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

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ABSTRACT

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Objective: To evaluate the *in vitro* interaction between methanolic extracts of *Terminalia catappa* (Combretaceae) (*T. catappa*) and *Carica papaya* (caricaceae) (*C. papaya*) leaves and certain known antimicrobial drugs like penicillin G (P), ampicillin (AMP), amoxycylav (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 problems[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].

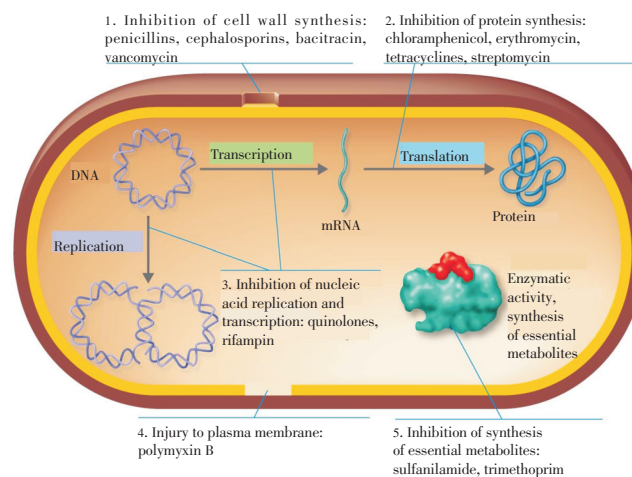


Figure 1. The action of antimicrobial drugs.

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

Plants antimicrobials have been found to be synergistic

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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 present study different pathogenic bacteria causing diseases 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. *Staphylococcus aureus* (*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 urinary tract infection is Gram negative bacteria that belong to the family *Enterobacteriaceae*.

Terminalia catappa L. (Desi badam) (*T. catappa*) 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, eye problems, 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 L. (Papaya) (*C. 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.

The present study was focused on the synergistic activity of two plant extracts with eleven antibiotics.

2. Materials and methods

2.1. Chemicals

Nutrient broth, Sabouraud dextrose broth, Muller 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. Ten grams 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 5000 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 5000 rpm for 10 min, the supernatant was collected and the solvents were evaporated and the dry extract was stored at 4 °C 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 *S. aureus* ATCC25923, *S. epidermidis* ATCC12228, *Bacillus megaterium* (*B. megaterium*) ATCC9885, *B. subtilis* ATCC6633, *Micrococcus flavus* (*M. flavus*) ATCC10240 and five Gram negative bacteria *Proteus morgani* (*P. morgani*) NCIM2040, *Proteus vulgaris* (*P. vulgaris*) NCIM2857, *Enterobacter aerogenes* (*E. aerogenes*) ATCC13048, *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 °C.

2.4.2. Antibiotics used

All antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, India) *viz.* penicillin G (P 10 units/disc), ampicillin (AMP 10 µg/disc), amoxyclav (AMC 30 µg/disc), cephalothin (CEP 30 µg/disc), polymyxin B (PB 300 units/disc), rifampicin (RIF 5 µg/disc), amikacin (AK 30 µg/disc), nilidixic acid (NA 30 µg/disc), gentamicin (GEN 10 µg/disc), chloramphenicol (C 30 µg/disc), ofloxacin (OF 5 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 μ L 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 μ L 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 $^{\circ}$ C. 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 \pm SEM.

3. Results

Antimicrobial mechanisms of the drugs used here were variable. 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 antimicrobial potential of the individually plant extracts

