under vacuum pressure below 45°C in rotatory evaporator. The semi-solid extract was collected in a glass vial and divided into two parts. One part was the methanolic extract (E1) andthe second part was used for refractionation with different solvents such as N-hexane (E2), chloroform (E3), ethylacetate (E4), butanol (E5) and aqueous extract (E6).

Bacterial Collection

Clinical strains were collected from Rehman Medical Institute (RMI) and Northwest General Hospital Peshawar and ATCC strain number 23922 were obtained from Agriculture University Peshawar.

Disc Diffusion Susceptibility Assay

This was a method which indicated the susceptibility of the organism to the tested antibiotic by clear zone of inhibited growth around the filter paper discs. The nutrient agar medium plates was allowed to solidify for about 24-48 hrs at 37°C to check sterility. The bacteria were streaked on plates in Laminar flow hood. For making the discs, Whatman filter paper No.1 (6 mm) was used which was sterilized in autoclave. Crude extract solutions and solutions of fractions at different concentrations i.e. 1, 4, 8, 12, 16, 20, 24, 30 mg/ml were prepared in 5% DMSO. Sterile

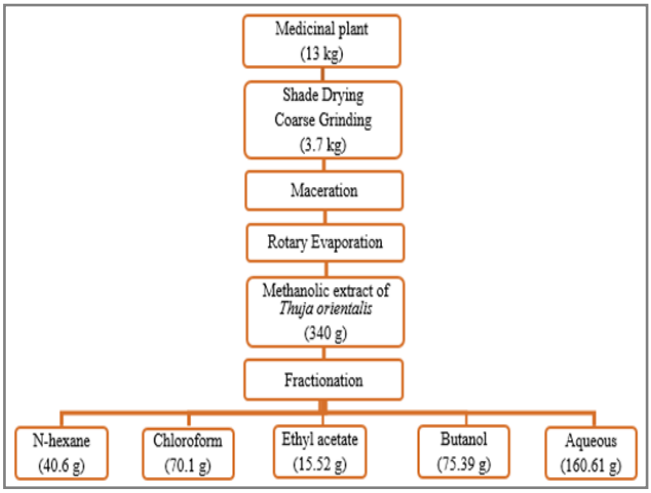

Figure: Plant extraction and fractionation process.

6 mm discs were impregnated with 50 µl of the extract and placed on the surface of agar plates inoculated with microbial culture. Ciprofloxacin (5 µg/disc) served as positive control and DMSO 6 µl served as negative control. Inoculated plates were then incubated at 37°C for 18-24 hrs. The next day zones of inhibition were measured in mm around the discs. Reference table used to interpret bacteria as sensitive (S), intermediate or resistant (R) according to clinical and laboratory standard institute (CLSI) guidelines of National

Committee for Clinical Laboratory Standards (NCCLS)[11].

Minimum Inhibitory Concentration (MIC)

It was the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

Table: Interpretation and Zone of Diameter (mm)

Bacteria	Interpretation and Zone of Diameter (mm)		
	Sensitive (S)	Intermediate	Resistant (R)
Escherichia coli	≥21	16-20	≤15

McFarland 0.5 Barium Sulphate Turbidity Standard

0.5 McFarland standard was prepared by adding 0.05 ml of barium chloride dihydrate (BaCl2) to 9.95 ml of sulphuric acid (1%H_2SO_4) with constant mixing. Bacterial colonies were transferred to 3-4 ml of sterile normal saline solution. This inoculum was then matched with 0.5 McFarland standard[11].

96-Well Microdilution Tray

96-well plates were loaded with 100 µl (0.1 ml) of two fold dilutions of extracts into each well. The extracts were serially diluted two fold in nutrient broth in order to make different concentrations i.e. 24 mg/ml, 12 mg/ml, 6 mg/ml, 3 mg/ml, 1.5 mg/ml, 0.75 mg/ml. Each well was filled with 5 µl bacterial inoculum (1.5×10^5 CFU/ml). Plates without any plant extracts served as growth control. The trays were incubated at 37°C for 18-24 hrs. The highest dilution of extract that showed no visible bacterial growth and turbidity was considered as MIC[11].

