Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 6, Issue, 04, pp. 7299-7304, April, 2016

Full Length Research Article

IN VITRO, INTERACTION OF SOME ANTIBIOTICS WITH DIFFERENT FRUIT EXTRACTS ON SOME PATHOGENIC BACTERIAL STRAINS

Abboud El Kichaoi, Mahmoud El-Hindi, Farida Mosleh, Ashraf Shafie and *Tarek Elbashiti

Microbiology Lab, Department of Biotechnology, Islamic University-Gaza, Gaza Strip, Palestine

ARTICLE INFO

Article History:

Received 19th January, 2016
Received in revised form
21st February, 2016
Accepted 08th March, 2016
Published online 27th April, 2016

Key Words:

Synergistic Activity,
Fruit Extracts,
Disc-diffusion Method,
Antibiotics,
Pathogenic Bacteria.

ABSTRACT

Objective: To evaluate *in vitro*, the synergistic effects of bioactive extracts of *Punica granatum* (Pomegranate) "pulp", *Actinidia deliciosa* (Kiwifruit) "peel" and *Citrus maxima* (Pomelo) "pulp" with different antibiotics against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Methods: The extraction was carried out using the soxhlet in presence of ethanol, methanol or water. Evaluation of synergistic activity was carried out using disc diffusion method. Results: The results pointed that some of crude extracts showed a synergistic activity against the tested bacteria, and some extract showed no synergistic effect. The highest synergistic effect was observed with streptomycin and ciprofloxancin. Conclusion: Obviously the wide use of antibiotics in the treatment of bacterial infections has led to spread of resistant strains so we need to find a source of bioactive substances that could possess broad spectrum of activity when it is combined with antibiotic to be able resist the pathogenic microorganism.

INTRODUCTION

The spread of bacterial infections and appearance of resistance strains encouraged a lot of studies in many countries to create more realistic solutions in an attempt to control of these infections and their causes (Adwan *et al.*, 2010; Masoud and Gouda, 2012). Antibiotic resistance in multi-drug resistance bacteria is becoming a major problem in the treatment of many infections (Ghanda and Rakholiya, 2011; Masoud and Gouda, 2012; Blesson *et al.*, 2014). Infection caused by bacteria is considered one of the factors that cause of morbidity and mortality worldwide (Masoud and Gouda, 2012; Tiwari *et al.*, 2015). So there is an urgent need to find ways to overcome the antibiotic resistance bacteria and control of diseases that result from them. The problem that led to the aggravation and allow the emergence of antibiotic-resistant bacteria are the use of antibiotics to prevent disease before it occur and inappropriate use of antibiotics for the treatment of the common viral infections (Masoud and Gouda, 2012). Which have led to impair in bacterial equilibrium in the body as a result eliminate beneficial bacteria in the body and the evolution of resistant strains.

Corresponding author: Tarek Elbashiti,
Microbiology Lab, Department of Biotechnology, Islamic University-Gaza, Gaza Strip, Palestine.

Many plant extracts showed its effectiveness as antibacterial, antifungal and against many pathogens (Nascimento *et al.*, 2000; Mahesh and Satish, 2008; Akinsulire *et al.*, 2008; Khan *et al.*, 2009). This may due to that plant extract contain natural inhibitor compounds that affected on growth of microorganisms by different mechanisms (Adwan and Mhanna, 2008). They generally produce many secondary metabolites such as alkaloid, flavonoids, tannins and phenolic compounds which are play an important role in control many diseases as sources of microbicides, pesticides and many pharmaceutical drugs (Avasthi *et al.*, 2010; Paiva *et al.*, 2010). Many of the plant such as clove, garlic, mustard, onion have been proved to be effective antimicrobial agents (Benkeblia, 2004; Mukhtar *et al.,* 2012). About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer (Bhalodia *et al.*, 2011). Some Palestinian plants possess great ability against human pathogenic bacteria (Adwan and Mhanna, 2008). Few studies have found combining antimicrobial agent with crude plant extracts increase the efficiency of them against *S. aureus*, *P. aeruginosa* and *E. coli* and thus the possibility of reducing the negative effect resulting from the frequent use of antibiotics (Adwan and Mhanna, 2008).

