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Synergistic/Antagonistic Interactions of Antibiotics and Hot Aqueous *Tinospora Cordifolia* Extracts

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ABSTRACT

Present study analyzes antibacterial and synergistic potential of different parts of *Tinospora cordifolia* hot aqueous extracts against three environmental isolates and three MTCC reference strains of *K. pneumoniae*, *E. coli* and *P. aeruginosa*. Antibacterial activity was observed by agar dilution method in triplicates. Synergistic potential of extracts was observed with seven different antibiotics named as Ceftiaxone (CTR), Kanamycin (K), Gentamycin (GEN), Chloramphenicol (C), Cefotaxime/Cephotaxime (CTX), Tetracycline (TE) and Nalidixic Acid (NA). Results revealed that no extracts shows any antibacterial activity, while synergistic activity was observed with various antibiotics with plant extracts. This concluds that plant extract contains antibiotic resistance inhibitor compound in it. Results show maximum synergistic activity with chloramphenicol against *P. aeruginosa* with all four aqueous extracts. While no synergistic or antagonistic activity was observed against *K. pneumoniae* with hot aqueous fruit and leaf extract with any antibiotics used. Antagonistic activity of hot aqueous leaf extract was observed with Ceftiaxone against *E. coli* followed by Cefotaxime against MTCC *K. pneumonia*e. While synergistic activity was observed with Chloramphenicol against *P. aeruginosa*, MTCC *P. aeruginosa* and Ceftiaxone against *P. aeruginosa*. It will be a great achievement for the researchers to test different combination of antibiotics and plant extracts without any confirmatory consideration of their individual effect *in-vitro*. This kind of synergistic work can lead to great evolution in pharmaceutical industries.

Keywords: Synergism, antagonistic, antibiotics, *K. pneumoniae*, *E. coli*, *P. aeruginosa.*

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INTRODUCTION

In any case of any infection/ disease antibiotics are being provided vigorously which causes threat for treatment by providing resistant bacterial strains in the environment. Bacteria are becoming more pathogenic by developing multidrug resistance (MDR). Sharma et al[8] reported ampicillin, cephotaxime, tetracycline and chloramphenicol resistance in *K. pneumoniae, E. coli, E. cloacae* and *P. aeruginosa.* Despite of tremendous progress of mankind, MDR strains are increasing rapidly due to indiscriminate, unsystematic and arbitrary use of antibiotics, which is a leading threat for biotic environment worldwide. Medicinal plants and their bioactive compounds have been used as healers from prehistoric times worldwide and their medicinal properties are also reported in Ayurveda and Unani systems of medicine. Scientifically testing them for their antibacterial and synergistic activity can provide a new alternative to synthesize new drugs or a combination of plant extract and antibiotic or they can be a novel drug themselves. Present study focused on *T. cordifolia* sequentially extracted aqueous extracts, for their antibacterial and synergistic activity. It is an important medicinal plant used to treat various ailments.

MATERIALS AND METHODS

Leaf, fruit, root and stem of *T. cordifolia* growing on neem (*Azadirechta indica*) was identified from the book "Medicinal plants of India- an Encyclopaedia" by Sharma[7] and "Indian medicinal plants a compendium of 500 species" by Warrier, Nambiar, Ramankutty[9]. Disease free fresh plant material was collected from Durg-Bhilai region to be used for preparation of crude extracts.

Preparation of crude extract: Fresh plant parts were washed with running tap water followed by surface sterilization with 0.01% $HgCl_2$ (Nahunnaro, 2008). Plant parts were shade dried and pulverized separately to prepare sequential crude extract using four solvents *viz.*, petroleum ether, ethanol, chloroform and distilled water respectively. Chopped 25 g of plant parts was dispensed in 300 ml of solvent for extraction using soxhlet apparatus at boiling point of used solvent till clear/ colourless solvent drops out and solvent obtained after extraction was evaporated till dryness at 46^0C and yield was recorded (Johnson et al, 2008). Assessment of antibacterial activity of plant extracts was done by agar dilution method (CLSI standard[3]).

Assessment of antibacterial and synergistic/Antagonistic activity: Antibacterial activity of plant extracts was performed using agar dilution method (CLSI standard[2]) while antibacterial activity of antibiotics was performed using disc diffusion method (Andrews[1]). Synergistic/Antagonistic activity between plant extract and seven antibiotics *viz.*, Chloramphenicol (C), Kanamycin (K), Ceftriaxone (CTX), Cephotaxime (CTR), Nalidixic acid (NA), Gentamycin

(G) and Tetracycline (TE) was assessed using Mueller Hinton Agar (Zhang et al; Shanthi and Nelson[6]).