Synergistic Antimicrobial Assays

Subsequently by doing a checkerboard titration, the combination action of both TO extract and ciprofloxacin on each isolate was also studied to assess the synergistic activity. Each organism was tested six timesand the mean minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) were obtained to report the synergism. The antibacterial activity was interpreted as one of the following categories; synergy; indifference; additive effect or antagonism[12].

Formula to Determine Synergy (MIC and FIC)

MIC_A in

$$FIC^*_A = \frac{\text{Combination}}{\text{MIC}}$$

MIC_B in

$$FIC_B = \frac{\text{Combination}}{\text{MIC}_B}$$

Where A and B are the two antimicrobial under investigation.

$$FICI = FIC_A + FIC_B$$

$$\text{Mean FICI} = \frac{\text{Sum of FICI Calculated}}{\text{Number of FICI Calculated}}$$

Interpretation

Synergy = mean FICI <0.5,

Partial Synergy/Addition = mean FICI >0.5 <1.0,

Indifference = mean FICI >1 - <2.0,

Antagonism = mean FICI >2.0.

RESULTS

Antimicrobial activities of TO fractions were observed in this study alone and in combination with ciprofloxacin against *E.coli*. The results of M.C.E (E1) fraction against *E.coli* (clinical and ATTC 25922 strains) alone and with ciprofloxacin in combination, showed synergistic effect at different concentrations. Their mean difference ranges from (27.33 ± 1.52, 30.66 ± 0.57 to 34.66 ± 0.57) mm against E.coli in combination with ciprofloxacin. N-hexane (E2) fraction results showed synergistic effect against different concentrations along with indifference effect at 12mg/ml against both E.coli (clinical and ATTCC strain 25922). The TO chloroform fraction (E3) results showed both synergistic and indifference effect at different concentrations against *E.coli* (clinical and ATCC 25922 strain). Their highest zone of inhibition ranges from 28.67 ± 1.53, 28.67 ± 5.13, 29 ± 2, 31.33 ± 4.73, 33.67 ± 1.15 to 34.67 ± 1.15 against *E.coli* (ATCC 25922). At 1, 4, 16 mg/ml concentrations of chloroform fraction showed no antibacterial activity. The Ethyl acetate fraction (E4) showed

different results at different concentrations against *E.coli* (clinical and ATCC 25922 strain) in combination with antibiotic. It only showed synergistic effect at 24mg/ml with zone of inhibition 31.67 ± 0.58 mm. At 12, 20, 30, 36mg/ml

Table-I: Antimicrobial activity (Zone of Inhibition) of different fractions of thujaorientalis and its synergistic effect with ciprofloxacin in mm.

