

Efficiency of Egyptian Plant Extracts on the Antibiotic Susceptible and Resistant Pathogenic bacterial Isolates

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SUCCESSIVE leaf extracts of three Egyptian plant species: *Datura stramonium* L., *Withania somnifera* (L.) Dun. in DC. and *Ziziphus spina-christi* (L.) Willd were studied for their antimicrobial activity against susceptible and Multi-Drug Resistant (MDR) bacteria isolated from chickens. Four bacterial spp. namely: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp. were isolated from 463 different chicken samples. The sensitivity of these bacterial isolates was tested against 11 types of antibiotics: Ampicillin, Amoxicillin, Cefaclor, Gentamicin, Amikacin, Tobramycin, Ciprofloxacin, Nalidixic acid, Erythromycin, Tetracycline and Vancomycin. The isolates of *E. coli*, *S. aureus*, *P. aeruginosa* and *Salmonella* sp. showed notable bacterial resistance against the used antibiotics with percentages: (68.18%), (59.09%), (54.54%) and (54.54%); respectively. Chloroform extract of *Datura stramonium* showed the highest antibacterial activity against all the studied bacterial isolates with MICs values varying from 75.0 to 0.037 mg/ml. In conclusion, the present work indicated the potentiality of *Datura stramonium* chloroform extract as a promising national source for drug industry to produce antibiotics to be used against pathogenic multidrug resistant bacteria.

Keywords: *Datura stramonium*, Plant extract, Resistant-bacteria, Egyptian species.

Introduction

For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002 and Loeraa et al., 2007).

The pathogens resistant to multiple antibiotics are considered as multi-drug resistant pathogens (Davies, 1997). The multi-drug resistant bacteria cause financial and economic implications, treatment failure and rapid invasion of these pathogenic bacteria to new persons. In fact, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Although, new generations of antibiotics were produced by pharmacological companies, the drug resistance has been increased (Tenover, 2006 and Chovanová et

al., 2013). This situation has forced the scientists attention towards natural and herbal products to develop better quality drugs with improved antibacterial activities (Bako et al., 2005; Sibanda & Okoh, 2007; Bocanegra-Garcia et al., 2009 and Saranraj, 2014).

Djeussi et al. (2013), provided useful baseline information for the potential use of some edible plants in the fight against both sensitive and multidrug-resistant (MDR) phenotypes. Some of the most important types of multiple drug resistant gram positive bacteria that have been encountered are: *Staphylococcus aureus* and *Streptococcus pneumoniae*. Also as serious gram negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Several authors among them: Iwu et al. (1999), Nathan (2004) and Mahady (2005) were claimed that the use of plant extracts and phytochemicals with known antimicrobial activities are relatively safer than synthetic alternatives. Among the

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recommended biologically active compounds are alkaloids, flavonoids, isoflavonoids, tannins, glycosides and phenolic compounds (Evans *et al.*, 2002; Romero, 2005 and Shah *et al.*, 2015). Recent efforts were done by several authors among them: Jahanet *al.* (2011), they studied the antibacterial activity of ethanolic-leaf extracts of *Syzygium cumini*, *Ocimum sanctum*, *Lawsonia inermis*, *Zizyphus mauritiana* and *Ficus religiosa* against antibiotic resistant and sensitive *Staphylococcus aureus* strains isolated from different samples collected from patients. As well Reddy (2009) found that *Datura stramonium* extracts showed significant antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusarium* species. Choudhary *et al.* (1995) and Awadh *et al.* (2001) revealed that *Withania somnifera* has a potential antimicrobial agent, with antifungal activity, and moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria strains. Amer & Abdelmohsen (2014), studied the antibacterial activity of four common-edible Solanaceae species namely: *Capsicum frutescens* L., *Lycopersicon esculentum* Mill. *Solanum melongena* L. and *Solanum tuberosum* L. against 25 isolates of antibiotic resistant bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*), the lipid fraction of all the studied species showed the highest antibacterial activity.

