https://dio: 10.21608/ bfszu .2021.59167.1061 BFSZU 4(2021) 1-11

ASRT

Bulletin of Faculty of Science - Zagazig University
https://bfszu.journals.ekb.eg

FS-ZU

Studies on the inhibitory activity of some Egyptian natural plant extracts against multidrug resistant bacteria

Mohamed T. Shaaban[1], Mohamed F. Ghaly[2], Sara M. Fahmi[1]*
[1]Department of Botany and Microbiology, Faculty of Science, Menoufia University, Menoufia, Egypt,
[2]Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, 44519 Egypt,

ARTICLE HISTORY
Received: January 2021
Revised: March 2021
Accepted: April 2021

KEY WORDS
Antibacterial activity,
Natural plant extracts,
Multidrug resistant bacteria

Abstract

The emergence of types of bacteria resistant to antibiotics is a common and most dangerous problem in the current era. In this study 50 samples from diabetic patients including diabetic foot (n = 36), wounds (n = 9), and bedsores (n = 5) were isolated and grew well on (nutrient agar, blood agar, and MacConkey gar) media. Ten patient's specimens (20%) had no bacterial growth. The sensitivity of 40 bacterial isolates to 7 antibiotics was performed. A microscopic examination of 40 bacterial isolates by Gram stained film was performed. Morphological and biochemical identifications of selected MDR bacteria were evaluated. The four MDR pathogenic bacteria are named *S. aureus* W, *P. aeruginosa* DF, *K. pneumonia* D, and *K. pneumonia* BS. Determination of MIC and MBC of the 3 strongest antibiotics against 4 multidrug resistant pathogenic bacteria. Antibacterial activities of six types of natural plant extracts were studied against tested pathogenic bacteria. Clove, garlic, and cinnamon boiling water extract showed inhibitory activity against MDR bacteria.

***Correspondence: rody.sheref_2013@yahoo.com, 002 01021614802.**

1. INTRODUCTION

The spreading of microorganisms resistant to antimicrobial agents in current years makes it necessary to lock for alternative antimicrobial drugs. Resistant bacteria are known that are not affected by antibacterial drugs as a result of exposure to the antibiotic for a long time due to the changing of bacterial membranes, or due to genetic modifications **(Cloete, 2003)**. It is necessary to isolate, purify, and identify the MDR strains for studying its antibiotic resistance properties and searching for effective antimicrobial therapy. **(Harvey and Champe, 2012)**. Antibiotic is antimicrobial agent has the strength to inhibit or kill the growth of microorganism while doesn't cause harm to the human cell. It is important to examine the susceptibility of the microorganism to the antibiotic to select the most effective one for the treatment. A few methods used for evaluating antibiotics include the filter paper disc (Kirby-Bauer) method **(Bauer *et al.*, 1966)**, agar and broth dilution method,

and the dilution method **(Owuama, 2015)**. Minimum inhibitory concentration (MIC) is defined as the lowest concentration of antimicrobial therapy that inhibits microbial growth, as well as Minimum bactericidal concentration (MBC) known as the lowest concentration of antimicrobial therapy required to kill microorganism **(Andrews, 2001)**. Methods that have been used in the determination of MIC and MBC are Macro-dilution and microdilution methods **(Lambert and Pearson, 2000; Eucast, 2003)**. Usually, the dilution method (DM) the way for the determination of MIC and MBC **(CLSI, 2012)**.

Medicinal plants that rich in many active compounds like simple phenols, flavonoids, and alkaloids it was found that they have high inhibitory activity against pathogenic microorganisms and can be used as an alternate therapy for microbial infections **(Robert *et al.*, 2011; Leon *et al.*, 2001)**. The existence of the hydroxyl group of phenolic compounds makes these compounds effective as antimicrobial agents; these groups can inhibit the enzyme action of the microorganism. In Egypt, many plants are used today in folk medicine and are sold at herbal vendors and shops **(Abdel-Azim *et al.*, 2011)**. Many medicinal herbs were used by the ancient Egyptians were to treat various diseases. They applied their medicaments in many forms such as powder, pill, and cream form **(Bogers *et al.*, 2006)**. The present study was undertaken to (i) isolate, collect, and identify multidrug resistant bacteria from Egyptian diabetic patients. (ii) Determination of MIC and MBC of the three strongest antibiotics as follows, imipenem, norfloxacin, and nitrofurantoin. (iii) Evaluate the antibacterial activity of some natural medicinal plant extracts.

