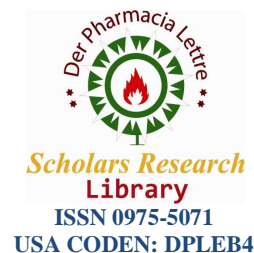




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Effect of methanol extract of *Moringa oleifera* leaves on antibacterial activity of β -lactam antibiotics against some pathogenic bacteria

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ABSTRACT

The decreasing effectiveness of traditional antibiotics against resistant bacteria is a global public health. Combination between plant extract and antibiotics is one of the most important tools in the increasing of the effectiveness of many traditional antibiotics against pathogenic bacteria. The purpose of the current investigation to study the interaction between methanol extract of *Moringa oleifera* leaves (MML) and β -lactam antibiotics by agar diffusion method. Obtained results showed that MML (1.0 g/ml) had negative antibacterial activity against all tested strains. While its combinations with β -lactam antibiotics exhibited different interactions against them. Furthermore, the combinations of MML/imipenem, cefepime, ceftazidime or piperacillin exhibited indifferent interaction against *Klebsiella* sp., antagonistic interaction against *E. coli* & *Pseudomonas* sp. and synergistic interaction against *Acinetobacter* sp.. Thus, MML could be used as source for resistance-modifying agents against infectious multi-drug resistant *Acinetobacter* sp.

Key words: *Moringa oleifera*, Beta-lactam, pathogenic bacteria, Interaction

INTRODUCTION

Excessive and irrational use of antibiotics is the foremost causes of distribution of bacterial resistance to antibiotics over the world, which led to decrease the effectiveness of many typical antibiotics against pathogenic bacteria[1]. The continuous evolution of this problem has necessitated the research for novel antimicrobial compounds. In the last few decades, a large number of investigations maintained to the importance of medicinal plants as a rich source of natural antimicrobials, which could increase the effectiveness of many antibiotics by synergistic interaction against pathogenic microorganisms[2-6].

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. It is grow commercially in Egypt and other countries of Africa, America and Asia[7]. According to several commentaries, the leaves of *Moringa oleifera* contained a wide variety of nutrients including; vitamins, minerals, amino acids, flavonoids, fatty acids, phenols, ascorbic acid and carotenoids. The leaves extracts have different medicinal benefits, they used as a natural anti-inflammatory, anti-hypertensive, diuretic, antioxidant and anti-diabetic[8,9, 10,11]. Furthermore, *Moringa oleifera* extracts suggested by many recent investigations as resistance-modifying agents against a wide array of pathogens[8,12-15]. The present study was designed to investigate the interactions between the methanol extract of *Moringa oleifera* leaves(MML) and β -lactam antibiotics against some pathogenic bacteria.

MATERIALS AND METHODS

Clinical bacteria and plant material

Clinical strains of *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp. and *Pseudomonas* sp. were obtained from Al Borg Laboratories, Mohandeseen, Giza, Egypt during January, 2014. Clinical strains were confirmed their identification before study using the key proposed by [16]. The tested cultures were maintained on nutrient agar slants at 4°C throughout the study and used as stock cultures. *M. oleifera* leaves powder was purchased from National Research Center, Dokki, Egypt.

Media and antibiotics

Nutrient agar medium (NA), Muller-Hinton agar medium (MHA), antibiotic disks including: ampicillin (10µg); imipenem (10µg); aztreonam (10µg); piperacillin/tazobactam (100/10µg); ampicillin/sulbactam (10/10µg); meropenem (10µg); cefixime (5µg); piperacillin (100µg); ceftazidime (30µg); ceftriaxone (30µg); cefepime (30µg); cefotaxime (10µg); cefoperazone (75µg); cefoperazone/sulbactam (75/30µg), cefuroxime (30µg) and oxacillin (1µg) were purchased from Oxoid Ltd. Co., and methanol was purchased from Sigma Chemicals.

Preparation of *M. oleifera* leaves extract and bacterial inoculum

One hundred gram of *M. oleifera* leaves powder transferred into 1000ml glass bottle contained 500ml methanol, closed tightly, protected from light by aluminum foil, incubated for 2h on rotary shaker (180rpm) at room temperature, and stored in refrigerator for 5 days. Afterward, the suspension was filtered using Whatman filter paper 1 and concentrated to 1.0g/ml using vacuum rotary evaporator (Heidolph, Germany) adjusted at 55°C. The methanol extract of *M. oleifera* leaves (MML) was protected from light by aluminum foil and stored in refrigerator until used. The suspension of each tested strain was prepared by direct colony suspension method according to [17].