Leaf extracts of *Zizyphus spina-christi* possess antibacterial activities against *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *E. coli*, *K. pneumonia*, *Brucella melitensis*, *Bordetella bronchiseptica* and *P. aeruginosa* (Motamedi *et al.*, 2009).

The present study is an attempt to estimate the antibacterial efficiency of *Datura stramonium* L., *Withania somnifera* (L.) Dun. in DC. (Family: Solanaceae) and *Zizyphus spina-christi* (L.) Willd. (Family: Rhamnaceae) against selected sensitive and multi-drug bacteria spp. isolated from chickens.

Material and Methods

Material

Plant material

Fresh plant material of *Datura stramonium* L., *Withania somnifera* (L.) Dun. in DC. (Family: Solanaceae) and *Zizyphus spina-christi* (L.) Willd.

(Family: Rhamnaceae) were collected from Cairo University Experimental Farm. Leafy branches of each plant were air-dried in shade, and then subjected to drying oven at 40°C to constant weight. The dried material was powdered and kept in plastic bags, and subjected later to extraction.

Bacterial samples

The studied bacterial isolates were collected from chicken organs (liver, lung and heart) from different live diseased chickens in Egypt. All samples used were collected under aseptic condition and safety precautions out lined earlier by: Roskey & Hamdy (1972), Swayne *et al.* (1998), Rodgers *et al.* (1999), Lee (2003), Kitai *et al.* (2005) and Middleton *et al.* (2005). The collected samples then marked and kept in an ice box and transferred to the laboratory as soon as possible for further investigation.

Antibiotic discs (Oxoid and Pfizer)

The antibiotic used belonged to Penicillins group were Ampicillin (AM, 10 mcg), Amoxicillin (AX, 25 mcg), Cephalosporines group were Cefaclor (CEC, 30 mcg), Aminoglycoside group were Gentamicin (CN, 10 mcg), Amikacin (AK, 30 mcg), Tobramycin (TOB, 10 mcg), Quinolones group were Ciprofloxacin (CIP, 5 mcg), Nalidixic acid (NA, 30 mcg), Macrolids group were Erythromycin (E, 15 mcg), Tetracyclines (BS) were Tetracycline (TE, 30 mcg), Glycopeptides were Vancomycin (VA, 30 mcg). The diameters of inhibition zones were measured. Inhibition zones were determined according to National Committee for Clinical Laboratory Standards NCCLS (2002).

Methods

Preparation of plant extracts

Ethanolic leaf extracts of the three selected plants were prepared as 100 g of the air dried powdered leaves of each plant were soaked in 100 ml of 70% ethanol for 72 h. The mixture was stirred after every 24 h then filtered. The ethanolic filtrate obtained was concentrated at 30 °C and then stored at 4 °C. The prepared plant extracts were screened for their antimicrobial activity. Water extract was prepared as: 100g of the air-dried leaves soaked in 100ml distilled water for 72 h. Then filtered and kept in refrigerators. For successive extraction: fifty grams of the air-dried powder plant leaves was extracted successively using the following solvents: petroleum ether, chloroform, methanol and water by using a Soxhlet extractor until colorless extract obtained on the top of the

extractor (Alade & Irobi, 1993). Extracts were concentrated under reduced pressure using rotary evaporator and dissolved in dimethyl sulfoxide (DMSO), and subjected to antimicrobial activity assay according to Thippeswamy et al. (2011).

Identification of bacterial isolates

Identification of the collected gram +ve &-ve isolates were carried out according to Bergey's Manual of Systematic Bacteriology (1989) and York et al. (2000).

Preparation of inocula

According to Ericsson & Sherris (1971), Inoculums standardized to give density 104 colony-forming units (CFU)/spot on the agar. Random four or five colonies of a pure culture were selected to avoid an atypical variant. The inoculums may be prepared by emulsifying overnight colonies from an agar medium or by diluting a broth culture. The broth used tested before application for its antagonistic to the agent tested. A 0.5McFar-land standard used for visual comparison to adjust the suspension to a density equivalent to approximately 108 CFU/mL. Alternatively, inocula can be adjusted photometrically. The bacterial suspensions were diluted with 0.85% saline or broth to give 107CFU/ml. Plates must be inoculated within 30 min of standardizing the inoculum, to avoid changes in inoculums density.

