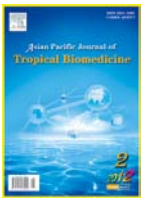
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

In vitro interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains

Kalpna Rakholiya, Sumitra Chanda*

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised from 5 July 2012
Accepted 11 August 2012
Available online 28 August 2012

Keywords:
Carica papaya
Terminalia catappa
Plant extracts
Synergistic effects
Antimicrobial agents
Disc-diffusion method

ABSTRACT

Objective: To evaluate the *in vitro* interaction between methanolic extracts of *Terminalia catappa* (*T. catappa*) (Combretaceae) and *Carica papaya* (*C. papaya*) (caricaceae) leaves and certain known antimicrobial drugs like penicillin G (P), ampicillin (AMP), amoxyclav (AMC), cephalothin (CEP), polymyxin B (PB), rifampicin (RIF), amikacin (AK), nilidixic acid (NA), gentamicin (GEN), chloramphenicol (C), ofloxacin (OF) against five Gram positive and five Gram negative bacteria. **Methods:** Evaluation of synergy interaction between plant extracts and antimicrobial agents was carried out using disc diffusion method. **Results:** The results of this study showed that there is an increased activity in case of combination of methanolic plant extracts and test antimicrobial agents. The more potent result was that the synergism between methanolic extract of *C. papaya* and antibiotics showed highest and strong synergistic effect against tested bacterial strains; though methanolic extract of *C. papaya* alone was not showing any antibacterial activity. **Conclusions:** These results indicate that combination between plant extract and the antibiotics could be useful in fighting emerging drug-resistance microorganisms.

1. Introduction

Today, the ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problem[1]. Thus, there is an urgent need for novel antimicrobials and/or new approaches to combat these problems[2]. Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. Antibiotics that work today may not work tomorrow. Antibiotic mechanism includes inhibition of cell wall synthesis, cell membrane function, protein and nucleic acid synthesis, and inhibition of specific enzyme system (Figure 1). Therefore, drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported. Therefore, combination therapy is often profitable for patients with serious infections caused by drug-resistant pathogens[3].

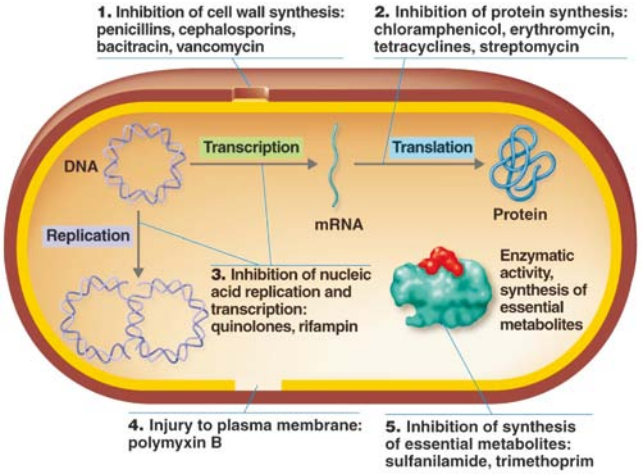


Figure 1. The action of antimicrobial drugs.

Synergistic effect occurs when the effect of two drugs together is greater than the effect of either alone. Indifference occurs when the effect of two drugs together is

*Corresponding author: Sumitra Chanda, Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India.
E-mail: svchanda@gmail.com
Financial support No. 37-524/2009 (SR)

less than the effect of either alone. Antagonism occurs when two drugs together has no effect.

The present study was focused on the synergistic activity of two plant extracts with eleven antibiotics. Plants antimicrobials have been found to be synergistic enhancers i.e. alone they may not have any antimicrobial properties, but when they are taken concurrently with standard drugs they enhance the effect of that drug[4]. Synergistic effects resulting from the combination of antibiotics with various plant extracts has been studied and experimented by a number of other scientists[5,6], delaying the emergence of bacterial resistance also[7].