RESULTS AND DISCUSSIONS

Antibiotic susceptibility of all bacterial strains is shown (Table 1) to compare results with combination of antibiotics with various plant extracts. This comparison will help to confirm their synergistic or antagonistic potential with plant extract. (Table 2) shows results obtained as synergism or antagonism and their efficiencies. Antagonistic activity of hot aqueous fruit extract (HAFE) observed against *E. coli* with Ceftiaxone, Cefotaxime and Nalidixic Acid respectively. Synergistic activity of HAFE was observed with Chloramphenicol, Nalidixic Acid and Gentamicin against *P. aeruginosa.*

Table 1: Antibiotic susceptibility profiling of environmental isolates and MTCC *E. coli* (3221), MTCC *K. pneumoniae* (9544), MTCC *P. aeruginosa* (3163), *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

S. No.	Antibiotics	Zone of inhibition (mm)					
		MTCC *E. coli*	MTCC *K. pneumoniae*	MTCC *P. aeruginosa*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
1.	CTR30	23	27	18.5	30	16	12
2.	TE 30	20	22	11	21	21	10
3.	CTX 30	23	27	13	26	14	15
4.	K 30	16.5	15	-	18	17	5
5.	C 30	27	28	-	23	25	5
6.	NA 30	18	19	-	22	18	19
7.	GEN 10	13	15	17	15	15	18

Table 2: synergistic and antagonistic activity of aqueous hot extracts with seven antibiotics chloramphenicol (C), tetracycline (TE), ceftiaxone (CTR), kanamycin (K), cefotaxime (CTX), nalidixic acid (NA) and gentamycin (GEN)

Extracts	Antibiotics	Zone of inhibition (mm)					
		Isolated Bacteria			MTCC Reference Strain		
		EC	KP	PA	EC*	KP*	PA*
HAFE	CTR 30	$14^{\#\#}$	12	16	19	$14.5^{\#\#}$	21
	TE 30	18	18	12	20	19.5	10
	CTX 30	$19^{\#}$	12	13.5	27	$14^{\#\#}$	17
	K 30	21	20	9	17	17	8^{+}
	C 30	21	22	27^{++}	25	$23^{\#}$	10^{+}
	NA 30	$16^{\#}$	15	24^{+}	19	$14^{\#}$	21^{++}
	GEN 10	17	17	24^{+}	17	15	20
HALE	CTR 30	$17^{\#\#}$	18.5	20.5^{++}	$18^{\#}$	$15^{\#\#}$	20
	TE 30	28^{+}	18.5	13	17	20	9
	CTX 30	21^{+}	16	21^{+}	$17.5^{\#}$	$16^{\#\#}$	16

	K 30	22	21.5	12^{+}	15	20^{+}	-
	C 30	23.5	21	29^{++++++}	$20.5^{\#}$	$23^{\#}$	12^{++}
	NA 30	$17^{\#}$	16	24^{+}	17	20	-
	GEN 10	18	17	23^{+}	18^{+}	17	17.5
HARE	CTR 30	$14^{\#\#}$	12	18^{+}	$13.5^{\#\#}$	$17^{\#\#}$	20
	TE 30	19	$16^{\#}$	9	$13^{\#}$	20	9
	CTX 30	$18.5^{\#}$	12	12	$15^{\#}$	$16^{\#\#}$	17