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments and enhance inhibitory effect of some antibiotics. The present study aims to assess the impact of local plant extracts alone or combined with antibiotics on some pathogenic microorganisms to finding new antimicrobial agents that have real effect against human pathogenic microorganisms especially drug-resistant bacteria as natural alternatives to synthetic compounds and their antimicrobial agents could have broad spectrum of activity when it is combined with antibiotics.

MATERIALS AND METHODS

Chemicals

Table1. Media and chemicals that used and their manufactures

Chemicals	Manufactured by	purchased from
Mueller Hinton agar	Liofilchem	Italy
Mueller Hinton Broth	Liofilchem	Italy
Dimethyl Sulphoxide (DMSO).	Applichem	Germany
80%Methanol, 80%Ethanol, Distilled water	CHEM Limited	Indian

Plant collection

The fruits used in this study (*Punica granatum*, *Actinidia deliciosa* and *Citrus maxima*) were collected from felids.

Extraction

The used parts from these fruits were pulp from (Pomegranate and Pomelo) and peel from (Kiwi fruit). Pulp and peel were separated from fruits and used as fresh and also air dried for 3 days. Then the fresh and dried parts cut into small pieces and 30 grams of these pieces extracted in a Soxhlet extractor by using 300 ml of 80%ethanol, 80%methanol, and water. The extraction time was eight hours for each solvent. The resulting extracts were evaporated using oven temperature 37°C for 3 days. Then all extracts were dissolved in DMSO to a final concentration 200mg/ml and all samples were maintained at -4°C until the usage time (Abeysinghe and Weeraddana, 2011).

Microorganisms

The microorganisms which have been used in this study are the bacteria (***Staphylococcus aureus, Escherichia coli*** and ***Pseudomonas aeruginosa***), which were isolated from clinical samples delivered from El-Shifa Hospital in Gaza strip.

Antimicrobial test

Agar disc- diffusion method

Agar disc-diffusion method was carried out to determine the antimicrobial activity.

A suspension of inoculum of overnight culture was introduced to MHA (cooled to 40 - 45°C) swirl gently to mix well. After solidification, sterile filter paper discs approximately 6mm in diameter were impregnated with stock extracts and placed on the surface agar plate. Incubation period of 24h at 37°C for bacteria. The antimicrobial activity was evaluated by measuring zones of inhibition of microbial growth surrounding the plant extracts (Sharma, 2011; ElKichaoi *et al*., 2015).

Antibiotic sensitivity test

Antibiotic sensitivity test was performed by disc diffusion method. By using sterile forceps, the selected of antibiotics (Table 2) were put on the surface of plate. The plates were incubated at 37^0C for 18 h. Then the zones of inhibition were measured in millimeter by using ruler (Elbashiti *et al*., 2011).

Table 2. The antibiotics and their potencies

Antibiotics	Potency
Ciprofloxacin	5µg
Cefazolin	30 µg
Amikacin	30mcg
Bacitracin	10 µg
Nalidixic acid	30 µg

Synergistic assay

The antibiotic discs of 6mm in diameter saturated with 20 µl stock extract placed on the surface agar plate. The plates were incubated at 37^0C for 24 h. The diameters of cleared zones were measured and compared with that of antibiotic alone and the extract a lone **(**Elbashiti *et al*., 2011; Jouda *et al.,* 2016).

RESULTS

Bioactivity of fruit extract alone by disc diffusion method

The results in Table 3 showed the antimicrobial activity by disc diffusion method against tested microorganisms. For *S. aureus*, the water extract of pomegranate pulp " fresh and dried " showed the highest effect with a 23 mm zone of inhibition followed by ethanolic extract of pomegranate pulp "fresh" and "dried" with a 15mm and 10mm zone of inhibition, respectively. Kiwipeel "dried" and "fresh" extracted by ethanol and methanol showed moderate activity. No antimicrobial activity was observed by pomelo "pulp" extracted by ethanol, methanol and water. For *E. coli*, the water extract of pomegranate pulp "dried" was showed the highest effect with a 20 mm zone of inhibition followed by pomegranate pulp "fresh" with a 17mm zone of inhibition.