In the presence study diseases causing different pathogenic bacteria were used. *Klebsiella pneumoniae* (*K. pneumoniae*) are widely distributed in hospitals and are increasingly being isolated from community acquired infections[8,9]. *Staphylococcus epidermidis* (*S. epidermidis*) is a major cause of nosocomial infections, including sepsis in premature infants and is resistant to phagocytosis due to ability to produce an exo-polysaccharide. *S. epidermidis* strains are often resistant to antibiotics including penicillin, amoxicillin, and methicillin. *S. aureus* is one of the commonest and most important Gram-positive hospital-acquired organisms. It has a high propensity to colonize abnormal skin surfaces and open wounds. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, to life-threatening diseases such as pneumonia, meningitis. *S. aureus* remains one of the five most common causes of nosocomial infections, often causing postsurgical wound infections[10,11]. *Bacillus subtilis* (*B. subtilis*) is responsible for causing food borne gastroenteritis[12]. The organisms like *Enterobacter*, *Klebsiella*, *Proteus* and *Shigella* species are implicated to cause severe infections in human, as they are found in multiple environmental habitats[13]. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae.

Terminalia catappa (*T. catappa*) L. (Desi badam) belongs to the family Combretaceae. The leaves are used in the treatment of leprosy and for reducing travel nausea, to get rid of intestinal parasites; treat eye problems, for wounds and to stop bleeding during teeth extraction. Juice of the leaves is used in the preparation of the ointment for scabies, and other cutaneous diseases and also useful in headache and colic[14].

Carica papaya (C. papaya) L. (Papaya) belongs to the family of Caricaceae. This plant produce natural compounds in leaf bark and twig tissues that possess anti-tumour and pesticidal properties. Fresh, green leaf is an antiseptic, whilst the brown, dried leaf is best as a tonic and blood purifier.

2. Materials and methods

2.1. Chemicals

Nutrient broth, Sabouraud Dextrose Broth, Mueller Hinton Agar No. 2 and Sabouraud Dextrose Agar were obtained from Hi-Media, Mumbai, India; petroleum ether, acetone, methanol, etc were obtained from Merck, India.

2.2. Plant collection

The leaves of *T. catappa* L. (PSN291) and *C. papaya* L. (PSN314) were collected in August, 2010 from Rajkot, Gujarat, India and identified by comparison with specimens available at the Herbarium of the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves were washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in air tight bottles.

2.3. Extraction

The dried powder of two plant leaves was extracted individually by cold percolation method[15] using different organic solvents like petroleum ether, acetone and methanol. 10 g of dried powder was taken in 100 mL of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5 000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 mL of solvent (acetone and methanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5 000 rpm for 10 min, the supernatant was collected and the solvents were evaporated and the dry extract was stored at 4 ℃ in air tight bottles. The residues were weighed to obtain the extraction yield.

2.4. Antimicrobial susceptibility test

2.4.1. Microorganisms

The ten disease causing bacterial strains were taken into consideration, Five Gram positive bacteria *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Staphylococcus epidermidis* (*S. epidermidis*) ATCC12228, *Bacillus megaterium* (*B. megaterium*) ATCC9885, *Bacillus subtilis* (*B. subtilis*) ATCC6633, *Micrococcus flavus* (*M. flavus*) ATCC10240) and five Gram negative bacteria *Proteus morganii* (*P. morganii*) NCIM2040, *Proteus vulgaris* (*P. vulgaris*) NCIM2857, *Enterobacter aerogenes* (*E. aerogenes*) ATCC13048, *Klebsiella pneumoniae* (*K. pneumoniae*) NCIM2719, *Proteus mirabilis* (*P. mirabilis*) NCIM2241. All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4 ℃.

2.4.2. Antibiotics used

All antibiotics were purchase from Hi-Media Laboratory Pvt. Ltd., (Mumbai, India) viz. penicillin G (P10 units/disc), ampicillin (AMP10 μg/disc), amoxyclav (AMC30 μg/disc), cephalothin (CEP30 μg/disc), polymyxin B (PB300 units/disc), rifampicin (RIF5 μg/disc), amikacin (AK30 μg/disc),

nilidixic acid (NA30 μg/disc), gentamicin (GEN10 μg/disc), chloramphenicol (C30 μg/disc), ofloxacin (OF5 μg/disc).