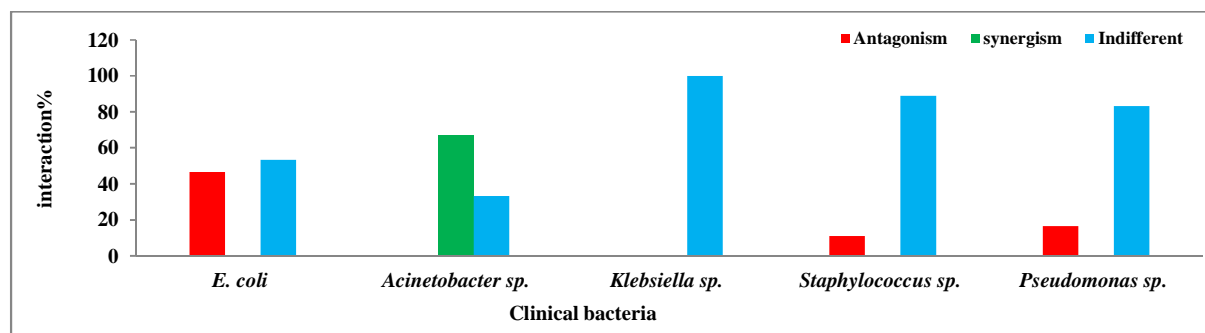
Antibacterial activity of MML and its interaction with β -lactam antibiotics

The antibacterial activity of MML (1.0g/ml) against each tested strain was studied by agar well diffusion method [18] using 200µL/well and using methanol absolute as control. The interaction between MML and β -lactam antibiotics against each tested strain was studied by disk diffusion method on MHA contained 1% MML as follow: One milliliter of MML (1.0g/ml) was added to sterile MHA previously melted and cooled to 45-55 °C, shook well and poured in sterile Petri dishes. A sterile cotton swab dipped into the bacterial suspension was spread on the surface MHA plates supplemented with 1% MML. Sensitivity of clinical strains to different β -lactam antibiotics was studied on MHA (control) and MHA plates supplemented with 1% MML according to Clinical and Laboratory Standards Institute [19]. The interaction was expressed as synergistic if the increasing of zone diameter around the antibiotic disk in MHA supplemented with 1% MML was $\geq 19\%$ compared to control (antibiotic alone), an indifferent if the change of zone diameter around the antibiotic disk in MHA supplemented with 1% MML was $< 19\%$ and an antagonistic if the decreasing of zone diameter around the antibiotic disk in MHA supplemented with 1% MML was $\geq 19\%$ compared to control [20].

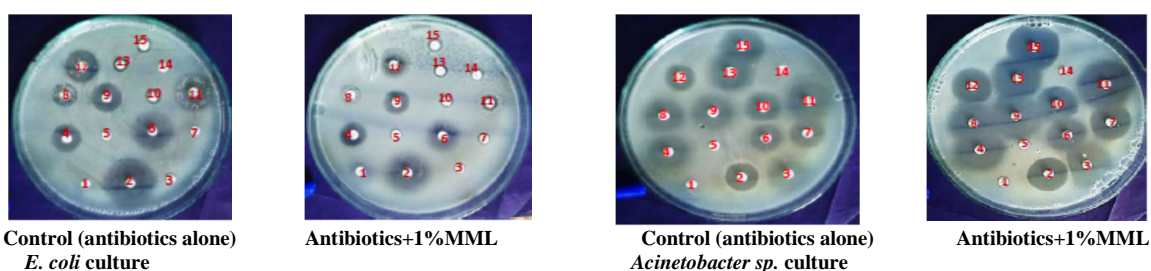
RESULTS

The present study was conducted to obtain preliminary information on the interaction between MML and β -lactam antibiotics against some clinical bacteria by agar diffusion method. The primary sensitivity screening of MML against various clinical strains showed that the tested extract had a negative antimicrobial activity against various tested strains. While, its combinations with β -lactam antibiotics gave different interactions according to the type of tested antibiotics (Fig.,1).

Notable indifferent interaction of MML/ β -lactam antibiotic combinations was observed against *Klebsiella* sp. (100%), followed by *Staphylococcus* sp., *Pseudomonas* sp., *E. coli* and *Acinetobacter* sp., which reached to 88.89, 83.33, 53.33 and 33.33%, respectively (Fig.,1). The maximum antagonistic interactions between MML and β -lactam antibiotics was found against *E. coli*, which reached to 46.67%, followed by *Pseudomonas* sp. and *Staphylococcus* sp., which reached to 16.67 and 11.11%, respectively (Fig.,1). On the other hand, the synergistic interaction between MML and β -lactam antibiotics was only observed against *Acinetobacter* sp., which reached to 66.67% of all tested combinations (Fig.,1).