The ethanolic extract of pomegranate pulp "fresh" and "dried" also showed a good activity with a zone of inhibition (14mm). No antimicrobial activity was observed by pomelo "pulp" extracted by water at concentration of 200mg /ml. For *P. aeruginosa*, the ethanolic extract of pomegranate pulp "dried" showed the highest effect (with a 25mm zone of inhibition) followed by ethanolic extract of pomegranate pulp "fresh" with a 22mm zone of inhibition.

Table 3. The antimicrobial and synergistic effect of plant extracts, extracted by Ethanol "mm"

M//O	A.B	A.B alone	R1		R2		K1		K2		B1		B2	
			EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B
Ec	CIP5	11	14	7	14	7	9	ND	6	-	7	ND	7	ND
	KZ30	-		-		7		9		7		9		ND
	S10	-		7		7		11		10		11		ND
	Ak30	21		11		7		ND		=		ND		ND
	NO30	11		7		8		11		13		11		ND
Sa	CIP5	7	15	12	10	10	8	9	6	-	-	7	-	7
	KZ30	6		13		-		10		-		-		11
	S10	7		25		-		8		-		7		7
	Ak30	-		ND		ND		8		-		-		7
	NO30	6		8		10		10		9		-		8
Pa	CIP5	11	13	ND	11	ND	6	ND	6	6	7	ND	7	7
	KZ30	-		ND		ND		ND		8		ND		-
	S10	-		ND		ND		ND		8		ND		7
	Ak30	21		ND		ND		ND		8		ND		-
	NO30	12		ND		ND		ND		10		ND		-

Table 4. The antimicrobial and synergistic effect of plant extracts, extracted by Methanol "mm"

M//O	A.B	A.B alone	R1		R2		K1		K2		B1		B2	
			EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B
Ec	CIP5	11	7	9	10	9	7	ND	6	ND	9	ND	9	ND
	KZ30	-		8		-		9		-		8		ND
	S10	-		7		7		10		9		21		10
	Ak30	21		10		7		ND		ND		ND		ND
	NO30	11		12		10		9		9		12		15
Sa	CIP5	7	7	7	7	8	7	7	7	ND	7	7	7	7
	KZ30	6		-		-		10		ND		14		-
	S10	7		9		-		7		ND		8		8
	Ak30	14		18		18		ND		ND		ND		ND
	NO30	6		7		8		10		ND		9		8
Pa	CIP5	11	22	ND	25	9	6	ND	6	6	7	ND	7	ND
	KZ30	-		ND		-		ND		8		ND		ND
	S10	-		ND		7		ND		8		ND		ND
	Ak30	20		ND		-		ND		8		ND		ND
	NO30	11		ND		9		ND		10		ND		ND

Table 5. The antimicrobial and synergistic effect of plant extracts, extracted by Water "mm"

M//O	A.B	A.B alone	R1		R2		K1		K2		B1		B2	
			EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B	EX Alone	EX+ A.B
Ec	CIP5	11	17	19	20	20	8	23	8	13	-	ND	-	ND
	KZ30	-		-		12		17		ND		12		11
	S10	-		8		9		10		10		9		ND
	Ak30	21		ND		ND		ND		ND		ND		ND
	NO30	11		14		14		13		15		10		13
Sa	CIP5	7	23	22	23	20	10	8	10	7	-	ND	-	ND
	KZ30	6		22		22		9		10		ND		ND
	S10	-		20		20		8		7		ND		ND
	Ak30	14		23		23		7		6		ND		ND
	NO30	-		23		23		12		11		ND		ND
Pa	CIP5	11	18	20	16	30	8	6	8	7	8	ND	7	ND
	KZ30	-		20		22		10		10		-		7
	S10	-		19		12		9		8		-		7
	Ak30	21		23		26		8		7		ND		ND