2.4.3. Antimicrobial test

Antibacterial activity of the methanolic extract of *T. catappa* and *C. papaya* with eleven standard antibiotics was assessed against 5 Gram positive bacteria and 5 Gram negative bacteria by using agar disc diffusion method[16,17]. The Petri plates were prepared by pouring 20 mL of sterilized molten Mueller Hinton agar (MHA) seeded with 200 μL test culture containing 1×10^8 cfu/mL as McFarland 0.5 turbidity standard. Plates were allowed to solidify. Sterile filter paper discs (6 mm) were impregnated with 20 μL of each drug separately and allowed to saturate for 30 min. and were placed on the surface of the agar plates which had previously been inoculated with tested microorganisms respectively. All plates were incubated for 24 h at 37 ℃. Results were recorded by measuring the zone of inhibition appearing around the discs. All the tests were performed in triplicate and the mean values are presented. Dimethyl sulfoxide (DMSO) was used as negative control.

2.5. Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

3. Results

Antimicrobial mechanism of the drugs used here were variable i.e. their mechanism was either inhibition of cell wall synthesis or damage to the cytoplasmic membrane or inhibit nucleic acid and protein synthesis or inhibition of specific enzyme system. The data pertaining to the

Table 1.
Antibacterial activity of methanol extract of *T. catappa* and *C. papaya* leaves and different antibiotics (n=3).

Microorganisms		Zone of inhibition (mm)* (extracts+antibiotics)												
		ET	EC	P	AMP	AMC	CEP	PB	RIF	AK	NA	GEN	C	OF
Gram positive bacteria	*Micrococcus flavus* ATCC10240	14.5 ± 0.3	NZ	24 ± 0	36 ± 0	23 ± 0	30 ± 0	ND	30 ± 0	22 ± 0	NZ	22 ± 0	25 ± 0	19 ± 0
	Bacillus megaterium ATCC9885	9.0 ± 0.0	NZ	NZ	NZ	13 ± 0	12 ± 0	ND	14 ± 0	25 ± 0	18 ± 0	21 ± 0	13 ± 0	19 ± 0
	Bacillus subtilis ATCC6633	NZ	NZ	NZ	11± 0	16 ± 0	36 ± 0	ND	11 ± 0	14 ± 0	20 ± 0	17 ± 0	22 ± 0	30 ± 0
	Staphylococcus aureus ATCC25923	12.0 ± 0.0	NZ	23 ± 0	21 ± 0	21 ± 0	25 ± 0	ND	24 ± 0	17 ± 0	12 ± 0	15 ± 0	21 ± 0	23 ± 0
	Staphylococcus epidermidis ATCC12228	12.0 ± 0.0	NZ	NZ	14 ± 0	11 ± 0	26 ± 0	ND	30 ± 0	18 ± 0	14± 0	17 ± 0	18 ± 0	22 ± 0
Gram negative bacteria	*Proteus morganii* NCIM2040	15.0 ± 0.0	NZ	ND	25 ± 0	24 ± 0	26 ± 0	12 ± 0	28 ± 0	23 ± 0	NZ	20 ± 0	25 ± 0	23 ± 0
	Proteus vulgaris NCIM2857	14.0 ± 0.0	NZ	ND	7 ± 0	13 ± 0	8 ± 0	NZ	17 ± 0	28 ± 0	34 ± 0	23 ± 0	27 ± 0	40 ± 0
	Klebsiella pneumoniae NCIM2719	11.5 ± 0.3	NZ	ND	34 ± 0	28 ± 0	40 ± 0	16 ± 0	30 ± 0	28 ± 0	14 ± 0	24 ± 0	41 ± 0	26 ± 0
	Proteus mirabilis NCIM2241	9.0 ± 0.0	NZ	ND	20 ± 0	19 ± 0	31 ± 0	12 ± 0	13 ± 0	23 ± 0	19 ± 0	19 ± 0	15 ± 0	24 ± 0
	Enterobacter aerogenes ATCC13048	11.0 ± 0.0	NZ	ND	17 ± 0	11 ± 0	NZ	11 ± 0	NZ	23 ± 0	25 ± 0	20 ± 0	27 ± 0	31 ± 0

ET = Methanolic extract of *T. catappa*; EC = Methanolic extract of *C. papaya*; Penicillin G (P); ampicillin (AMP); amoxyclav (AMC); cephalothin (CEP); polymyxin B (PB); rifampicin (RIF); amikacin (AK); nilidixic acid (NA); gentamicin (GEN); chloramphenicol (C); ofloxacin (OF); ND = not done; NZ = no zone of inhibition; * The values are Mean ± SEM.