Fractions	Microorganism	Conc.		Stnd. Cip. Disc	T.O & Cip Disc		Interp.
E1 (M.C.E)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	28.33 ± 0.57	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28.33 ± 0.57	4 + cip	-	Nil
		8 mg/ml	18 ± 1	28.33 ± 0.57	8 + cip	32.33 ± 0.57	S
		12 mg/ml	18 ± 1	28.33 ± 0.57	12 + cip	34.66 ± 0.57	S
		16 mg/ml	0.00 ± 0.00	28.33 ± 0.57	16 + cip	-	Nil
		20 mg/ml	16 ± 1	28.33 ± 0.57	20 + cip	31 ± 1	S
		24 mg/ml	16.66 ± 0.57	28.33 ± 0.57	24 + cip	32 ± 1	S
		30 mg/ml	16.66 ± 0.57	28 ± 1	30 + cip	30.66 ± 0.57	S
		36 mg/ml	18.33 ± 0.57	28 ± 1	36 + cip	31.66 ± 0.57	S
	E.coli (Clinical)	1 mg/ml	0.00 ± 0.00	28.33 ± 1.52	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28.33 ± 1.52	4 + cip	-	Nil
		8 mg/ml	8.33 ± 4.04	28.33 ± 1.52	8 + cip	31 ± 1	S
		12 mg/ml	9 ± 5.19	28.33 ± 1.52	12 + cip	31 ± 1	S
		16 mg/ml	0.00 ± 0.00	28 ± 1	16 + cip	-	Nil
		20 mg/ml	16 ± 1	28 ± 1	20 + cip	31 ± 1	S
		24 mg/ml	11.33 ± 4.72	28 ± 1	24 + cip	27.33 ± 1.52	A
		30 mg/ml	0.00 ± 0.00	28 ± 1.73	30 + cip	30.66 ± 0.57	S
		36 mg/ml	8,33 ± 4.04	28 ± 1.73	36 + cip	31.66 ± 0.57	S
E2 (N-hexane fraction)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	28 ± 1	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28 ± 1	4 + cip	-	Nil
		8 mg/ml	13.66 ± 1.52	28 ± 1	8 + cip	27.33 ± 3.78	A
		12 mg/ml	14.33 ± 1.15	28 ± 1	12 + cip	28.33 ± 3.78	I
		16 mg/ml	0.00 ± 0.00	28 ± 1	16 + cip	-	Nil
		20 mg/ml	15.66 ± 0.57	28 ± 1	20 + cip	32 ± 1	S
		24 mg/ml	16.66 ± 0.57	28 ± 1	24 + cip	33.33 ± 0.57	S
		30 mg/ml	17.66 ± 0.57	28 ± 1	30 + cip	35.33 ± 0.57	S
		36 mg/ml	18.66 ± 0.57	28 ± 1	36 + cip	36.33 ± 0.57	S
	E.coli (Clinical)	1 mg/ml	0.00 ± 0.00	27.66 ± 1.52	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.66 ± 1.52	4 + cip	-	Nil
		8 mg/ml	0.00 ± 0.00	27.66 ± 1.52	8 + cip	27.33 ± 3.78	Nil
		12 mg/ml	9.33 ± 2.08	27.66 ± 1.52	12 + cip	28.33 ± 3.78	I
		16 mg/ml	6.66 ± 1.15	27.66 ± 1.15	16 + cip	-	Nil
		20 mg/ml	8.66 ± 3.78	27.66 ± 1.15	20 + cip	32 ± 1	S
		24 mg/ml	10 ± 6.92	27.66 ± 1.15	24 + cip	33.33 ± 0.57	S
		30 mg/ml	10 ± 6.92	28 ± 1	30 + cip	35.33 ± 0.57	S
		36 mg/ml	9.66 ± 6.35	28 ± 1	36 + cip	36.33 ± 0.57	S
E3 (Chloroform fraction)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	27.67 ± 1.15	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.67 ± 1.15	4 + cip	-	Nil
		8 mg/ml	13.67 ± 1.53	27.67 ± 1.15	8 + cip	28.67 ± 5.13	I
		12 mg/ml	14.33 ± 2.08	27.67 ± 1.15	12 + cip	31.33 ± 4.73	S
		16 mg/ml	6.66 ± 1.15	28 ± 1	16 + cip	-	Nil
		20 mg/ml	15.67 ± 1.53	28 ± 1	20 + cip	28.67 ± 1.53	I
		24 mg/ml	16.67 ± 0.57	28 ± 1	24 + cip	29 ± 2	I
		30 mg/ml	17.00 ± 1	27.67 ± 0.58	30 + cip	33.67 ± 1.15	S
		36 mg/ml	18.33 ± 1.15	27.67 ± 0.58	36 + cip	34.67 ± 1.15	S
	E.coli (Clinical)	1 mg/ml	0.00 ± 0.00	28 ± 1	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28 ± 1	4 + cip	-	Nil
		8 mg/ml	9.33 ± 2.08	28 ± 1	8 + cip	28.67 ± 5.13	I
		12 mg/ml	9.00 ± 1.73	28 ± 1	12 + cip	31.33 ± 4.73	S
		16 mg/ml	0.00 ± 0.00	28 ± 1.73	16 + cip	-	Nil
		20 mg/ml	6.67 ± 3.06	28 ± 1.73	20 + cip	28.67 ± 1.53	I
		24 mg/ml	0 ± 0	28 ± 1.73	24 + cip	29 ± 02	I
		30 mg/ml	9.33 ± 1.53	26.33 ± 1.53	30 + cip	33.67 ± 1.15	S
		36 mg/ml	10.67 ± 1.15	26.33 ± 1.53	36 + cip	34.67 ± 1.15	S