M/Os: Microorganisms, A.B: Antibiotic, EX: Extract, Ea: E.coli, Sa: S. aureus, Pa: P. aeruginosa, CIP5: Ciprofloxacin KZ30: Cefazolin, AK30: Amikacin, BA10: Bacitracin, NA30: Nalidixic Acid, R1:Pomegranate pulp fresh R1:Pomegranate pulp dried, k1: Kiwi peel fresh, k1: Kiwi peel dried, P1:pomelo pulp fresh, P2: pomelo pulp dried, ND: Not determine

The water extract of pomegranate pulp "fresh" and "dried" showed good activity with a 18mm and 16mm zone of inhibition, respectively.

The synergistic activity of fruit extracts with antibiotics

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 (Rakholiya and Chanda, 2011).

Against *E. coli*

Ethanolic extract and antibiotics

As shown in Table 3 Pomegranate pulp "fresh and dried" didn't show any synergistic effect when was added to tested antibiotics. Kiwi fruit "Fresh" showed synergistic effect only when was added to streptomycin with Inhibition zone 11mm. Kiwi fruit "dried" showed synergistic effect when was added to novobiocin. Antagonistic effect was observed when Kiwi fruit "dried" was combined with ciprofloxacin and amikacin. Pomelo "Fresh" showed synergistic effect only when was added to streptomycin and Cefazolin with Inhibition zone 11mm and 9mm, Respectively. Pomelo "dried" didn't determine in this study.

Methanolic extract and antibiotics

Pomegranate pulp "fresh" showed negligible or weak synergistic effect when was added to novobiocin and bacitracin with Inhibition zone 12mm and 8mm, Respectively. Pomegranate pulp "dried" didn't show any synergistic effect when was added to tested antibiotics. Antagonistic effect was observed when Pomegranate pulp "dried" was combined with bacitracin. Pomegranate pulp "dried" when was added to novobiocin ciprofloxacin, streptomycin, amikacin showed indifferent effects. Kiwi fruit "Fresh" showed synergistic effect when was added to streptomycin and bacitracin with Inhibition zone 10mm and 9mm, Respectively. Kiwi fruit "dried" showed synergistic effect only when was added to streptomycin with Inhibition zone 9mm. Pomelo "Fresh" showed the highest synergistic effect when was added to streptomycin with Inhibition zone 21mm. Pomelo "dried" showed the highest synergistic effect when was added to novobiocin with Inhibition zone 15mm. Pomelo "fresh" showed negligible weak synergistic effect when was added to novobiocin (Table 4).

Water extract and antibiotics

Pomegranate pulp "fresh" showed highest synergistic effect when combined with Ciprofloxacin with Inhibition zone 19mm. Antagonistic effect was observed when Pomegranate pulp "fresh" was combined with bacitracin. Kiwi fruit "fresh" showed the highest synergistic effect when combined with Ciprofloxacin with Inhibition zone 23mm. Followed by bacitracin with Inhibition zone 17mm. The combining between Kiwi fruit "fresh" with the rest antibiotics also showed an acceptable results. Also Kiwi fruit "dried" showed synergistic effect when combined with Novobiocin, Streptomycin and Ciprofloxacin.

Pomelo "fresh" showed the highest synergistic effect when was added to bacitracin with Inhibition zone 12mm. Followed by with Streptomycin with Inhibition zone 9mm. Pomelo "dried" showed the highest synergistic effect when was added to novobiocin with Inhibition zone 13mm. Followed by bacitracin with Inhibition zone 11mm (Table5).