Table 2.
Synergistic activity of methanolic extract of *T. catappa* leaves with different standard antibiotics against bacteria (n=3).

Microorganisms		Zone of inhibition (mm)* (antibiotics + methanolic extract of *T. catappa*)										
		P	AMP	AMC	CEP	PB	RIF	AK	NA	GEN	C	OF
Gram positive bacteria	*M. flavus* ATCC10240	27.5 ± 0.0(I)	28.0 ± 0.0(A)	24.0 ± 0.0(I)	34.0 ± 0.0(I)	ND	30.0 ± 0.0(I)	23.0 ± 0.0(I)	14.0 ± 0.0(I)	20.5 ± 0.3(I)	24.5 ± 03(I)	16.5 ± 0.3(A)
	B. megaterium ATCC9885	0.0 ± 0.0(A)	13.0 ± 0.0(S)	16.0 ± 0.0(I)	16.0 ± 0.0(I)	ND	17.0 ± 0.0(I)	24.5 ± 0.3(I)	18.0 ± 0.0(I)	19.5 ± 0.3(I)	15.0 ± 0.3(I)	20.0 ± 0.0(I)
	B. subtilis ATCC6633	10.0 ± 0.0(S)	14.0 ± 0.0(S)	16.5 ± 0.0(I)	37.0 ± 0.0(S)	ND	15.0 ± 0.0(S)	15.5 ± 0.0(S)	21.0 ± 0.0(I)	16.0 ± 0.3(I)	24.5 ± 0.3(S)	31.0 ± 0.6(S)
	S. aureus ATCC25923	24.0 ± 0.0(I)	24.0 ± 0.0(I)	23.0 ± 0.0(I)	25.0 ± 0.0(I)	ND	25.0 ± 0.0(I)	17.0 ± 0.0(I)	12.0 ± 0.0(I)	15.0 ± 0.0(I)	21.0 ± 0.0(I)	21.0 ± 0.0(I)
	S. epidermidis ATCC12228	10.0 ± 0.0(A)	15.0 ± 0.0(I)	12.5 ± 0.3(I)	26.0 ± 0.0(I)	ND	31.0 ± 0.0(I)	19.5 ± 0.3(I)	14.0 ± 0.0(I)	16.0 ± 0.0(I)	18.0 ± 0.0(I)	21.0 ± 0.0(I)
Gram negative bacteria	*P. morganii* NCIM2040	ND	26.0 ± 0.0(I)	25.0 ± 0.0(I)	28.0 ± 1.4(I)	10.0 ± 0.0(I)	31.0 ± 0.0(I)	23.5 ± 0.3(I)	14.0 ± 0.0(I)	20.0 ± 0.0(I)	26.5 ± 0.3(I)	23.0 ± 0.6(I)
	P. vulgaris NCIM2857	ND	13.0 ± 0.0(I)	14.0 ± 0.0(I)	14.0 ± 0.0(I)	10.0 ± 0.0(I)	19.0 ± 0.0(I)	28.0 ± 0.0(I)	35.0 ± 0.0(I)	22.5 ± 0.3(I)	28.0 ± 0.0(I)	40.0 ± 0.3(I)
	K. pneumoniae NCIM2719	ND	36.0 ± 0.0(I)	30.0 ± 0.0(I)	42.5 ± 0.3(I)	15.0 ± 0.0(I)	34.0 ± 0.0(I)	30.0 ± 0.0(I)	15.0 ± 0.0(I)	24.0 ± 0.3(I)	43.0 ± 0.0(I)	24.0 ± 0.3(I)
	*P. mirabilis*NCIM2241	ND	21.5 ± 0.0(I)	20.0 ± 0.0(I)	33.5 ± 0.3(I)	11.0 ± 0.0(I)	15.0 ± 0.0(I)	25.0 ± 0.0(I)	20.0 ± 0.0(I)	19.0 ± 0.0(I)	14.0 ± 0.0(I)	25.5 ± 0.3(I)
	E. aerogenes ATCC13048	ND	16.5 ± 0.3(I)	11.0 ± 0.0(I)	10.0 ± 0.0(I)	11.0 ± 0.0(I)	11.0 ± 0.0(I)	24.5 ± 0.6(I)	26.5 ± 0.6(I)	19.5 ± 0.6(I)	30.0 ± 0.0(I)	34.0 ± 0.0(I)