E4 (Ethyl Acetate fraction)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	28.00 ± 0.00	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28.00 ± 0.00	4 + cip	-	Nil
		8 mg/ml	7.00 ± 7.00	28.00 ± 0.00	8 + cip	27.00 ± 3.61	A
		12 mg/ml	6.67 ± 6.11	28.00 ± 0.00	12 + cip	28.33 ± 4.04	I
		16 mg/ml	0.00 ± 0.00	28.67 ± 0.58	16 + cip	-	Nil
		20 mg/ml	11.67 ± 0.58	28.67 ± 0.58	20 + cip	30.67 ± 0.58	I
		24 mg/ml	12.67 ± 0.58	28.67 ± 0.58	24 + cip	31.67 ± 0.58	S
		30 mg/ml	12.67 ± 0.58	27.00 ± 1.00	30 + cip	29.00 ± 1.00	I
		36 mg/ml	14.67 ± 2.08	27.00 ± 1.00	36 + cip	30.00 ± 1.00	I
	E.coli (Clinical)	1 mg/ml	0.00 ± 0.00	28.00 ± 0.00	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	28.00 ± 0.00	4 + cip	-	Nil
		8 mg/ml	9.67 ± 6.35	28.00 ± 0.00	8 + cip	27.00 ± 3.61	A
		12 mg/ml	10.00 ± 6.93	28.00 ± 0.00	12 + cip	28.33 ± 4.04	I
		16 mg/ml	0.00 ± 0.00	28.67 ± 0.58	16 + cip	-	Nil
		20 mg/ml	7.67 ± 4.93	28.67 ± 0.58	20 + cip	30.67 ± 0.58	I
		24 mg/ml	6.67 ± 6.43	28.67 ± 0.58	24 + cip	31.67 ± 0.58	S
		30 mg/ml	6.33 ± 5.51	27.67 ± 0.58	30 + cip	29.00 ± 1.00	I
		36 mg/ml	6.33 ± 5.51	27.67 ± 0.58	36 + cip	30.00 ± 1.00	I
E5 (Butanol fraction)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	27.67 ± 2.52	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.67 ± 2.52	4 + cip	-	Nil
		8 mg/ml	9.67 ± 6.35	27.67 ± 2.52	8 + cip	26.67 ± 4.04	A
		12 mg/ml	10.33 ± 7.51	27.67 ± 2.52	12 + cip	27.00 ± 5.00	I
		16 mg/ml	0.00 ± 0.00	27.33 ± 0.58	16 + cip	-	Nil
		20 mg/ml	10.00 ± 1.00	27.33 ± 0.58	20 + cip	30.00 ± 1.00	S
		24 mg/ml	13.00 ± 1.00	27.33 ± 0.58	24 + cip	31.00 ± 1.00	S
		30 mg/ml	14.33 ± 0.58	28.00 ± 1.00	30 + cip	30.00 ± 1.00	I
		36 mg/ml	15.33 ± 0.58	28.00 ± 1.00	36 + cip	31.00 ± 1.00	S
	E.coli (Clinical)	1 mg/ml	0.67 ± 1.15	27.67 ± 2.52	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.67 ± 2.52	4 + cip	-	Nil
		8 mg/ml	7.00 ± 6.08	27.67 ± 2.52	8 + cip	26.67 ± 4.04	A
		12 mg/ml	6.67 ± 6.66	27.67 ± 2.52	12 + cip	27.00 ± 5.00	I
		16 mg/ml	0.00 ± 0.00	27.33 ± 0.58	16 + cip	-	Nil
		20 mg/ml	9.67 ± 1.15	27.33 ± 0.58	20 + cip	30.67 ± 0.58	S
		24 mg/ml	11.00 ± 1.73	27.33 ± 0.58	24 + cip	31.00 ± 1.00	S
		30 mg/ml	9.33 ± 5.71	28.00 ± 1.00	30 + cip	30.00 ± 1.00	I
		36 mg/ml	7.33 ± 6.35	28.00 ± 1.00	36 + cip	31.00 ± 1.00	S
E6 (Aqueous fraction)	E.coli (ATCC 25922)	1 mg/ml	0.00 ± 0.00	27.67 ± 0.58	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.67 ± 0.58	4 + cip	-	Nil
		8 mg/ml	9.00 ± 8.66	27.67 ± 0.58	8 + cip	30.00 ± 1.00	S
		12 mg/ml	10.67 ± 4.73	27.67 ± 0.58	12 + cip	30.67 ± 1.53	S
		16 mg/ml	0.00 ± 0.00	27.67 ± 1.53	16 + cip	-	Nil
		20 mg/ml	0.00 ± 0.00	27.67 ± 1.53	20 + cip	29.67 ± 0.58	S
		24 mg/ml	8.33 ± 4.04	27.67 ± 1.53	24 + cip	39.33 ± 1.15	S
		30 mg/ml	11.33 ± 0.58	27.67 ± 1.53	30 + cip	30.33 ± 0.58	S
		36 mg/ml	12.33 ± 0.58	27.67 ± 1.53	36 + cip	32.00 ± 0.00	S
	E.coli (Clinical)	1 mg/ml	0.00 ± 0.00	27.67 ± 0.58	1 + cip	-	Nil
		4 mg/ml	0.00 ± 0.00	27.67 ± 0.58	4 + cip	-	Nil
		8 mg/ml	0.00 ± 0.00	27.67 ± 0.58	8 + cip	29.00 ± 1.00	I
		12 mg/ml	8.33 ± 2.08	27.67 ± 0.58	12 + cip	30.00 ± 1.00	S
		16 mg/ml	0.00 ± 0.00	28.33 ± 0.58	16 + cip	-	Nil
		20 mg/ml	0.00 ± 0.00	28.33 ± 0.58	20 + cip	39.33 ± 0.58	S
		24 mg/ml	8.33 ± 4.04	28.33 ± 0.58	24 + cip	29.33 ± 1.15	I
		30 mg/ml	8.67 ± 1.53	27.33 ± 2.08	30 + cip	30.00 ± 1.00	S
		36 mg/ml	6.67 ± 5.86	27.33 ± 2.08	36 + cip	30.33 ± 1.15	S