Fig.(1): Interaction percentage between MML and β -lactam antibiotics against some clinical bacteriaTable (1_a): Effect of MML¹ on antibacterial activity of β -lactam antibiotics against some clinical bacteria

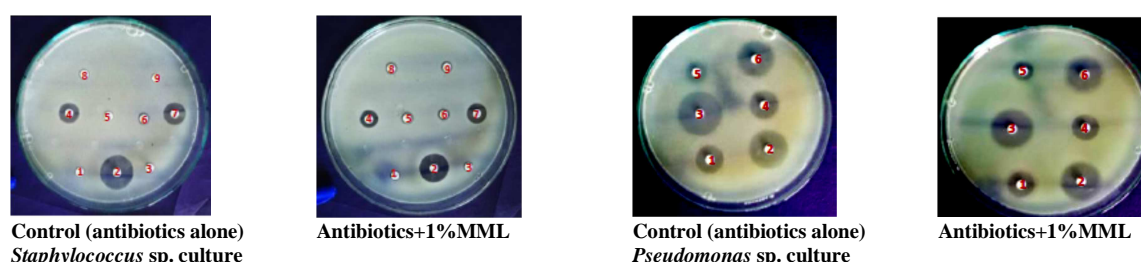
No.	Antibiotics (Con./disk)	Clinical isolates					
		<i>E. coli</i>		<i>Acinetobacter</i>		<i>Klebsiella</i>	
		Control ²	A/MML ³	Control ²	A/MML ³	Control ²	A/MML ³
		Inhibition zone (mm)					
1	Ampicillin (10μg)	0	0	0	0	0	0
2	Imipenem (10μg)	35	25	22	29	29	29
3	Aztreonam (10μg)	0	0	29	30	0	0
4	Pipera/Tazo (100/10μg)	25	20	20	35	25	25
5	Amp/sulba (10/10μg)	0	0	0	0	0	0
6	Meropenem (10μg)	30	24	20	25	23	22
7	Cefixime (5μg)	0	0	23	29	0	0
8	Piperacillin (100μg)	19	12	23	30	0	0
9	Ceftazidime (30μg)	24	19	20	35	10	10
10	Ceftriaxone (30μg)	10	10	25	25	0	0
11	Cefepime (30μg)	30	12	20	34	20	19
12	Cefotaxime (10μg)	24	19	20	27	0	0
13	Cefop/sulb (75/30μg)	10	10	16	20	20	20
14	Cefuroxime (30μg)	0	0	0	0	0	0
15	Cefoperazone (75μg)	0	0	29	35	10	10

(1): Methanol extract of *Moringa oleifera* leaves (1%), (2): Antibiotics alone, (3): Antibiotics + MMLFig. (2): The interaction between MML and β -lactam antibiotics against *E. coli* and *Acinetobacter sp.*

In case of *E. coli*, combinations of MML/imipenem, pipera/tazo, meropenem, piperacillin, ceftazidime, cefepime or cefotaxime decreased the inhibition zone of tested antibiotics from 35, 25, 30, 19, 24, 30 and 24mm to 25, 20, 24, 12, 19, 12, and 19mm, respectively. While, the antagonistic interactions against *Pseudomonas sp.* was detected by combination of MML/piperacillin, cefepime, imipenem, meropenem or ceftazidime, which decreased their antibacterial activities from 25, 35, 25, 15, 28 to 20, 28, 12, 13 and 22mm, respectively (Table, 1_{a,b} & Figs. 2,3).

Table (1_b): Effect of MML¹ on antibacterial activity of β -lactam antibiotics against some clinical bacteria

NO.	Antibiotics (Con./disk)	Staphylococcus		No.	Antibiotics (Con./disk)	Pseudomonas	
		Control ²	A/MML ³			Control ²	A/MML ³
		Inhibition zone (mm)				Inhibition zone (mm)	
1	Ampicillin (10µg)	0	0	1	Piperacillin (100µg)	25	20
2	Imipenem (10µg)	26	20	2	Pipe/Tazo (100/10µg)	30	30
3	Oxacillin (1µg)	0	0	3	Cefepime (30µg)	35	28
4	Pipe/Tazo(100/10µg)	15	15	4	Imipenem (10µg)	25	13
5	Ampi/sulb (10/10µg)	R	R	5	Meropenem (10µg)	15	12
6	Meropenem (10µg)	10	10	6	Ceftazidime (30µg)	28	22
7	Amox/Clav(20/10µg)	20	19				
8	Cefepime (30µg)	0	0				
9	Cefotaxime (30µg)	0	0				