Antimicrobial assay

It was carried out according to Perez et al. (1990). The antimicrobial activity of the investigated plant extracts were evaluated using agar well diffusion method. 0.1 ml (105 CFU/ml) of each bacterial suspension of different isolates to be tested was swabbed on the nutrient agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, 100 µl of the prepared plant extracts was added to each well in the agar plate. The plates were incubated aerobically in an upright position at 37±2 °C for 24-48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm). The diameters of the inhibition zones were interpreted according to NCCLS (2002), and the examined isolates were reported as susceptible, intermediate, or resistant to the antibiotic under test. The test was performed in triplicates with controls for each strain (media control, bacterial control, and extract control).

Determination of minimum inhibitory concentration (MIC) of selected isolates against antibiotics and selected plants & minimum bactericidal concentration (MBC)

Broth micro-dilution method was used to determine MIC and MBC according to NCCLS (1999) and Yu et al. (2004). MIC was determined using broth dilution method. Different concentration of Ampicillin (showed weak activity) and Ciprofloxacin (showed potent activity) antibiotics, ranging from 4 to 1024 µg/ml. Chloform extract was diluted to give the final concentrations of 75, 37.5, 18.8, 9.4, 4.7, 2.4, 1.2, 0.6, 0.3, 0.15, 0.075, 0.037 mg/ml. 100 µl of 105 CFU/ml of each of the tested bacterium were inoculated in tubes with equal volumes of nutrient broth and the tested plant extract. The tubes were incubated aerobically at 37°C for 24-48 h. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the tested extract that produced no visible growth (no turbidity) in the first 24 h when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 h at 37°C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC. MBC value was determined by sub culturing the test dilution which showed no visible turbidity on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37°C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC.

Results and Discussion

Out of the collected 463 different chicken samples, 22 samples were found to be positive for *E.coli*, 4 samples for *Staphylococcus aureus*, 20 samples for *Salmonella* sp. and finally 2 samples for *Pseudomonas aeruginosa*. These isolates were identified according to the recommended keys of Bergey's Manual of Systematic Bacteriology (1989) and York et al. (2000). In this study, sixteen bacterial isolates were selected as represented samples for further investigation. Identification of these isolates revealed that two Gram positive isolates were found to belong to *Staphylococcus aureus* and symbolized as: St1 and St2. The other 14 isolates were Gram negative: six isolates were found to belong to *E. coli* and were numbered: *E. coli* 1, *E. coli* 2, *E. coli* 3, *E. coli* 4, *E. coli* 5 and

E. coli 6. Additionally, two bacterial isolates were found to belong to *Pseudomonas aeruginosa* and symbolized as: Ps1 and Ps2. Furthermore, six isolates were identified as *Salmonella* sp. and given the symbols: S1, S2, S3, S4, S5 and S6.

The different bacterial isolates showed variable response towards the various tested antibiotics. The sensitivity pattern of the bacterial isolates to antibiotic are shown in Table 1. The 16 bacterial isolates were divided based on its antibiotic sensitivity into "Antibiotic-Resistant" and "Antibiotic-Sensitive". These bacterial isolates were screened for susceptibility to 11 types of antibiotics which are: Ampicillin, Amoxicillin, Cefaclor, Gentamicin, Amikacin, Tobramycin, Ciprofloxacin, Nalidixic acid, Erythromycin, Tetracycline and Vancomycin using disk diffusion method and the results are presented in Table 2.