Against *S. aureus*

Ethanolic extract and antibiotics

As shown in Table 4. Pomegranate pulp "fresh" showed the highest synergistic effect when was added to Streptomycin with inhibition zone 25mm. Pomegranate pulp "dried" showed indifferent effect when was added to novobiocin and ciprofloxacin. Antagonistic effect was observed when Pomegranate pulp "dried" was combined with bacitracin and streptomycin. The combining between Pomegranate pulp "fresh" and antibiotics Ciprofloxacin, bacitracin and novobiocin showed indifferent effects. Also the combining between Pomegranate pulp "dried" and novobiocin showed indifferent effects (Table3).

Kiwi fruit "fresh" showed the highest synergistic effect when was added to bacitracin and novobiocin with Inhibition zone 10mm and when added to ciprofloxacin showed weak or negligible synergistic effect. Kiwi fruit "dried" showed the highest synergistic effect when was added to novobiocin. Antagonistic effect was observed when Kiwi fruit "dried" was combined with ciprofloxacin, bacitracin and streptomycin. The combining between Kiwi fruit "fresh" and "streptomycin and nalidixic acid" showed indifferent effects. The combining between Kiwi fruit "dried" and nalidixic acid also showed antagonism effect. Pomelo "Fresh" didn't show any synergistic effect when was added to tested antibiotics. Pomelo "dried" showed the highest synergistic effect when was added to nalidixic acid with Inhibition zone 7mm followed by bacitracin and novobiocin (Table 3).

Methanolic extract and antibiotics

Pomegranate pulp "fresh and dried" didn't show any synergistic effect except when were added to amikacin with Inhibition zone 18mm. Antagonistic effect was observed when both Pomegranate pulp "fresh and dried" were combined with bacitracin. Pomegranate pulp "fresh" when was added to ciprofloxacin and novobiocin showed indifferent effects. Also indifferent effects were observed when Pomegranate pulp "dried" was combined with novobiocin. Kiwi fruit "fresh" showed the highest synergistic effect when was added to bacitracin and novobiocin with Inhibition zone 10mm. The combining between Kiwi fruit "fresh" and antibiotics ciprofloxacin and streptomycin showed indifferent effects. Pomelo "fresh" showed the highest synergistic effect when was added to bacitracin with Inhibition zone 14mm. Followed by novobiocin with Inhibition zone 9mm. When it was combined with antibiotics Streptomycin showed weak or negligible synergistic effects. Pomelo "dried" when was combined with antibiotics novobiocin showed weak or negligible synergistic effects.

Water extract and antibiotics

There was no any synergistic effect when was added both Pomegranate pulp "Fresh and dried" to tested antibiotics. The combining between both Pomegranate pulp "fresh and dried" with the rest antibiotics showed indifferent effects. Kiwi fruit "fresh" showed weak or negligible synergistic effects when combined with novobiocin Inhibition zone 12mm. Also Kiwi fruit "dried" showed weak or negligible synergistic effects when combined with Novobiocin Inhibition zone 11mm. Kiwi fruit "fresh and dried" when were combined with antibiotics " Streptomycin and nalidixic acid " combined with "Ciprofloxacin and bacitracin" showed indifferent effects.

Against *P. aeruginosa*

Ethanolic extract and antibiotics

As shown in Table5.The combining between both Pomegranate pulp "fresh and dried" and antibiotics didn't determine in this study. Kiwi fruit "dried" showed weak or negligible synergistic effect when was added to cefazolin and streptomycin with Inhibition zone 8mm, and when added to ciprofloxacin showed indifferent effect. The combining between Kiwi fruit "dried" nalidixic acid showed indifferent effect. Kiwi fruit "fresh" and Pomelo "Fresh" also didn't determine in this study. Pomelo "Dried" didn't show any synergistic effect when was added to tested antibiotics.

Methanolic extract and antibiotics

The combining between methanolic extracts "Fresh and dried" and antibiotics didn't determine in this study.