Penicillin G (P); ampicillin (AMP); amoxyclav (AMC); cephalothin (CEP); polymyxin B (PB); rifampicin (RIF); amikacin (AK); nilidixic acid (NA); gentamicin (GEN); chloramphenicol (C); ofloxacin (OF); I: indifferent; S: Synergism; A: antagonism; ND: not done; * The values are Mean ± SEM.

Table 3.
Synergistic activity of methanolic extract of *C. papaya* leaves with different standard antibiotics against bacteria (n=3).

Microorganisms		Zone of inhibition (mm)* (antibiotics + methanolic extract of *C. papaya*)										
		P	AMP	AMC	CEP	PB	RIF	AK	NA	GEN	C	OF
Gram positive bacteria	*M. flavus* ATCC10240	24.5 ± 0.3(S)	30.0 ± 0.0(A)	28.0 ± 0.0(S)	37.0 ± 0.0(S)	ND	30.5 ± 0.0(I)	26.0 ± 0.0(S)	8.0 ± 0.0(S)	23.0 ± 0.6(I)	25.5 ± 0.3(I)	22.5 ± 0.3(S)
	B. megaterium ATCC9885	0.0 ± 0.0	0.0 ± 0.0	14.0 ± 0.0(I)	14.0 ± 0.0(S)	ND	176.0 ± 0.0(S)	26.0 ± 0.6(I)	18.0 ± 0.0(I)	22.0 ± 0.3(I)	14.0 ± 0.0(I)	20.5 ± 0.3(I)
	*B. subtilis*ATCC6633	9.0 ± 0.0(S)	13.0 ± 0.0(S)	15.5 ± 0.3(I)	38.0 ± 0.6(S)	ND	13.5 ± 0.3(I)	16.5 ± 0.9(S)	21.0 ± 0.0(I)	16.0 ± 0.0(I)	25.0 ± 0.0(S)	35.0 ± 0.0(S)
	S. aureus ATCC25923	24.5 ± 0.3(S)	24.5 ± 0.3(S)	22.0 ± 0.6(S)	25.0 ± 0.0(S)	ND	25.0 ± 0.0(S)	17.5 ± 0.3(S)	11.0 ± 0.0(I)	18.0 ± 0.0(S)	22.0 ± 0.0(S)	23.5 ± 0.3(S)
	S. epidermidis ATCC12228	9.0 ± 0.0(S)	16.0 ± 0.0(S)	11.0 ± 0.0(S)	26.5 ± 0.0(S)	ND	30.5 ± 0.3(S)	21.0 ± 0.0(S)	14.0 ± 0.0(S)	19.0 ± 0.0(S)	20.5 ± 0.3(S)	24.0 ± 0.6(S)
Gram negative bacteria	*P. morganii* NCIM2040	ND	28.0 ± 0.0(S)	25.0 ± 0.0(S)	30.0 ± 0.3(S)	13.5 ± 0.3(S)	30.0 ± 0.0(S)	24.5 ± 0.3(S)	0.0 ± 0.0	22.5 ± 0.29(S)	26.0 ± 0.0(S)	24.0 ± 0.0(S)
	P. vulgaris NCIM2857	ND	8.0 ± 0.0(S)	11.5 ± 0.3 (I)	10.0 ± 0.0(S)	8.0 ± 0.0(S)	18.0 ± 0.0(S)	28.0 ± 0.0(S)	35.5 ± 0.0(S)	25.0 ± 0.0(S)	27.5 ± 0.3(S)	42.0 ± 0.0(S)
	K. pneumoniae NCIM2719	ND	36.5 ± 0.0(S)	30.0 ± 0.0(S)	41.5 ± 0.3(S)	16.0 ± 0.8(S)	34.0 ± 0.6(S)	30.0 ± 0.0(S)	14.5 ± 0.3 (S)	24.5 ± 0.9(S)	43.5 ± 0.3(S)	27.0 ± 0.0(S)
	P. mirabilis NCIM2241	ND	22.0 ± 0.0(S)	19.0 ± 0.0(I)	33.5 ± 0.3(I)	14.0 ± 0.0(I)	11.0 ± 0.0(I)	26.0 ± 0.0(I)	20.5 ± 0.1 (I)	21.0 ± 0.0(I)	14.5 ± 0.3(I)	26.5 ± 0.3(S)
	E. aerogenes ATCC13048	ND	16.0 ± 0.0(I)	0.0 ± 0.0(A)	9.0 ± 0.0(S)	12.0 ± 0.0	0.0 ± 0.0	26.0 ± 0.6(S)	28.5 ± 0.6 (S)	20.0 ± 0.0(S)	32.0 ± 0.0(S)	36.5 ± 0.3(S)