2. MATERIALS AND METHODES

Isolated bacteria were assembled from 50 patients who suffered from diabetes mellitus. From Zagazig University Hospitals.

The samples grew well on Nutrient agar and Blood agar according to **(Collee and Marr, 1989),** MacConkey agar according to **(Koneman *et al.*, 1997)**.

2.1. Antibiotic susceptibility test: The susceptibility of 40 bacterial isolates to 7 antibiotics was tested by standard disc diffusion technique according to **(CLSI 2012)**. The following antibiotic discs were used: imipenem (IPM 10 µg), neomycin (30 µg), cefoxitin (30 µg) nitrofurantoin (300 µg), cefepime (30 µg), and amoxicillin/clavulanic acid (30 µg) norfloxacin (10 µg).

2.2. Microscopic examination of bacterial isolates by (Gram stained film): Preparation of the bacterial smear according to **(Collee *et al.*, 1996).**

Gram stain by crystal violet solution, gram's iodine solution, decolorizing solution and safranin counterstain according to **(Rita *et al.*, 2017).** Using oil immersion lens according to **(Benson, 1998).**

2.3. Identification of bacterial isolates: The biochemical identification of MDR pathogenic bacteria was performed according to **(Trujillo and Goodfellow, 2012).**

2.4. Determination of the minimum inhibitory concentration (MIC) of the tested antibiotics: MIC of the tested antibiotics was done by preparation of stock solutions, that were prepared from the selected antibiotics (imipenem, norfloxacin, and nitrofurantoin,). According to (**Lorian, 2005)**.

A suitable dilution range was selected before preparing stock solutions. Doubling dilutions were chosen. The concentrations chosen were: 200 mg/ml, 100 mg\ml, 50 mg\ml, 5 mg\ml, 500 µg\ml, 250 µg\ml, 125 µg\ml, 12.5 µg\ml, 1.25 µg\ml, 0.125 µg\ml, 0.0125 µg\ml - Broth cultures were prepared from each of the 4 most

resistant isolates (isolates number 30, 31, 35, and 40). Tubes containing the broth were then set to 0.5 Mc Farland [0.05 ml 1% barium chloride, and 9.95 ml 1% sulfuric acid; this equals a cell density of 1 X 10^8 CFU\ml, **(Farland, 1987).**

Tubes were prepared for each strain of bacteria, each tube consists of one ml of the antibiotic's concentration just before the required one and one ml of the diluted broth of the tested isolate, this step is repeated for each strain and each one of the selected antibiotics. Tubes were incubated at 37ºC for 24 hours. After that, the tubes were noted for turbidity.

2.5. Measurement of the minimum bactericidal concentration (MBC) of the tested antibiotics: The tubes identical to the MIC concentration and the concentrations greater than it in which no turbidity had placed were centrifuged; all organisms were precipitated to the bottom of the tubes. After centrifugation the supernatant consisting of the antibiotic was poured off. Broth without antibiotic was added to the tubes and the organisms taken from the tubes were cultured on nutrient agar and incubated at 37ºC for 24 hours. The first concentration at which no growth had occurred was MBC **(Yamamoto, 2003)**.

2.6. Preparation of plant extracts: Some natural agents such as clove (*Syzygium aromaticum)*, garlic (*Alium sativum)*, cinnamon (*Cinnamomum zylanicum)*, moringa seeds, moringa green leaves, and moringa dry leaves (*Moringa oleifera)* extracts were studied for their inhibitory activity against MDR bacteria. The plant samples collected were put to dry in the oven at 45 ºC, then pulverized. The powders were kept in closed plastic containers ready for investigation. Seeds and fresh leaves were powdered just before the examination. The dried powdered plant material 20 gm of every plant was extracted with 50 ml of distilled boiled water for 10 minutes and cooled (**Izzo *et al.*, 1995**). The mixture was boiled in water bath for 15 min then left at room temperature. All extracts were sterilized by filtration; the filtrate was kept at 4ºC in the refrigerator till use according to **Egyptian Pharma Copoeia, (1984**).