Water extract and antibiotics

Pomegranate pulp "fresh" showed synergistic effect when was added to all tested antibiotics. The highest synergistic effect when it added to novobiocin with inhibition zone 22mm. The rest antibiotics gave acceptable results when combination with Pomegranate pulp "fresh". Also Pomegranate pulp "dried" showed synergistic effect when was added to all tested antibiotics expect streptomycin. The highest synergistic effect when it added to Ciprofloxacin with inhibition zone 30mm. The combining between Kiwi fruit "fresh" and antibiotics streptomycin, bacitracin and novobiocin showed weak or negligible synergistic effect. The combining between Kiwi fruit "dried" and antibiotics bacitracin and novobiocin antagonism effects. Pomelo " Fresh and dried" didn't show any synergistic effect when was added to tested antibiotics.

DISCUSSION

The discovery of antibiotics is one of the methods in combating bacterial infections that once ravaged humankind (Chanda and Rakholiya, 2011). Although antibiotics are one of the main methods used in resistance bacterial infections, the use of antibiotics has become threatened due to appearance of resistance strains. The first way to prevent the spread of antibiotic resistance bacteria and control of diseases is starting to publish campaigns titled "safe use of antibiotics".

The development and spread of resistance to antibiotics due to improper use of current antimicrobial drugs is a worldwide concern (Bhalodia *et al*., 2014).The problem of microbial resistance is increasing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to overcome this problem, for example, control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs (Nascimento *et al*., 2000). Many *in vitro*, studies have found important synergistic effects with significant decrease in the minimum inhibitory concentration "MIC" antibiotic when it interaction with different plant extracts against *S. aureus* strains (Adwan and Mhanna, 2008; Ahmed *et al*., 2009; Rakholiya and Chanda, 2011; Müller *et al*., 2013). Our study indicated that the combination extracts obtained from the selected fruits with antibiotics reported acceptable synergistic effects in tested bacteria and also pointed that there is a possibility for occurrence of antagonism.

The reason for occurrence synergistic effects may be referred to present bioactive compounds that can increase the sensitivity of bacterial cells to antibiotics. Due to present of numerous compounds within the crude extract and antibiotic may have interfered with the actions of one and may be considered reason for occurrence antagonism. Secondary metabolites in plant such as carotenoids, flavonoids, vitamin, alkaloids and pigments which have biological significance and may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modifications and decrease intracellular drug accumulation (Abeysinghe and Weeraddana, 2011). Antibiotic mechanism includes inhibition of cell membrane function, cell wall synthesis, specific enzyme system, protein and nucleic acid synthesis (Rakholiya and Chanda, 2011).

Synergism may be occur in different mechanism

- Crude extract and antibiotic may sequentially block a microbial metabolic pathway (Adwan and Mhanna, 2008).
- One of two drugs may affect the cell membrane and thus the second drug can enter easily (Adwan and Mhanna, 2008).

Understanding these mechanisms of action for known antimicrobial agents and bioactive fruits extracts may be lead to know the reasons of antagonism and synergism between them.

Conclusion

In conclusion, the results from these studies were encouraging to find new antimicrobial agents or new ways that are effective for the treatment of infectious diseases caused by test pathogenic microorganism especially drug-resistant bacteria. The parts of fruits "pulp and peel" that used in this study are generally thrown into environmental as waste. These waste can be exploited for some application as medical plants and enhance the inhibitory ability of some antibiotics resulting from combination of known antimicrobial agents and these parts of fruits.

Acknowledgements

The authors thank Department of biology and Biotechnology, Islamic University for providing excellent research facilities and El-Shifa Hospital for providing clinical bacteria strains.

REFERENCES

Abeysinghe Pushpa, D. and Weeraddana Chaminda De S. 2011. "Screening of Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water Extracts of Medicinal Plant, Avicennia *marina* for Antibacterial Activity against Antibiotic Resistant Bacteria Species, *Staphylococcus* and *Proteus*." *Journal of Pharmaceutical and Biomedical Sciences (JPBMS)* 11.11.

Adwan, G. and Mhanna, M. 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. *Middle-East Journal of Scientific Research*, 3(3), 134-139.

Adwan, G., Abu-Shanab, B. and Adwan, K. 2010. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug–resistant *Pseudomonas aeruginosa* strains. *Asian Pacific Journal of Tropical Medicine*, *3*(4), 266-269.