(1): Methanol extract of *Moringa oleifera* leaves (1%), (2): Antibiotics alone, (3): Antibiotics + MMLFig. (3): The interaction between MML and β -lactam antibiotics against *Staphylococcus sp.* *Pseudomonas sp.*

In case of *Staphylococcus sp.*, the antagonistic interaction was only observed with imipenem, which decreased its activity from 26 to 20mm. Data recorded in Table (1_{a&b}) showed that the synergistic interaction was only expressed against *Acinetobacter* by combining of MML with most tested β -lactam antibiotics, which increased the inhibition zone of piperacillin, meropenem, cefixime, piperacillin, ceftazidime, cefepime, cefotaxime, cefop/sulb or cefoperazone from 22, 20, 23, 23, 20, 20, 20, 16 and 29 to 29, 35, 25, 29, 35, 35, 34, 27, 20 and 35mm, respectively (Table, 1_{a,b} & Figs. 2,3).

In summary, MML could change the antibacterial activity of tested β -lactam antibiotics against clinical bacterial strains based on the type of both tested bacteria and the type of antibiotic used.

DISCUSSION

Previous studies focused only on the antimicrobial activity of *Moringa oleifera* extract against various pathogenic microorganisms [8,12,13,14, 15]. This is the first report concerning the interactions of combination between MML/ β -lactam antibiotics against some common pathogenic bacteria.

MML was found to have no inhibitory effect on various tested strains in the present study and this may be due that the antimicrobial of MML needs to increase its concentration above used. This result was corroborated by [23], who found that the antimicrobial activity of MML against different microorganisms was only observed at concentration over 2.5%. While, [21] noticed that there was no antimicrobial activity of MML against different Gram negative and positive bacteria. On the other hand, [22] reported that MML gave the highest antibacterial activity against various Gram negative strains, compared to other solvent extracts. [23] found that the antibacterial activity of MML against *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Shigella Flexneri* was observed at concentration of 0.6%.

Obtained results showed that the antimicrobial activity of MML is not essential to make an interaction with β -lactam antibiotics against target strain. In addition, different interactions of the same MML/ β -lactam antibiotic combination were detected against tested bacteria, for instance the combination of MML/imipenem, cefepime, ceftazidime or piperacillin gave indifferent interaction against *Klebsiella* sp., antagonistic interaction against *E. coli* & *Pseudomonas* sp. and synergistic interaction against *Acinetobacter* sp. These results may be due to that the MML contained a wide

variety of constitutions working by different mechanisms against tested strains and each individual interaction between MML and β -lactam antibiotics was influenced by some constitutions, which could change the activity of tested antibiotic depending on the sensitivity of tested bacteria to MML contents.

Previous studies revealed that the phytochemical analysis of MML contained a wide variety of tannins, saponins, alkaloids and phenols, which may be responsible for the interactions with antibiotics [14,23]. The interaction between MML and β -lactam antibiotics was only investigated by [24]. They found that the combination of MML/amoxicillin gave different interactions against tested strains; it had indifferent interaction against *Klebsiella pneumonia*, antagonistic interactions against *E. coli*, *Bacillus subtilis* & *Proteus vulgaris* and synergetic interaction against *Staphylococcus aureus*.

Combination between antibiotic and medicinal plant extracts became a useful tool in fighting emerging drug-resistance microorganisms but we must be approached with care since the combination may increase the antagonistic rather than synergistic. Recently, the health benefits of *Moringa oleifera* leaves as a dietary supplement were investigated by many researchers [8-11]. While, the antagonist properties of MML/ β -lactam combinations against some Gram negative bacteria in the current study revealed that the using of *Moringa* leaves as dietary supplement needs some precautions, because it may decrease the effectiveness of antibiotics against disease causing bacteria. On the other hand, the antagonist properties may make some protections to intestine flora in human and animal bodies.

CONCLUSION

Current study has successfully shown that the interactions of MML/ β -lactam combinations against some pathogenic bacteria. The synergetic interactions of MML with some β -lactam antibiotics against *Acinetobacter* sp. supported the importance of MML as a promising source of phytochemicals, which could increase the effectiveness of many traditional antibiotics against resistant *Acinetobacter* sp.. Further studies would be required to isolate the responsible phytochemicals for the synergistic interaction with β -lactam antibiotics and using them against Multi- drug resistant *Acinetobacter* sp..

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