3. RESULTS

3.1. Distribution of bacterial isolates among patients: About 50 bacterial isolates were collected from diabetic patients in Zagazig University Hospitals. Firstly, staining reactions and cultural characteristics of the clinical isolates on simple, enriched and selective media were carried out. Fifty samples were analyzed for the presence of pathogenic bacteria on nutrient agar, blood agar and MacConkey agar media. Out of 50 isolates, forty showed bacterial growth, while, ten showed no growth **Table (1).**

3.2. Antibiotics susceptibility of clinical bacterial isolates: The clinical bacterial isolates were tested for their susceptibility to antibiotic discs using disc diffusion method. The antibiotics, which used in this study, were as following imipenem IPM (10µg), amoxicillin clavulanic acid AMC (30µg), norfloxacin NOR (10µg), cefoxitin FOX (30µg), neomycin N (30µg), nitrofurantoin F (300µg) and cefepime FEP (30µg). The isolate No. 30, 31, 35, and 40 were resistant to all antibiotic groups **(Table 2).** The most effective antibiotic against the isolated clinical bacteria was imipenem, followed by norfloxacin and nitrofurantoin, and finally that the more resistant bacterial isolates were detected by cefepime.

3.3. Morphological characteristics and biochemical identification of bacterial isolates: The selected MDR bacteria were identified chemically

(sugar fermentation, catalase test, coagulase test, hemolysis test, oxidase test, citrate test, H_2S tes,t and in dole test), morphologically and physiologically according to identification protocols as *P. aeruginosa* DF, *Klebsiella pneumonia* D, *Klebsiella pneumonia* BS and *S. aureus* W **(Table 3).**

3.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to three strongest antibiotics for selected bacterial isolates: The most four multidrug resistant bacteria were cultivated with different concentrations of imipenem, norfloxacin, and nitrofurantoin antibiotics in nutrient broth. The results from **Table (4,5)** showed the minimum inhibitory concentrations (MIC) of the tested antibiotics and the minimum bactericidal concentrations (MBC).

As shown in **Table (4).** Imipenem had the highest MIC (5mg/ml) for *P. aeruginosa* followed by MIC (250 µg/ml) for *K. pneumoniae* 1 and. Then MIC (12.5µg/ml) for *K. pneumoniae* 2. Finally, the lowest MIC (1.25µg/ml) for *S. aureus*. Nitrofurantoin had the highest MIC (100mg/ml) for *P. aeruginosa*, *K. pneumoniae* 1 and *S. aureus* Then the lowest MIC (500µg/ml) for *K. pneumoniae* 2. Norfloxacin had MIC (5mg/ml) for *P. aeruginosa, K. pneumoniae* 1, *K. pneumoniae* 2 and *S. aureus*.

The results from **Table (5)** showed that imipenem had the highest MBC (100mg/ml) for *S. aureus, K. pneumoniae* 2 *and P. aeruginosa*. Then the lowest MBC (5mg/ml) for, *K. pneumonia* 1. Nitrofurantoin had the highest MBC (200mg/ml) for *S. aureus*. Then, lowest MBC (5mg/ml) for *K. pneumoniae* 2, and not detected for *K. pneumoniae* 1 *and P aeruginosa* at concentration (400mg/ml). Norfloxacin had the highest MBC (100mg/ml) for *S. aureus*. Then the lowest MBC (50mg/ml) for *K. pneumoniae* 2, and not detected for *K. pneumoniae* 1 *and P. aeruginosa* at the concentration (400mg/ml).

3.5. Antibacterial activities of some plants boiling water extract on MDR bacteria: The inhibitory effect of plant extracts against MDR bacteria is given in **(Table 6).** Cinnamon boiling water extract showed the higher inhibitory against all tested bacteria IZDs reached (8–13) mm followed by clove and garlic, while the inhibitory activity of moringa dry leaves, moringa seeds and moringa green leaves extracts were had no effect against the tested pathogenic bacteria.

Table (1): The Distribution of most clinical bacterial isolates among studied group of patients.