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	<i>M. flavus</i> ATCC10240	14.5 \pm 0.3	0 \pm 0	24.0 \pm 0.0	36.0 \pm 0.0	23.0 \pm 0.0	30.0 \pm 0.0	ND	30.0 \pm 0.0	22.0 \pm 0.0	0.0 \pm 0.0	22.0 \pm 0.0	25.0 \pm 0.0	19.0 \pm 0.0
	<i>B. megaterium</i> ATCC9885	9.0 \pm 0.0	0 \pm 0	NZ	NZ	13.0 \pm 0.0	12.0 \pm 0.0	ND	14.0 \pm 0.0	25.0 \pm 0.0	18.0 \pm 0.0	21.0 \pm 0.0	13.0 \pm 0.0	19.0 \pm 0.0
	<i>B. subtilis</i> ATCC6633	0.0 \pm 0.0	0 \pm 0	0.0 \pm 0.0	11.0 \pm 0.0	16.0 \pm 0.0	36.0 \pm 0.0	ND	11.0 \pm 0.0	14.0 \pm 0.0	20.0 \pm 0.0	17.0 \pm 0.0	22.0 \pm 0.0	30.0 \pm 0.0
	<i>S. aureus</i> ATCC25923	12.0 \pm 0.0	0 \pm 0	23.0 \pm 0.0	21.0 \pm 0.0	21.0 \pm 0.0	25.0 \pm 0.0	ND	24.0 \pm 0.0	17.0 \pm 0.0	12.0 \pm 0.0	15.0 \pm 0.0	21.0 \pm 0.0	23.0 \pm 0.0
	<i>S. epidermidis</i> ATCC12228	12.0 \pm 0.0	0 \pm 0	0.0 \pm 0.0	14.0 \pm 0.0	11.0 \pm 0.0	26.0 \pm 0.0	ND	30.0 \pm 0.0	18.0 \pm 0.0	14.0 \pm 0.0	17.0 \pm 0.0	18.0 \pm 0.0	22.0 \pm 0.0
Gram negative bacteria	<i>P. morganii</i> NCIM2040	15.0 \pm 0.0	0 \pm 0	ND	25.0 \pm 0.0	24.0 \pm 0.0	26.0 \pm 0.0	12.0 \pm 0.0	28.0 \pm 0.0	23.0 \pm 0.0	NZ	20.0 \pm 0.0	25.0 \pm 0.0	23.0 \pm 0.0
	<i>P. vulgaris</i> NCIM2857	14.0 \pm 0.0	0 \pm 0	ND	7.0 \pm 0.0	13.0 \pm 0.0	8.0 \pm 0.0	NZ	17.0 \pm 0.0	28.0 \pm 0.0	34.0 \pm 0.0	23.0 \pm 0.0	27.0 \pm 0.0	40.0 \pm 0.0
	<i>K. pneumoniae</i> NCIM2719	11.5 \pm 0.3	0 \pm 0	ND	34.0 \pm 0.0	28.0 \pm 0.0	40.0 \pm 0.0	16.0 \pm 0.0	30.0 \pm 0.0	28.0 \pm 0.0	14.0 \pm 0.0	24.0 \pm 0.0	41.0 \pm 0.0	26.0 \pm 0.0
	<i>P. mirabilis</i> NCIM2241	9.0 \pm 0.0	9 \pm 0	ND	20.0 \pm 0.0	19.0 \pm 0.0	31.0 \pm 0.0	12.0 \pm 0.0	13.0 \pm 0.0	23.0 \pm 0.0	19.0 \pm 0.0	19.0 \pm 0.0	15.0 \pm 0.0	24.0 \pm 0.0
	<i>E. aerogenes</i> ATCC13048	11.0 \pm 0.0	0 \pm 0	ND	17.0 \pm 0.0	11.0 \pm 0.0	NZ	11.0 \pm 0.0	NZ	23.0 \pm 0.0	25.0 \pm 0.0	20.0 \pm 0.0	27.0 \pm 0.0	31.0 \pm 0.0

ET = Methanolic extract of *T. catappa*; EC = Methanolic extract of *C. papaya*; Penicillin G (P); ampicillin (AMP); amoxycylav (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. Values are presented as mean \pm SEM. What is the difference between "0 \pm 0" and "NZ" in the table?