The results outlined in Table 2 indicated that, all the gram positive and gram negative bacterial isolates exhibited 100% antibiotic resistance against Ampicillin, Amoxycillin and Erythromycin. While, *Salmonella*, *E.coli* and *Pseudomonas* isolates showed high resistance against Vancomycin (87.5 %) and Tetracycline (81.25%) where the two *Staphylococcus* isolates were sensitive to Vancomycin and variant (sensitive or resistant) against Tetracycline. Cell wall synthesis is inhibited by β -lactams, such as Penicillins (Ampicillin, Amoxicillin) and Cephalosporins (Cefaclor), which inhibit peptidoglycan polymerization, and by Glycopeptides (Vancomycin), which combines with cell wall substrates. The sixteen bacterial isolates reacted variably against Nalidixic acid, but the majority showed resistance. Lower bacterial sensitivity was observed against Cefaclor and Tobramycin (31.25%) followed by Ciprofloxacin and Gentamicin (25%). *E. coli* 1-4 and St1 isolates showed resistance or intermediate reactions against Ciprofloxacin. Quinolones (Nalidixic acid, Ciprofloxacin) bind to a bacterial complex of DNA and DNA gyrase, blocking DNA replication, are effective on Gram-negative and some Gram-positive bacteria. The behavior of the isolates S1 and *E.coli* 1 and 2 and 6 and the gram +ve isolates St1 and St2, showed some resistance against Gentamycin. Amikacin was found to be able to exert an antibacterial effect on all the bacterial strains under study. Aminoglycosides (Amikacin, Gentamycin,

Tobramycin), Tetracyclines (Tetracycline) and Macrolide (Erythromycin) all interfere with ribosome function.

Similar observations have been reported by earlier studies among them: Tahnkiwale *et al.* (2002), Samy *et al.* (2003), Macedo & Santos (2005), Shittu & Lin (2006) and Orrett & Land (2006). While, Bhat *et al.* (1990) found that the variation in the antibiotic sensitivity pattern of the isolated organisms, may be related to several factors including differences in pH value, condition and time of incubation, composition and nature of the culture media, size of inoculum, source of isolated organism and perhaps differences in strain activity. Scherrer & Gerhardt (1971) also reported that the structure of the bacterial cell wall and the permeability of the cell membranes to different antibiotics may affect on variation in the antibiotic sensitivity.

In this study, the crude ethanol and water extracts of the studied bacterial species, showed no considerable effect against the bacterial isolates under investigation. While, the successive extracts of the studied plants using diethyl ether, chloroform, methanol and water were screened for their antimicrobial activity against 16 bacterial isolates. The results outlined in Table 3, illustrate that, chloroform extract of the studied spp. (*Datura stramonium*, *Withania somnifera* and *Ziziphus spina-christi*) showed high inhibition zones against all the studied bacterial isolates (13- 22 mm), followed by diethyl ether extract (ranged from 13- 20 mm). *D. stramonium* chloroform extract exhibited the highest inhibition (14-22 mm) against bacteria. Methanol and water extracts of these plant spp. showed the lowest inhibitory effect against the *E.coli* and Ps2 isolates (Table 3). Sastry & Rao (1994) studied the effectiveness of marine algae extracts, and claimed that methanol extract yielded higher antibacterial activity than n-hexane and ethyl acetate. Whereas others reported that algae chloroform extracts was better than methanol and benzene (Febles *et al.*, 1995). Lima *et al.* (2002) concluded that using organic solvents provides a higher efficiency in extracting compounds from algae for antimicrobial activities compared to water based method.

TABLE 1. The used antibiotics and its references inhibition zones. (Interpretation according to NCCLS 2002).

Antibiotic class	Antibiotics Name	Symbol	Disc Potency mg/disc	Interpretation		
				Diameter of the inhibition zone in millimeter (mm)		
				Resistant (R) = or <	Intermediate (I) From -To	Sensitive (S) = or >
Pencillins	Ampicillin	AM	10 mcg	≤13	14 - 18	≥19
Pencillins	Amoxycillin	AX	25 mcg	≤28	-	≥29
Cephalosporins	Cefaclor	CEC	30 mcg	≤13	14 - 16	≥17
Amino Glycoside	Amikacin	AK	30 mcg	≤14	15 - 16	≥17
Quinolones/Flouro-quinolones	Ciprofloxacin	CIP	5 mcg	≤15	16 - 20	≥21
Macrolids	Erythromycin	E	15 mcg	≤13	14 -22	≥23
Amino Glycoside	Gentamicin	CN	10 mcg	≤12	13 -14	≥15
Amino Glycoside	Tobramycin	TOB	10 mcg	≤12	13 -14	≥15
Quinolones/Flouro-quinolones	Nalidixic acid	NA	30 mcg	≤13	14 -18	≥19
Tetracyclines(BS)	Tetracycline	TE	30 mcg	≤14	15-18	≥19
Glycopeptides	Vancomycin	VA	30 mcg	-	-	≥ 18