No of patients	Age	Gender	Source of sample	Fasting Glucose level	Medication
1	42	Female	Diabetic foot	200	Insulin
2	54	Male	Diabetic foot	180	Insulin
3	73	Male	Diabetic foot	314	Tablet
4	57	Female	Bed sores	250	Insulin
5	50	Female	Diabetic foot	150	Insulin
6	67	Female	Diabetic foot	230	Insulin
7	80	Female	Wound	250	Insulin
8	60	Female	Diabetic foot	270	Insulin
9	48	Female	Wound	200	Insulin
10	55	Male	Wound	185	Insulin
11	63	Female	Diabetic foot	300	Insulin
12	60	Female	Diabetic foot	210	Insulin
13	53	Female	Diabetic foot	130	Tablet
14	46	Male	Diabetic foot	170	Tablet
15	68	Male	Diabetic foot	170	Insulin
16	60	Male	Diabetic foot	500	Insulin
17	61	Male	Wound	223	Insulin
18	71	Male	Bed sores	300	Insulin
19	67	Male	Diabetic foot	230	Insulin
20	60	Female	Wound	200	Insulin
21	65	Female	Diabetic foot	180	Insulin
22	58	Female	Diabetic foot	274	Insulin
23	52	Male	Diabetic foot	217	Insulin
24	81	Male	Diabetic foot	416	Insulin
25	74	Male	Bed sores	267	Insulin
26	66	Female	Diabetic foot	360	Insulin
27	48	Male	Diabetic foot	270	Insulin
28	74	Male	Diabetic foot	215	Insulin
29	62	Male	Diabetic foot	220	Insulin
30	58	Male	Diabetic foot	580	Insulin
31	58	Female	Diabetic foot	400	Insulin
32	60	Female	Bed sores	300	Insulin
33	73	Male	Wound	260	Insulin
34	60	Male	Diabetic foot	350	Insulin
35	56	Male	Diabetic foot	300	Insulin
36	57	Male	Wound	120	Tablet
37	72	Male	Diabetic foot	190	Insulin
38	70	Male	Diabetic foot	180	Tablet
39	60	Female	Diabetic foot	376	Insulin
40	58	Female	Bed sores	280	Insulin
41	75	Female	Wound	200	Insulin
42	66	Female	Diabetic foot	250	Insulin
43	60	Female	Diabetic foot	150	Tablet
44	54	Female	Diabetic foot	270	Insulin
45	58	Female	Diabetic foot	350	Insulin
46	62	Male	Diabetic foot	200	Insulin
47	50	Male	Diabetic foot	160	Tablet
48	44	Male	Wound	140	Tablet
49	62	Male	Diabetic foot	220	Insulin
50	66	Male	Diabetic foot	200	Insulin

Table (2): Antibiotics susceptibility test against bacterial isolates.

No of bacterial isolates	Antibiotics with IZDs mm							R	I	S
	IPM 10	AMC 30	NOR 10	FOX 30	N30	F300	FEP 30			
1	30 S	28 S	22 S	17 R	18 S	18 S	8 R	2	-	5
2	24 S	35 S	R	R	12 R	22 S	13 R	3	-	4
3	17 S	R	17 S	R	R	12 R	R	5	-	2
4	15 I	R	17 S	R	R	11 R	R	5	1	1
5	24 S	R	R	R	R	R	R	6	-	1
6	25 S	R	16 I	18 R	8 R	R	R	5	1	1
7	18 S	R	14 I	11 R	10 R	R	R	5	1	1
8	23 S	21 S	R	R	R	21S	R	4	-	3
9	25 S	R	17 S	14 R	18 S	R	R	4	-	3
10	27 S	28 S	18 S	R	15 I	23 S	R	2	1	4
11	33 S	15 R	21 S	12 R	9 R	17 S	R	4	-	3
12	33 S	26 S	19 S	26 S	20 S	12 R	14 R	2	-	5
13	21 S	15 R	R	R	R	17 S	R	5	--	2
14	28 S	10 R	R	16 R	15 I	18 S	R	4	1	2
15	25 S	10 R	R	14 R	16 I	17 S	R	4	1	2
16	35 S	20 S	15 I	R	R	R	R	4	1	2
17	R	14 R	23 S	R	R	22 S	R	5	-	2
18	27 S	25 S	R	R	R	17 S	R	4	-	3
19	R	R	18 S	R	R	R	R	6	-	1
20	24 S	R	R	R	R	R	R	6	-	1
21	30 S	15 R	R	R	13 R	R	R	6	-	1
22	27 S	R	R	R	R	R	R	6	-	1
23	35 S	32 S	26 S	24 S	R	20 S	18 R	2	-	5
24	17 S	R	R	R	R	R	R	6	-	1
25	34 S	R	31 S	R	18 I	R	R	4	1	2
26	30 S	27 S	24 S	22 S	16 I	24 S	18 R	1	1	5
27	18 S	R	R	R	18 S	14 R	R	5	-	2
28	35 S	24 S	R	R	R	20 S	R	4	-	3
29	30 S	13 R	13 I	R	R	R	R	5	1	1
30	R	R	R	R	R	R	R	7	-	-
31	R	R	R	R	R	R	R	7	-	-
32	28 S	R	R	R	R	R	R	6	-	1
33	20 S	R	18 S	R	16 I	R	16 R	5	1	1
34	12 R	R	17 S	R	R	R	R	6	-	1
35	R	R	R	R	R	R	R	7	-	-
36	16 S	R	18 S	R	18 S	15 R	R	4	-	3
37	26 S	R	R	24 S	R	R	R	5	-	2
38	18 S	R	16(I)	R	R	R	R	5	1	1
39	30 S	22 S	22 S	20 S	11 R	16 I	14 R	2	1	4
40	12 R	R	R	R	R	R	R	7	-	-
S	32	11	16	5	5	12	-			
I	1	-	5	-	6	1	-			
R	7	29	19	35	29	26	40			