Values are represented as Mean ± SEM; I = Indifference; S = Synergy; A = Antagonism; Cip = Ciprofloxacin; TO = Thujaorientalis; MCE = Methanolic Crude Extract

concentrations it showed indifference effect with zone of inhibition ranged from 28.33 ± 4.04, 29 ± 1.0, 30.67 ± 0.58 to 30 ± 1.0mm. Antagonist effect was observed at 8mg/ml concentration against *E.coli* with zone of inhibition 27.00 ± 3.61mm. Butanol fraction (E5) showed same results as

Ethyl acetate fraction (E4) at different concentration against *E.coli* alone and in combination with antimicrobial agent. Aqueous fraction of TO showed synergistic effect at different concentrations against *E.coli* ATCC 25922 strain. It showed no antibacterial activity at 1, 4, 16mg/ml concentration. Its highest zone of inhibition was shown at 24mg/ml with 39.33 ± 1.15mm zone of inhibition.

Evaluation of Synergistic Effect of Ciprofloxacin / Extracts

The FICI result showed antagonistic and in different effect through MIC against *E.coli* (ATCC 25922). FICI ranged from 1.24 to 3.25 (table-II).

Table-II: Fractional Inhibitory Concentration Index (FICI) for Thujaorientalis with Ciprofloxacin against E.coli.

Concentrations	MICa	MICc	FIC	FICI	Interpretation
M.C.E-Cip					
M.C.E. (mg/ml) Cip (µg/ml)	3 0.25	6 0.75	2 0.33	2.33	Antagonism
N-Hexane-Cip					
N-hexane (mg/ml) Cip (µg/ml)	6 0.25	3 0.187	0.5 0.74	1.24	Indifferent
Chloroform-Cip					
Chloroform (mg/ml) Cip (µg/ml)	6 0.25	3 0.375	0.5 1.5	2	Indifferent
E.A-Cip					
E.A (mg/ml) Cip (µg/ml)	3 0.25	3 0.187	1 0.74	1.74	Indifferent
Butanol-Cip					
Butanol (mg/ml) Cip (µg/ml)	6 0.25	1.5 0.75	0.25 3	3.25	Antagonism
Aqueous Cip					
Auqeous (mg/ml) Cip (µg/ml)	No MIC 0.25	1.5 0.187	1.5 0.74	2.24	Antagonism