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 <i>T. catappa</i>)											
	P	AMP	AMC	CEP	PB	RIF	AK	NA	GEN	C	OF	
Gram positive bacteria	<i>M. flavus</i> ATCC10240	27.5 \pm 0.0 (I)	28.0 \pm 0.0 (A)	24.0 \pm 0.0 (I)	34.0 \pm 0.0 (I)	ND	30.0 \pm 0.0 (I)	23.0 \pm 0.0 (I)	14.0 \pm 0.0 (I)	20.5 \pm 0.3 (I)	24.5 \pm 0.3 (I)	16.5 \pm 0.3 (A)
	<i>B. megaterium</i> ATCC9885	0.0 \pm 0.0 (A)	13.0 \pm 0.0 (S)	16.0 \pm 0.0 (I)	16.0 \pm 0.0 (I)	ND	17.0 \pm 0.0 (I)	24.5 \pm 0.3 (I)	18.0 \pm 0.0 (I)	19.5 \pm 0.3 (I)	15.0 \pm 0.3 (I)	20.0 \pm 0.0 (I)
	<i>B. subtilis</i> ATCC6633	10.0 \pm 0.0 (S)	14.0 \pm 0.0 (S)	16.5 \pm 0.0 (I)	37.0 \pm 0.0 (S)	ND	15.0 \pm 0.0 (S)	15.5 \pm 0.0 (S)	21.0 \pm 0.0 (I)	16.0 \pm 0.3 (I)	24.5 \pm 0.3 (S)	31.0 \pm 0.6 (S)
	<i>S. aureus</i> ATCC25923	24.0 \pm 0.0 (I)	24.0 \pm 0.0 (I)	23.0 \pm 0.0 (I)	25.0 \pm 0.0 (I)	ND	25.0 \pm 0.0 (I)	17.0 \pm 0.0 (I)	12.0 \pm 0.0 (I)	15.0 \pm 0.0 (I)	21.0 \pm 0.0 (I)	21.0 \pm 0.0 (I)
	<i>S. epidermidis</i> ATCC12228	10.0 \pm 0.0 (A)	15.0 \pm 0.0 (I)	12.5 \pm 0.3 (I)	26.0 \pm 0.0 (I)	ND	31.0 \pm 0.0 (I)	19.5 \pm 0.3 (I)	14.0 \pm 0.0 (I)	16.0 \pm 0.0 (I)	18.0 \pm 0.0 (I)	21.0 \pm 0.0 (I)
Gram negative bacteria	<i>P. morganii</i> NCIM2040	ND	26.0 \pm 0.0 (I)	25.0 \pm 0.0 (I)	28.0 \pm 1.4 (I)	10.0 \pm 0.0 (I)	31.0 \pm 0.0 (I)	23.5 \pm 0.3 (I)	14.0 \pm 0.0 (I)	20.0 \pm 0.0 (I)	26.5 \pm 0.3 (I)	23.0 \pm 0.6 (I)
	<i>P. vulgaris</i> NCIM2857	ND	13.0 \pm 0.0 (I)	14.0 \pm 0.0 (I)	14.0 \pm 0.0 (I)	10.0 \pm 0.0 (I)	19.0 \pm 0.0 (I)	28.0 \pm 0.0 (I)	35.0 \pm 0.0 (I)	22.5 \pm 0.3 (I)	28.0 \pm 0.0 (I)	40.0 \pm 0.3 (I)
	<i>K. pneumoniae</i> NCIM2719	ND	36.0 \pm 0.0 (I)	30.0 \pm 0.0 (I)	42.5 \pm 0.3 (I)	15.0 \pm 0.0 (I)	34 \pm 0 (I)	30 \pm 0 (I)	15 \pm 0 (I)	24 \pm 0.29 (I)	43 \pm 0 (I)	24.0 \pm 0.3 (I)
	<i>P. mirabilis</i> NCIM2241	ND	21.5 \pm 0.0 (I)	20.0 \pm 0.0 (I)	33.5 \pm 0.3 (I)	11.0 \pm 0.0 (I)	15 \pm 0 (I)	25 \pm 0 (I)	20 \pm 0 (I)	19 \pm 0 (I)	14 \pm 0 (I)	25.5 \pm 0.3 (I)
	<i>E. aerogenes</i> ATCC13048	ND	16.5 \pm 0.3 (I)	11.0 \pm 0.0 (I)	10.0 \pm 0.0 (I)	11.0 \pm 0.0 (I)	11 \pm 0 (I)	24.5 \pm 0.58 (I)	26.5 \pm 0.58 (I)	19.5 \pm 0.58 (I)	30 \pm 0 (I)	34.0 \pm 0.0 (I)