IPM= Imipenem**, AMC=** Amoxicillin clavulanic acid**, NOR=** Norfloxacin**, FOX=** Cefoxitin**, N=**Neomycin**, F=** Nitrofurantoin**, FEP=** Cefepime**, IZD=** Inhibition zoon diameter**, R=** Resistant**, I=** Intermediate**, S=** Sensitive

Table (3): Morphological characteristics and biochemical identification of bacterial isolates.

Test	*P. aeruginosa DF*	*K. pneumonia D*	*K. pneumonia BS*	*S. aureus W*
Gram stain	- ve	- ve	- ve	+ ve
Motility	+ ve	- ve	- ve	- ve
Growth on MacConky	+ ve	+ ve	+ ve	ND
Shape of cell	**Rod shape**	**Rod shape**	**Rod shape**	**Cocci shape**
Sugar fermentation reactions				
D-Glucose	+ ve	+AG	+AG	ND
Lactose	- ve	+ ve	+ ve	ND
Sucrose	- ve	+ ve	+ ve	ND
Maltose	- ve	+ ve	+ ve	ND
D-sorbitol	- ve	+ ve	+ ve	ND
D-Mannitol	+ ve	+ ve	+ ve	+ ve
D-Mannose	- ve	+ ve	+ ve	ND
Specific biochemical reactions				
B-hemolysis	ND	ND	ND	+ ve
Catalase	+ ve	+ ve	+ ve	+ ve
Coagulase	ND	ND	ND	+ ve
Oxidase	+ ve	- ve	- ve	- ve
Indole	- ve	- ve	- ve	- ve
Citrate	- ve	+ ve	+ ve	- ve
H_2S	- ve	- ve	- ve	- ve
Urease	+ ve	+ ve	+ ve	+ ve

Table (4): Determination MIC to three antibiotics for selected bacterial isolates.

Bacterial isolates	Antibiotic	Different concentrations of antibiotic and IZD (mm)								
		100 mg\l	**50 mg\l**	**5 mg\l**	**500 µg\l**	**250 µg\l**	**125 µg\l**	**12.5 µg\l**	**1.25 µg\l**	**0.125 µg\l**
P. aeruginosa DF	**IPM**	16±4.54	13±3.67	7±2.64	0	0	0	0	0	0
K. pneumonia D		23±2.54	21±1.00	16±2.00	9±2.64	7±0.64	0	0	0	0
K. pneumonia BS		26±2.64	25±0.74	20±2.43	15±1.00	13±2.64	11±3.54	9±2.00	0	0
S. aureus W		18±1.34	16±2.64	15±3.54	14±1.23	14±2.64	13±1.00	13±1.45	11±2.64	0
P. aeruginosa DF	**NOR**	25±2.00	20±3.76	13 ±1.67	0	0	0	0	0	0
K. pneumonia D		18±1.54	16±1.64	11±2.64	0	0	0	0	0	0
K. pneumonia BS		20±4.73	16±0.64	11±4.64	0	0	0	0	0	0
S. aureus W		16±2.64	15±1.66	13±0.84	0	0	0	0	0	0
P. aeruginosa DF	**F**	11±3.73	0	0	0	0	0	0	0	0
K. pneumonia D		17±1.64	0	0	0	0	0	0	0	0
K. pneumonia BS		19±4.32	18±1.64	16±1.00	18±2.64	0	0	0	0	0
S. aureus W		16±2.64	0	0	0	0	0	0	0	0

Table (5): MBC to three antibiotics for selected bacterial isolates.