Penicillin G (P); ampicillin (AMP); amoxyclav (AMC); cephalothin (CEP); polymyxin B (PB); rifampicin (RIF); amikacin (AK); nilidixic acid (NA); gentamicin (GEN); chloramphenicol (C); ofloxacin (OF); I: indifferent; S: Synergism; A: antagonism; ND: not done; * The values are Mean ± SEM.

antimicrobial potential of the individually plant extracts and eleven antibacterial drug against five Gram positive and five Gram negative bacteria is presented in Table 1. The methanolic extract of *T. catappa* showed maximum zone of inhibition against tested bacteria; while all the bacteria were resistant to methanolic extract of *C. papaya*. All the antibiotics showed activity against bacteria but to a varying level. Penicillin G, ampicillin, cephalothin, polymyxin B, rifampicin and nilidixic acid did not show any activity against some bacteria (Table 1).

Synergistic activity of methanolic extract of *T. catappa* leaves with different standard antibiotics against bacteria is shown in Table 2. The synergistic effect was found only against B. subtilis, when methanolic extract of *T. catappa* was combined with penicillin, ampicillin, cephalothin, rifampicin, amikacin, chloramphenicol, ofloxacin. This suggests the potential of this plant to improve the performance of penicillin, ampicillin, cephalothin, rifampicin, amikacin, chloramphenicol, ofloxacin. Similar synergistic effect of acetone extract of *Garcinia kola* (*G. kola*) seeds and chloramphenicol, amoxicillin and penicillin G was reported by Sibanda and Okoh[18]. Antagonistic effect was observed against *B. megaterium* and *S. epidermidis* when methanolic extract of *T. catappa* was combined with penicillin but when combined with ampicillin and ofloxacin, antagonism was observed against *M. flavus*. The remaining combination of methanolic extract of *T. catappa* and antibiotics showed indifferent effects.

Synergistic activity of methanolic extract of *C. papaya* leaves with different standard antibiotics against bacteria is shown in Table 3. Synergistic effect was found in almost all the antibiotics used against all the tested bacteria. The maximum synergistic effect was found in *C. papaya* with cephalothin and ofloxacin. Antagonistic effect was found in ampicillin and amoxyclav against *M. flavus* and *E. aerogenes* respectively. *S. epidermidis* and *K. pneumoniae* were the more susceptible Gram positive and Gram negative bacteria respectively. Generally, medicinal plants tend to be more effective against Gram-positive than Gram-negative bacteria[19]. This agreement contradicts our results because maximum synergistic effect was observed against Gram negative bacteria as compared to Gram positive bacteria. The synergy detected in this study as enumerated suggests that plant crude extracts is a blend of compounds that can enhance the activity of different antibiotics. Plants have been known to contain myriads of antimicrobial compounds[20].