TABLE 2. Susceptibility test of the collected 16 bacterial isolates against standard antibiotics.

Antibiotics Bacterial isolates	Antibiotics										
	NA	AX	E	AM	VA	CIP	AK	TE	CN	TOB	CEC
<i>Salmonella</i> sp											
S1	R	R	R	R	R	I	S	R	R	R	S
S2	S	R	R	R	R	S	S	R	S	S	S
S3	R	R	R	R	R	S	S	I	S	S	S
S4	R	R	R	R	R	S	S	R	S	S	S
S5	S	R	R	R	R	S	S	R	S	S	S
S6	S	R	R	R	R	S	S	R	S	S	S
<i>E. coli</i>											
<i>E. coli</i> 1	R	R	R	R	R	R	S	R	I	R	R
<i>E. coli</i> 2	R	R	R	R	R	R	S	R	R	R	R
<i>E. coli</i> 3	R	R	R	R	R	R	S	R	S	S	S
<i>E. coli</i> 4	R	R	R	R	R	I	R	R	S	S	S
<i>E. coli</i> 5	S	R	R	R	R	S	S	S	S	S	R
<i>E. coli</i> 6	R	R	R	R	R	S	S	R	R	I	S
<i>S. aureus</i>											
St1	R	R	R	R	I	R	S	R	R	R	S
St 2	R	R	R	R	S	S	S	I	I	R	S
<i>P. aeruginosa</i>											
Ps1	S	R	R	R	R	S	S	R	S	S	R
Ps2	I	R	R	R	R	S	S	R	S	S	R

The diameters of the inhibition zones were interpreted according to NCCLS (2002), and the examined isolates were reported as susceptible, intermediate, or resistant to the antibiotic under test. R: Resistant, I: Intermediate, S: Sensitive.

TABLE 3. Antimicrobial activity (in mm) of successive extracts of the studied spp. against the tested bacterial isolate .

Studied sp.	<i>Ziziphus- spina christi</i>				<i>Withania somnifera</i>				<i>Datura stramonium</i>			
	Solvent used				Solvent used				Solvent used			
Bacterial isolates	E	C	M	W	E	C	M	W	E	C	M	W
<u><i>Salmonella</i> sp</u>												
S1	15	16	16	16	17	16	15	17	16	15	16	16
S2	19	20	15	17	19	20	15	17	17	22	15	17
S3	17	20	15	16	16	18	16	16	17	17	17	16
S4	20	19	19.5	17	18	15	14	17	17	20	15	18
S5	18	17	16	18	18	18	20	17	17	20	20	17
S6	16	17	15	16	17	17	16	18	16	18	15	17
<u><i>E. coli</i></u>												
<i>E. coli</i> 1	16	17	15	10	17	16	6	10	15	18	10	6
<i>E. coli</i> 2	16	16	8	8	17	16	8	6	17	17	11	10
<i>E. coli</i> 3	16	15	8	6	17	17	6	11	17	18	9	8
<i>E. coli</i> 4	19	13	6	6	19	13	6	6	17	20	11	6
<i>E. coli</i> 5	13	15	10	8	16	16	6	6	14	14	11	8
<i>E. coli</i> 6	15	15	6	6	15	15	6	6	18	20	10	6
<u><i>S. aureus</i></u>												
St1	17	20	17	19	19	20	18	21	15	20	19	21
St 2	17	17	17	18	15	14	17	17	15	15	19	19
<u><i>P. aeurogenosa</i></u>												
Ps1	15	16	15	19	18	16	16	16	16	17	14	20
Ps2	16	16	12	6	17	19	12	6	18	18	13	11

E: Diethyl ether extract , C : Chloroform extract, M: Methanol extract and W :Water extract.