Ahmed, Z., Khan, S. S., Khan, M., Tanveer, A. and Lone, Z. A. 2009. Synergistic Effect of Salvadora persica extracts tetracycline and penicillin against *Staphylococcus aureus*. *Afr J Basic Appl Sci*, *2*(1-2), 25-29.

Akinsulire, O. R., Aibin, I. E., Adenipekun, T., Adelowotan, T. and Odugbemi, T. 2008. *In vitro* antimicrobial activity of crude extracts from plants Bryophyllum pinnatum and Kalanchoe crenata. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(3), 338-344.

Avasthi, S., Gautam, A. K. and Bhadauria, R. 2010. Antifungal activity of plant products against Aspergillus niger: A potential application in the control of a spoilage fungus. *In Biological Forum—An International Journal* (Vol. 2, No. 1, pp. 53-55).

Benkeblia, N. 2004. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). *LWT-Food Science and Technology*, *37*(2), 263-268.

Bhalodia, N. R. and Shukla, V. J. 2011. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *Journal of advanced pharmaceutical technology and research*, 2(2), 104.

Blesson, J., Saji, C. V., Nivya, R. M. and Kumar, R. 2014. Synergistic antibacterial activity of natural plant extracts and antibiotics against methicillin resistant staphylococcus aureus (MRSA). World journal of pharmacy and pharmaceutical sciences, 03, 741-763.

Chanda, S. and Rakholiya, K. 2011. Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. *Microbiol Book Series, 520-529.*

Elbashiti, T. A., Elmanama, A. A. and Masad, A. A. 2011. The antibacterial and synergistic effects of some Palestinian plant extracts on Escherichia coli and Staphylococcus aureus. *Funct Plant Sci Biotechnol*, *5*, 57-62.

Elkichaoi, A., El-Hindi, M., Mosleh, F. and Elbashiti, T. 2015. The Antimicrobial Effects of the Fruit Extracts of *Punica granatum, Actinidia deliciosa* and *Citrus maxima* on Some Human Pathogenic Microorganisms. American International Journal of Biology, 3 (2), 1-18.

Jouda, M., Elbashiti, T., Masad, A. and Albayoumi, M. 2016. The Antibacterial Effect of Some Medicinal Plant Extracts and Their Synergistic Effect With Antibiotics. World Journal of Pharmacy and Pharmaceutical Sciences, 5 (2), 23-33.

Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A. A., Ali, S. M. and Khan, A. U. 2009. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin.*Molecules*, *14*(2), 586-597.

Mahesh, B. and Satish, S. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World journal of agricultural sciences*, *4*(5), 839-843.

Masoud, E. A. and Gouda, H. A. 2012. Effect of some natural plant extracts against gram negative bacteria in Njran Area, Saudi Arabia. *Egypt Acad J Biolog Sci,* 4, 85-92.

Mukhtar, S. and Ghori, I. 2012. Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *Escherichia coli* ATCC 25922 and *Bacillus subtilis* DSm 3256. Int. J. Appl. Biol. Pharm. Technol, 3(2), 131-135.

Nascimento, G. G., Locatelli, J., Freitas, P. C. and Silva, G. L. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*(4), 247-256.

Paiva, P. M. G., Gomes, F. S., Napoleão, T. H., Sá, R. A., Correia, M. T. S. and Coelho, L. C. B. B. 2010. Antimicrobial activity of secondary metabolites and lectins from plants. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, *1*, 396-406.

Rakholiya, K. and Chanda, S. 2012. *In vitro* interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), S876-S880.

Sharma, A. 2011. Antibacterial activity of ethanolic extracts of some arid zone plants. *Int J Pharm Tech Res*, *3*, 283-286.

Tiwari, V., Roy, R. and Tiwari, M. 2015. Antimicrobial active herbal compounds against Acinetobacter baumannii and other pathogens. *Frontiers in microbiology, 6.*

****