4. Discussion

Synergistic effects resulting from the combination of antibiotics with various plant extracts has been studied and experimented by a number of scientists. The methanolic extract of *T. catappa* and *C. papaya* showed synergistic effect with though *C. papaya* showed better synergistic activity. This suggests the potential of these plants to improve the performance of the antibiotics evaluated. The synergistic effect of methanolic extract of *C. papaya* was with all eleven antibiotics, while that of *T. catappa* was with seven antibiotics. The methanolic extract of *C. papaya* with antimicrobial agents possesses synergistic properties which act against some pathogenic organisms as compared to individual extract. These results indicate that *C. papaya* extract contain natural inhibitors working by different mechanisms.

A number of *in vitro* studies have reported the use of plant extracts in combination with antibiotics against some resistant strains[21-23]. Adwan *et al*[24] investigated *in vitro* interaction between ethanolic extracts of *Rhus coriaria* (seed), *Sacropoterium spinosum* (seed), *Rosa damascene* (flower) and certain known antimicrobial drugs including oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium and enrofloxacin. Synergy testing of these extracts and antibiotics was carried out against 3 multidrug-resistant *Pseudomonas aeruginosa* strains. The synergy between *R. coriaria* and antibiotics showed a high decrease in MIC and a strong bactericidal activity. These results indicated that combination between *R. coriaria* extract and antibiotics could be useful in fighting emerging drug-resistant *P. aeruginosa*. Toroglu[25] investigated *in-vitro* synergistic effects of different spices and herbs (*Rosmarinus officinalis*, *Coriandrum sativum*, *Micromeria fruticosa*, *Cumium cyminum*, *Mentha piperita*) with gentamicin, cephalothin, ceftriaxone and nystatin against 13 microbial species. This study suggested that essential oils of tested spices and herbs could protect some bacterial strains and the combination of plant extract with antibiotics further reduced drug resistance. The synergistic effects obtained could lead to new choices for the treatment of infectious diseases

Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug[26].

The use of antimicrobial agents displaying synergy is one of the well established indications for combination antimicrobial therapy. Combinations of antimicrobials that demonstrate an *in vitro* synergistic effect against infecting strains are more likely to result in successful therapeutic result. In addition, combinations of agents that exhibit synergy or partial synergy could potentially improve the outcome for patients with difficult to treat infections[27]. Thus, evidence of *in vitro* synergism could be useful in selecting most favourable combinations of antimicrobials for the practical therapy of serious bacterial infections. Our results revealed that the combined used of plant extracts and antibiotics could be useful in treatment of infectious diseases and useful in fighting emerging drug resistance problem however *in vivo* experiments are needed to confirm the bacterial protection using this combination.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgement

The authors thank Prof. S.P. Singh, Head, Department of Biosciences, Saurashtra University for providing excellent research facilities; and University Grants Commission, New Delhi, India [F. No. 37-524/2009 (SR)] for providing financial support in the form of a Major Research Project.

References

[1] Albuquerque WF, Macrae A, Sousa OV, Vieira GHF, Vieira RHSF. Multiple drug resistant *Staphylococcus aureus* strains isolated from a fish market and from fish handlers. *Braz J Microbiol* 2007; **38**: 131-134.

[2] Liu IX, Durham DG, Richards RM. Baicalin synergy with β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* other β-lactam-resistant strains of *S. aureus*. *J Pharm Pharmacol* 2000; **52**: 361-366.

[3] Dawis MA, Isenberg HD, France KA, Jenkins SG. *In vitro* activity of gatifloxacin alone and incombination with cefepime, meropenem, piperacillin and gentamicin against multidrug-resistant organisms. *J Antimicrob Chemother* 2003; **51**: 1203-1211.

[4] Kamatou GPP, van Zyl RL, van Vuuren SF, Viljoen AM. Chemical composition, leaf trichome types and biological activities of the essential oils of four related salvia species indigenous to Southern Africa. *J Ess Oil Res* 2006; **18**: 72-79.

[5] Ibezim EC, Esimone CO, Nnamani PO, Onyishi IV, Brown SA, Obodo CE. *In vitro* study of the interaction between some flouroquinolones and extracts of *Kola nitida* seed. *Afr J Biotechnol* 2006; **5**: 1781-1784.