Since the studied bacterial isolates were sensitive to almost all the tested plant extracts, this indicates that these extracts might have different modes of action than that of the tested antibiotics. This observation agrees with the hypothesis of Ellof (1998) who expected that plant extracts showing target sites other than those used by antibiotics will be active

against drug-resistant microbial pathogens. *D. stramonium* chloroform extract was the most efficient against the sixteen bacterial sensitive and multi drug resistant isolates followed by diethyl ether of the same plant (Table 3). Janssen et al. (1987) and Lee et al. (2004) suggested that the antimicrobial activity of the plant extract may be due to the effect

on the internal contents of the bacteria which cause the inhibition of growth or killing of the pathogenic bacteria. These result from a Solanaceae plant was supported by the work of Gandhiappan & Rengasamy (2012), as they reported that the methanolic leaves extract from six Solanum species (*S. anguivi*, *S. nigrum*, *S. pubescens*, *S. surratense*, *S. torvum*, *S. swartz* and *S. trilobatum*) showed moderate activity against human pathogenic bacteria such as *Staphylococcus aureus* MTCC 96, *Micrococcus luteus* ATCC 4698, *Vibrio cholerae* ATCC14035 and *Klebsiella pneumoniae* MTCC 109. Parameswari et al. (2012), also mentioned that methanolic extracts of *Solanum nigrum* showed highest antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, compared to ethanol extract. Recently, Amer & Abdelmohsen (2014), studied the efficiency of ether extract of the four common-edible Solanaceae species namely: *Capsicum frutescens* L., *Lycopersicon esculentum* Mill., *Solanum melongena* L. and *Solanum tuberosum* L. against the antibiotic resistant bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* the results showed high inhibition activity.

Ampicillin and Ciprofloxacin were selected to estimate the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the tested isolates (Table 2). Ciprofloxacin, is the antibiotic of Quinolones group, the behavior of organisms towards its effect varied. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of Ciprofloxacin showed the highest activity against all the tested bacteria (Table 4). While, Ampicillin, is an antibiotic of Pencillins group. The drug showed the highest activity against all the tested isolates of *Salmonella* sp. except S3 with the lowest (MIC) and (MBC) recorded values (128 µg/ml, Table 4). The antibiotic showed variable inhibitory effects on *E. coli*, *S. aureus* and *P. aeruginosa* isolates with values ranging from 64 to 512 µg/ml as outlined in Table 4.

This study revealed that the chloroform extract out of the studied extracts showed the highest efficiency against bacterial growth, which indicated the efficiency of *Datura* leaves to reduce the growth of the studied bacterial spp. with MICs values varying from 75.0 to 0.037 mg/ml. The results of MICs of *D. stramonium* chloroform extract and Ciprofloxacin, gave highest inhibitory activity, showed similar antibacterial effect against all studied sensitive and drug resistant bacterial isolates. The minimum bactericidal concentration (MBC) was considered as the lowest concentration of the extract associated with no bacterial culture. The fact that the extract, exerted effect at low concentrations might be due to solubility of the extract (Green wood, 1983) or simply sometimes the antimicrobial agent is more effective when used at lower concentrations than at higher ones (Prescott et al., 1993). This fact correlated with the obtained results as low concentrations of the studied *D. stramonium* extract showed great effectiveness towards all the tested bacterial species.

According to Alam (2009), the development of resistance to chemotherapeutic agents by the microorganisms has appeared to be a continuous process since the discovery of antibiotics. So every antibiotic has certain life span regarding its efficacy. The antibacterial activities significantly differed depending on taxonomic characteristics of the plant species as well as biological characteristics of the tested bacteria which may explain the variations in the antibacterial activity of the tested plant extracts. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would be generally expected that a much greater number would be active against Gram-positive than Gram-negative bacteria (MC-Cutcheon et al., 1992). The demonstration of antibacterial activity against both Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan et al., 2001). This fact may be applicable to the results obtained from the studied *D. stramonium* extract, which showed great effectiveness towards all the tested bacterial species.