Bacterial isolates	Antibiotic	MBC
***K. pneumonia* D**	IPM	5 mg\ml
	NOR	ND
	F	ND
***P. aeruginosa* DF**	IPM	100 mg\ml
	NOR	ND
	F	ND
***S. aureus* W**	IPM	100 mg\ml
	NOR	200 mg\ml
	F	100 mg\ml
***K. pneumonia* BS**	IPM	100 mg\l
	NOR	5 mg\l
	F	5 mg\ml

Table (6): Antibacterial activities of boiling water plant extracts on bacterial isolates.

Plant extracts	IZD (mm)			
	P. aeruginosa	*K. pneumonia* 1	*K. pneumonia* 2	*S. aureus*
Clove	8±2.51	0	0	10±2.64
Garlic	0	10±0.47	0	7±0.64
Cinnamon	8±1.00	10±1.00	13±1.00	12±1.00
Moringa green leaves	0	0	0	0
Moringa dry leaves	0	0	0	0
Moringa seeds	0	0	0	0
Ginger	0	0	0	0
Aloe vera	0	0	0	0

4. DISCUSSION

In clinical practice worldwide, infectious diseases related to antibiotic resistance is a major threat to human health. Antibiotic resistance can affect anyone, of any age **(Khalifa *et al.,* 2019)**. So, there is a need for the discover of safe and nontoxic natural products with the antimicrobial capacity to be utilized as alternative medication in general health. Out of 50 bacterial isolates, 4 only were resistant to all antibiotic groups this supported later work in this respect **(Abdel-Shafi, 2013)**. Selected multidrug resistant bacteria of this study were identified herein as belonging to *S. aureus, P. aeruginosa, K. pneumonia*, and later published work showed the prevalence of similar drug resistant bacteria **(Abeer *et al.,* 2020).**

Antibiotic susceptibility testing is the measurement of the susceptibility of bacteria to the antibiotic. It is used due to the resistance of bacteria to some antibiotics. **(Leekha *et al.,* 2011).**

From our results, the most effective antibiotic against the isolated clinical bacteria was imipenem, this agreement with the study of **Helmut *et al.,* (1985)** they showed that the activity of imipenem is high against *S. aureus*, streptococci, *Bacteroides, P. aeruginosa* and *S. epidermidis*. Imipenem was previously reported to be the most effective and similar effectiveness of these antibiotic were also reported (**Abeer *et al.,* 2020).**

Many natural plant extracts used as flavoring and seasoning agents in food have been used therapeutically for centuries **(Vinoth *et al.,* 2011).** Antibacterial activities of cinnamon, onion garlic, and cloves and their active compounds have been studied since the end of the last century **(Jagadeesh *et al.,* 2011).** Cinnamon contains mainly cinnamaldehyde (50.5%), cinnamyl acetate (8.7%), eugenol (4.7%), methoxy cinnamaldehyde (MOCA) and cinnamic acid **(Charu *et al.,* 2008)**. Allicin is the active compound present in garlic, which is a thiosuffinate compound reported for its highest antimicrobial activity **(Chung *et al.,* 2007).** Clove is characterized by the presence of eugenol and other phenolic compounds that Characterized by their antimicrobial activity **(Mittal *et al.,* 2014).**

In our study, six plant extracts were tested for their inhibitory activities against MDR pathogenic bacteria. Our results showed that the boiling water plant extracts of cinnamon, clove and garlic, had antibacterial activity against MDR bacteria, while, moringa green leaves, moringa dry leaves and moringa seeds had no antibacterial activity. Our results agree with **Avijit *et al.,* (2018)**, they showed that the ethanol extract of garlic, clove and cinnamon was highly active against *S. aureus, Shigella spp., B. cereus Serratia spp., E. coli, S. typhi and Acine-tobacter spp.* and *Klebsiella spp.* Furthe-rmore, **Maha *et al.,* (2012)**, demonstrated that alcoholic extract of clove, cinnamon and thyme showed antibacterial activity against

S. aureus, B. subtilis, E. coli, P. aeruginosa, S. typhi, C. albicans and *A. niger.*

5. CONCLUSIONS

According to the obtained results, it can be concluded that cinnamon could be used as an antibacterial agent. It can be efficiently and successfully used as a safe, natural product. It can be prepared with a low cost.