Penicillin G (P); ampicillin (AMP); amoxycylav (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; Values are presented as mean \pm 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 <i>C. papaya</i>)											
	P	AMP	AMC	CEP	PB	RIF	AK	NA	GEN	C	OF	
Gram positive bacteria	<i>M. flavus</i> ATCC10240	24.5 \pm 0.3 (S)	30.0 \pm 0.0 (A)	28.0 \pm 0.0 (S)	37.0 \pm 0.0 (S)	ND	30.5 \pm 0.0 (I)	26.0 \pm 0.0 (S)	8.0 \pm 0.0 (S)	23.0 \pm 0.6 (I)	25.5 \pm 0.3 (I)	22.5 \pm 0.3 (S)
	<i>B. megaterium</i> ATCC9885	0.0 \pm 0.0	0.0 \pm 0.0	14.0 \pm 0.0 (I)	14.0 \pm 0.0 (S)	ND	176.0 \pm 0.0 (S)	26.0 \pm 0.6 (I)	18.0 \pm 0.0 (I)	22.0 \pm 0.3 (I)	14.0 \pm 0.0 (I)	20.5 \pm 0.3 (I)
	<i>B. subtilis</i> ATCC6633	9.0 \pm 0.0 (S)	13.0 \pm 0.0 (S)	15.5 \pm 0.3 (I)	38.0 \pm 0.6 (S)	ND	13.5 \pm 0.3 (I)	16.5 \pm 0.9 (S)	21.0 \pm 0.0 (I)	16.0 \pm 0.0 (I)	25.0 \pm 0.0 (S)	35.0 \pm 0.0 (S)
	<i>S. aureus</i> ATCC25923	24.5 \pm 0.3 (S)	24.5 \pm 0.3 (S)	22.0 \pm 0.6 (S)	25.0 \pm 0.0 (S)	ND	25.0 \pm 0.0 (S)	17.5 \pm 0.3 (S)	11.0 \pm 0.0 (I)	18.0 \pm 0.0 (S)	22.0 \pm 0.0 (S)	23.5 \pm 0.3 (S)
	<i>S. epidermidis</i> ATCC12228	9.0 \pm 0.0 (S)	16.0 \pm 0.0 (S)	11.0 \pm 0.0 (S)	26.5 \pm 0.0 (S)	ND	30.5 \pm 0.3 (S)	21.0 \pm 0.0 (S)	14.0 \pm 0.0 (S)	19.0 \pm 0.0 (S)	20.5 \pm 0.3 (S)	24.0 \pm 0.6 (S)
Gram negative bacteria	<i>P. morganii</i> NCIM2040	ND	28.0 \pm 0.0 (S)	25.0 \pm 0.0 (S)	30.0 \pm 0.3 (S)	13.5 \pm 0.3 (S)	30.0 \pm 0.0 (S)	24.5 \pm 0.3 (S)	0.0 \pm 0.0	22.5 \pm 0.29 (S)	26.0 \pm 0.0 (S)	24.0 \pm 0.0 (S)
	<i>P. vulgaris</i> NCIM2857	ND	8.0 \pm 0.0 (S)	11.5 \pm 0.3 (I)	10.0 \pm 0.0 (S)	8.0 \pm 0.0 (S)	18.0 \pm 0.0 (S)	28.0 \pm 0.0 (S)	35.5 \pm 0.0 (S)	25.0 \pm 0.0 (S)	27.5 \pm 0.3 (S)	42.0 \pm 0.0 (S)
	<i>K. pneumoniae</i> NCIM2719	ND	36.5 \pm 0.0 (S)	30.0 \pm 0.0 (S)	41.5 \pm 0.3 (S)	16.0 \pm 0.8 (S)	34.0 \pm 0.6 (S)	30.0 \pm 0.0 (S)	14.5 \pm 0.3 (S)	24.5 \pm 0.9 (S)	43.5 \pm 0.3 (S)	27.0 \pm 0.0 (S)
	<i>P. mirabilis</i> NCIM2241	ND	22.0 \pm 0.0 (S)	19.0 \pm 0.0 (I)	33.5 \pm 0.3 (I)	14.0 \pm 0.0 (I)	11.0 \pm 0.0 (I)	26.0 \pm 0.0 (I)	20.5 \pm 0.1 (I)	21.0 \pm 0.0 (I)	14.5 \pm 0.3 (I)	26.5 \pm 0.3 (S)
	<i>E. aerogenes</i> ATCC13048	ND	16.0 \pm 0.0 (I)	0.0 \pm 0.0 (A)	9.0 \pm 0.0 (S)	12.0 \pm 0.0	0.0 \pm 0.0	26.0 \pm 0.6 (S)	28.5 \pm 0.6 (S)	20.0 \pm 0.0 (S)	32.0 \pm 0.0 (S)	36.5 \pm 0.3 (S)

Penicillin G (P); ampicillin (AMP); amoxycylav (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; Values are presented as mean \pm SEM.

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 against *B. subtilis*. Similar synergistic effect of acetone extract of *Garcinia 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 amoxycylav against *M. flavus* and *E. aerogenes* respectively. *S. epidermidis* and *K. pneumoniae* were the more susceptible to 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* (*R. coriaria*) (seed), *Sacropoterium spinosum* (seed), *Rosa damascene* (flower) and certain known antimicrobial drugs including oxytetracycline HCl, penicillin G, cephalixin, sulfadimethoxine sodium and enrofloxacin. Synergy testing of these extracts and antibiotics was carried out against 3 multidrug-resistant *Pseudomonas aeruginosa* (*P. 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*, *Cuminum 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. Although 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 statement

We declare that we have no conflict of interest.

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