[6] Esimone CO, Okoye FBC, Nworu CS, Agubata CO. *In vitro* interaction between caffeine and some penicillin antibiotics against *Staphylococcus aureus*. *Trop J Pharm Res* 2008; **7**: 969-974.

[7] Chambers HF. General principles of antimicrobial therapy. In: Bruton LL (ed). *Goodman and Gilman's Pharmacologiced Basis of Therapeutics*. 11th Ed. USA: McGraw Hill; 2006, p. 1102-1104.

[8] Khan AU, Musharraf A. Plasmid mediated multiple antibiotic resistances in *Proteus mirabilis* isolated from patients with urinary tract infection. *Med Sci Mont* 2004; **10**: 598-602.

[9] Akram M, Shahid M, Khan AU. Etiology and antibiotics resistance pattern of community acquired urinary infections in J N M C Hospital Aligarh India. *Ann Clin Microbiol Antimicrob* 2007; **6**: 4.

[10] Kluytmans J, Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997; **10**: 505-520.

[11] Chanda S, Vyas BRM, Vaghasiya Y, Patel H. Global resistance trends and the potential impact of Methicillin Resistant *Staphylococcus aureus* (MRSA) and its solutions. In: Mendez-Vilas A. (ed). *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. 2nd Series. Spain: Formatex; 2010, p. 444-450.

[12] Bai NR, Christi RM, Kala TC. Antimicrobial potency of the marine alga, *Valoniopsis pachynema* (mar.) boery. *Plant Arch* 2010; **10**: 699-701.

[13] Maleki S, Seyyednejad SM, Damabi NM, Motamedi H. Antibacterial activity of the fruits of Iranian torilis leptophylla against some clinical pathogens. *Pak J Biol Sci* 2008; **11**: 1286-1289.

[14] Anjaria J, Parabia M, Dwivedi S. *Ethanovet Heritage Indian Ethnoveterinary Medicine – An Overview*, Ahmedabad: Pathik Enterprise; 2002, p. 420.

[15] Parekh J, Chanda S. *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fructicosa* Kurz. Flower (Lythraceae). *Braz J Microbiol* 2007; **38**: 204-207.

[16] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; **45**: 493-496.

[17] Vaghasiya YK, Parekh JP, Shukla VJ, Chanda SV. Antimicrobial and anti-inflammatory screening of four Indian medicinal plants. *Lat Am J Pharm* 2011; **30**: 661-666.

[18] Sibanda T, Okoh AI. *In vitro* evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics. *Afr J Biotech* 2008; **7**: 1672-1678.

[19] Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. *Pak J Bot* 2008; **40**: 1349-1358.

[20] Wadhwa S, Bairagi M, Bhatt G, Panday M, Porwal A. Antimicrobial activity of essential oils of *Trachyspermum ammi*. *Int J Pharm Biol Arch* 2010; **1**: 131-133.

[21] Chatterjee SK, Bhattacharjee I, Chandra G. *In vitro* synergistic effect of doxycycline & ofloxacin in combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. *Indian J Med Res* 2009; **130**: 475-478.

[22] Espina L, Somolinos M, Loran S, Conchello P, Garcia D, Pagan R. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control* 2011; **22**: 896-902.

[23] Lowa WL, Martin C, Hill DJ, Kenwarda MA. Antimicrobial efficacy of silver ions in combination with tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Int J Antimicrob Agents* 2011; **37**: 162-165.

[24] Adwan G, Abu-Shanab B, Adwan K. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. *Asian Pac J Trop Med* 2010; **3**: 266-269.

[25] Toroglu S. *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. *J Environ Biol* 2011; **32**: 23-29.

[26] Kamatou GPP, van Zyl RL, van Vuuren SF, Viljoen AM. Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* Species indigenous to Southern Africa. *J Ess Oil Res* 2006; **18**: 72-79.

[27] Song W, Woo HJ, Kim JS, Lee KM. *In vitro* activity of β-lactams in combination with other antimicrobial agents against resistant strains of *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2003; **21**: 8-12.