6. REFERENCES

Abdel-Azim, N.S.; Shams, K.A.; Shahat, A.A.; El Missiry, M.M.; Ismail, S.I.; Hammouda, F.M. (2011): Egyptian herbal drug industry: challenges and future prospects. Res J Med Plant, 5: 136–144.

Abdel-Shafi, S.; Osman, A.; Enan, G.; El-Nemer, M.; Sitohy, M. (2016): Antibacterial activity of methylated egg white proteins against pathogenic G+ and G− bacteria matching antibiotics. Springer Plus, 5 (1) 983.

Abdel-Shafi, S.; Ouda, S.M.; Elbalat, I.; Enan, G. (2013): Characterization and Identification of Multidrug Resistant Bacteria from Some Egyptian Patients. Biotechnology; 12:65-73.

Abeer, F.; Mohamed, F.; and Ahmed, S. (2020): Identification of multidrug resistant bacterial isolates from Egyptian hospitals environments and molecular detection of their resistance genes. Bioscience research.17 (3): 2015-2024.

Andrews, J.M. (2001): Determination of minimum inhibitory concentrations. Antimicrob. Chemother. 48(6):5-16.

Avijit, B.; Maruf, A.; Tamanna, Z.; Suvamoy, D. (2018): Antibacterial Activity of Allium Sativum, Syzygium Aromaticum, and Cinnamomum Zeylanicum against Food Borne Pathogens in Vitro, Journal of Pharmacy and Biological Sciences, 13: 68-73.

Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; and Turk, M. (1966): Antibiotic susceptibility testing by a standerized single disk method. Am. J. of clin. Pathol, (45): 493-496.

Benson, H.J. (1998): Microscope Slide Techniques (Bacterial Morphology). In: Microbiological Applications Laboratory Manual in Ge010neral Microbiology. 7th ed. Boston, California St. Louis Missouri, 49-70.

Bogers, R.J.; Craker, L.E.; Lange, D. (2006): Medicinal and aromatic plants: agricultural, commercial, ecological, legal, pharmacological and social aspects. Berlin, Heidelberg: Springer, 17: 1573-4544.

Charu, G.; Amar, P.; Ramesh, C.; and Archana, K. (2008): Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. African Journal of Microbiology Research, 2(9), 247-251.

Chung, I.; Kwon, S.H.; Shim, S.-T.; And Kyung, K.H. (2007): Journal of food science, 72, M434-M440.

Cloete, T.E.; (2003): Resistance mechanisms of bacteria to antimicrobial compounds. Int. Biodeterant. Biodegradat, 51: 277-282.

CLSI, Clinical and Laboratory Standards Institute (2012): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Collee, J.G.; and Marr, W. (1989): Culture containers and culture media. In "Practical Medical Microbiology", 13th ed. Vol. (2). Collee, J. G; Duguid, J. P; Fraser, A. G; and Marmion, B. P. (eds), Churchill Livingstone. New York. 100-120.

Collee, J.G.; Marr, W.; Fraser, A.G.; Marmion, B.P.; and Simmons, A. (1996): Specimen collection, culture containers and media.: Mackie and Mac Carthey Practical medical microbiology. Churchill Livingstone, New York, London, Tokyo. 95-111.

Egyptain Pharma Copoeia (1984): The Pharmaceutical Press, Vol. I. El Atki, Y.; Aouam, I.; El Kamari, F.; Taroq, A.; Nayme, K.; Timinouni, M.; Lyoussi, B.; and Abdellaoui, A. (2019): Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J Adv Pharm Technol Res. 10(2):63-67.

El Atki, Y.; Aouam, I.; El Kamari, F.; Taroq, A.; Nayme, K.; Timinouni, M.; Lyoussi, B.; and Abdellaoui, A. (2019): Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J Adv Pharm Technol Res, 10(2):63-67.