M.C.E = Methanolic Crude Extract; Cip = Ciprofloxacin; E.A = Ethyl Acetate

DISCUSSION

The main purpose of this study was to evaluate the effectiveness of TO against *E.coli* alone and in combination with ciprofloxacin. Both TO and ciprofloxacin showed sensitivity against *E.coli* when they were used alone. When they were given in combination synergistic effect was produced by disc diffusion method but MIC method showed no synergism. Leaves of TO produce the essential oil that has its active ingredient: Alpha-thujone, which is effective as anti-fungal and insecticidal agent[13]. In traditional medicines it is also used for the treatment of blood diseases, gastrointestinal upsets, chronic cough, asthma, skin diseases and many more conditions[14]. Furthermore, the phytochemicals in TO have significant hepatoprotective activity as reported by Dubey and Batra[15]. The different parts of the TO plant are used for different purposes for example leaves are used as antipyretic, anti-diuretic and as astringent. Seeds are used as aperients, laxative, lenitive, sedative and different nervous disorder like insomnia. Scalds and pustules are treated by bark part of the plant[16]. TO also has very good antibacterial activity[10]. The medicinal properties of TO may be due to the presence of one or more of the active constituents of the plant i.e. terpenoids, flavonoids, alkaloids. Therefore, the preliminary screening of TO for antimicrobial activity and presence of phytochemicals was used as basis for further development of research on the plant.

Based on our results it is important to understand that clinical use of medicinal plants

combined with the existing antimicrobial agents is a subject of exploration in order to improve the efficacy of the drugs and to resolve the problem of drug resistance. In Pakistan 3.5 is the average number of drug per prescription in which 76% are antibiotics[17]. Antibiotic resistance is one of the emerging health problems[18]. Therefore, importance of identifying new and effective antimicrobial agents cannot be ignored in view evolving resistance to available therapies[19]. In search for effective techniques, modern medical practices would fail if it remains in isolation by ignoring the value of medicinal plants which have been used primitively. Hence, it is important to investigate the therapeutic uses of medicinal plants as well and its clinical application either alone or in combination with allopathic drugs[20]. Medicinal plants have been an important source of medicinal agents since long. The rationalization of using medicinal plants combined with the existing antimicrobial agents is an effective method to overcome resistance. Simultaneous administration of two or more drugs or an antibiotic with a medicinal plant has shown to provide effective treatment for the resistant pathogens[21].

A study was performed in Nepal by Sah and colleagues, to find out the sensitivity of gram positive (*Staphylococcus aureus* & *streptococcus Spp.*) and gram negative bacteria (*E.coli* & *Pseudomonas aeruginosa*) against TO. The antibacterial activity of TO was determined by Agar well diffusion and disc diffusion method. Prominent antibacterial activity of TO was found against all the four bacteria by both methods[10]. Jain and Garg conducted a study to check the antimicrobial activity of TO against six bacteria (*Bacilis subtilis, Corynaebacteriumdiphtheriae, Staphylococous aureus Salmonella typhi Shigella sp, Escherichia coli*) and 5 fungi (*Aspergillus niger A. fumigatus Rhizopusoryzae, Fusarium psidi* and *Curvularia lunate*). Profound antimicrobial activity was detected against all the bacteria and fungi. It was notedthat TO possess more antibacterial activity than antifungal[22].

Eltayeb and Hamid also conducted a research in order to find out the sensitivity of various bacteria including *E.coli* to TO. The results revealed the sensitivity of all the bacteria tested including *E.coli* to TO[23]. In another study Jasuja *et al* tested the antibacterial effect of TO against *E.coli* and found that the extract of TO was able to inhibit the growth of *E.coli* significantly[24]. Similar results were also reported by Belilli *et al*, Shah and Qadir, Jahan *et al*, Jirovets *et al*, and Tsiri *et al*[25]. The results of all the cited study are in accordance with the results of our study.

LIMITATION OF STUDY

As this study was based on a single center, therefore, further research studies should be conducted at a wide level, so that the results may become more valid.

RECOMMENDATION

Thujaoriental is used in combination with ciprofloxacin showed synergism by disc diffusion method against *E.coli* and should therefore be investigated as potential anti-bacterial agent.

CONCLUSION

By disc diffusion method this study showed synergistic effect of Thujaoriental is in combination with ciprofloxacin against *Escherichia coli* while no synergism was found through minimum inhibitory concentration.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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