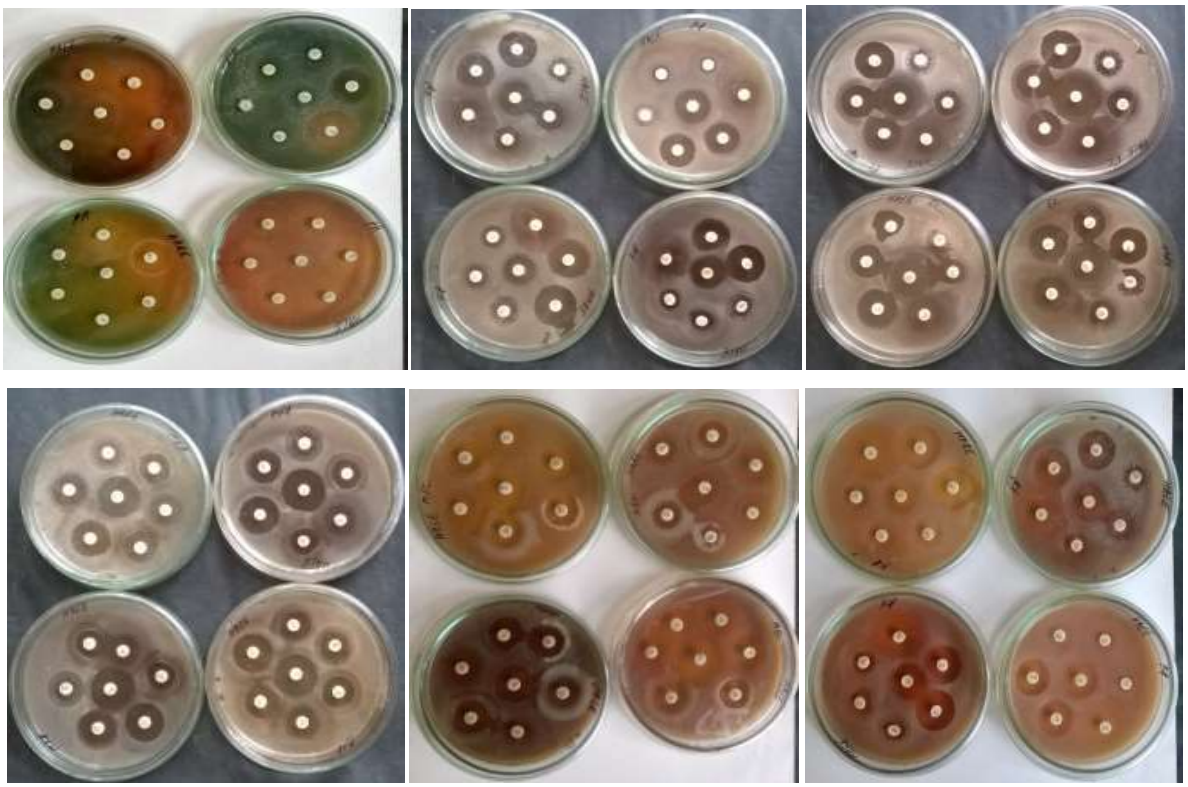

Figure 1: Synergistic interaction of various antibiotics and plant extracts against tested organisms

Antagonistic activity of HAFE observed against MTCC *K. pneumoniae* with Ceftiaxone, Cefotaxime, Chloramphenicol and Nalidixic Acid respectively. Synergistic activity observed with Nalidixic Acid, Chloramphenicol, Kanamicin and HAFE against MTCC *P. aeruginosa* respectively. While no synergistic or antagonistic activity observed against *K. pneumoniae* and MTCC *E. coli* (Figure 1). Antagonistic activity of HALE with Ceftiaxone against *E. coli*, with Cefotaxime against MTCC *K. pneumonia*e observed, while synergistic activity observed with Chloramphenicol against *P. aeruginosa*, MTCC *P. aeruginosa* and Ceftiaxone against *P. aeruginosa*. Maximum synergistic activity was observed with Chloramphenicol and HASE followed by HALE, HARE and HAFE respectively against MTCC *P. aeruginosa* and *P. aeruginosa.* Kanamycin with HASE and Nalidixic Acid with HARE and HASE showed synergistic activity against MTCC *P. aeruginosa.* While maximum antagonistic activity observed

with Nalidixic Acid and Cefotaxime against *P. aeruginosa.* Any hot aqueous plant extracts does not show antibacterial activity but all of them possess synergistic as well as antagonistic activity. Every hot extract showed antagonistic activity with Ceftiaxone against *E. coli* while every extract showed synergistic activity with Chloramphenicol against *P. aeruginosa* and MTCC *P. aruginosa*. Every hot extract showed antagonistic activity with Cefotaxime against MTCC *K. pneumoniae*. Maximum synergistic activity was observed against MTCC *P. aeruginosa* with various antibiotics while no antagonistic activity was observed against it. This kind of combined interactions can be used for treating diseases and reduce side effects of their combination.

CONCLUSION

Knowledge of phytoconstituents extracted with different solvent fractions of plants having medicinal property could be used to increase their use to treat diseases and somewhat it can be useful for taking them synergistically with some antibiotics for better results. Knowledge of antagonistic activities could reduce the side effects and treat disease efficiently. It will be a great achievement for the researchers to test different combination of antibiotics and plant extracts without any confirmatory consideration of their individual effect *in-vitro*. This kind of synergistic work can lead to great evolution in pharmaceutical industries.

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