Eucast (2003): Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. Clin. Microbiol. Infect. 6(9):509-515.

Farland, M.C. (1987): Standardization of bacterial culture for disc diffusion assay. Journal of American Medical Association, 49: 1176-1178.

Harvey, R.A.; and Champe, D.C. (2012): Lippincott's Illustrated Reviews in Microbiology. Lippincott Williams and Wilkins, USA, 19-22.

Helmut, K.; Lynn, G.; Jon, G.; Frederick, M. (1985): Antibacterial Activity of Imipenem: The First Thienamycin Antibiotic. Reviews of Infectious Diseases, 7(3): 389–410.

Izzo, A. A.; Di Garlo, G. Biscardi, D. De Fusco, R. Mascolo, N. Borrell, F.; Capasso, F.; Fasulo, M. P. and Autore, G. (1995): "Biological screening of Italian medicinal plants for antibacterial activity". Phytotherapy. Research. 9, 281-286.

Jagadeesh, A.; Rupa, A.; Indumathi, J.; Srujan, R.; and Sravanthi, M. (2011): Research. 5. Study on the antimicrobial activity and minimum inhibitory concentration of essential oils of spices. Veterinary World, 4(7), 311-316.

Khalifa, H.O.; Soliman, A.M.; Ahmed, A.M.; et al. (2019): High Prevalence of Antimicrobial Resistance in Gram-Negative Bacteria Isolated from Clinical Settings in Egypt: Recalling for Judicious Use of Conventional Antimicrobials in Developing Nations. Microb Drug Resist, 25:371-385.

Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C.; and Winn, W.C. (1997): Introduction to microbiology part I: The role of the microbiology laboratory in the diagnosis of infection diseases. Guidelines to practice and management in "Color atlas and text book of diagnosis microbiology". Fifth edition. Philadelphia**.** 69-120.

Lambert, R.J.; Pearson, J. (2000): Susceptibility testing: Accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. J. Appl. Microbiol, 88(5):784-790.

Leekha, S.; Terrell, C.L.; Edson, R.S. (2011): General principles of antimicrobial therapy". Mayo Clinic Proceedings, 86 (2): 156–67.

Leon, J.; Rojo, E.; and Sanchezerrano, J. (2001): Wound signaling in plants. Journal of Experimental Botany, 52: 1-9.

Lorian, V. (2005): Antibiotics in laboratory medicine. Books in laboratory medicine. In: Lorian, V. editions (5th ed.), Published by Lippincott Williams & Wilkins; P: 1-6.

Maha, M.; Ismail, T.M.; Essam, A.F.M; and Fathia, E.M. (2012): Screening for the Antimicrobial Activities of Alcoholic and Aqueous Extracts of Some Common Spices in Egypt. International Journal of Microbiological Research 3 (3): 200-207.

Mittal, M.; Gupta, N.; Parashar, P.; Mehra, P.; Khatri, M (2014): Phytochemical evaluation and pharmacological activity of *syzygium aromaticum*. Int J Pharm Sci, (6): 67-72.

Owuama, C.I. (2015): Microbiology Laboratory manual. Microtrend Digital Press, Yola. ISBN 978-978-943-328-2.

Rita, B.; Jackie, R.; Donald, P. (2009): Differential Staining of Bacteria: Gram Stain. Current protocols in microbiology, 15: 1-8.

Robert, C. M. (2011): Discovering new antimicrobial agents. International Journal of Antimicrobial Agents, 37: 2-9.

Trujillo, M. E.; Goodfellow, M. (2012): Genus I. Brevibacterium Breed 1953a. 13AL emend. Colins, Jones, Keddie and Sneath 1980. In Bergeys Manual of Systematic Bacteriology, 2nd edn.vol. 5: The Actinobacteria, 685–700 .

Vinoth, K.; Amala, B.; Veera, V.; Sudheer, C.; and Ramesh, N. (2010): Biopotency of Allium sativum. Linn (Garlic) in Lead Acetate Intoxicated Male Albino Rats. Der Pharmacia Sinica, 1 (2): 1-4.

Yamamoto, L.G. (2003): Inhibitory and bactericidal principles MIC&MB. Journal of biological sciences. (4): 1-8.