TABLE 4. Different concentrations of Ampicillin (AM) and Ciprofloxacin (CIP) against tested bacterial isolates with reference to MIC and MBC.

Bacterial isolates	Different Ampicillin and Ciprofloxacin antibiotic conc. µg/ml												MIC			MBC						
	1024		512		256		128		64		32		16		8		4		AM	CIP	AM	CIP
	AM	CIP	AM	CIP	AM	CIP	AM	CIP	AM	CIP	AM	CIP	AM	CIP	AM	CIP	AM	CIP				
<i>Salmonella</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	128	-
S4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>E.coli</i> 1	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	64	-
<i>E. coli</i> 2	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	256	-
<i>E. coli</i> 3	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	512	-
<i>E. coli</i> 4	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	256	-
<i>E. coli</i> 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64	-
<i>E. coli</i> 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
St1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64	-
St2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64	-
<i>P.aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps1	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	128	-
Ps2	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	256	-

MIC : Minimum Inhibition Concentration , MBC: Minimum Bacterial Concentration, + : showing growth , - : No growth

Reference

- Alade, P.I. and Irobi, O.N. (1993) Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. *Journal of Ethnopharmacology*, **39**,171-174.
- Alam, S. (2009) Antimicrobial activity of natural products from medicinal plants. *Global Journal of Medical Sciences*, **7**,176.
- Amer, W. M. and Abdelmohsen, G. (2014) Lipid of some edible Solanaceae species; and its activity against some antibiotic resistant pathogenic bacteria. *World Journal of Pharmaceutical Research*, **3**(3), 3511-3527.
- Awadh, N.A., Julicch, W.D., Kusnick, C. and Lindequist, U. (2001) Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *Journal of Ethnopharmacology*, **74**, 173-179.
- Bako, S.P., Bakfur, M.J., John, I. and Bala, E.I. (2005) Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *International Journal of Botany*, **1**(2),147–50.
- Bergey's Manual of Systematic Bacteriology (1989) Williams, S.T., Sharpe, M.E., Holt, J.G. (Ed.), pp399. The Williams and Wilkins Co. Baltimore. Vol. 4.
- Bhat, G., Nagesha, C.N., Pirvathi, C.N., Joseph, K.M. and Shivananda, P.G. (1990) Drugs resistance and penicillinase activity in skin isolated *Staphylococcus aureus*. *Indian Journal of Dermatology, Venereology and Leprology*, **56**, 371-4.
- Bocanegra-Garcia, V., Camacho-Corona, M., Ramirez-Cabrera, M. and Garza-Gonzalez, G.R. (2009) The bioactivity of plant extracts against representative bacterial pathogens of the lower respiratory tract. *Biomed Central Research Notes*, **2**, 95.
- Choudhary, M.I., Dur-e-Shahwar, N.A., Parveen, Z., Jabbar, A., Ali, I. and Atta-ur-Rahman, A. (1995) Antifungal steroidal lactones from *Withania coagulance*. *Phytochemistry*, **40**, 1243-1246.
- Chovanová, R., Mikulášová, M. and Vaverková, Š. (2013) *In vitro* antibacterial and antibiotic resistance modifying effect of bioactive plant extracts on methicillin-resistant *Staphylococcus epidermidis*. *International Journal of Microbiology*, **2013**:760969.
- Cohen, M.L. (1992) Epidemiology of drug resistance, implications for a post-antimicrobial era. *Science*, **257**,1050-1057.
- Davies, J.E. (1997) Origins, acquisition and dissemination of antibiotic resistance determinants. *Ciba Foundation symposium*, **207**, 15-35.
- Djeussi, D.E., Noumedem, J.A.K., Seukep, J.A., Fankam, A.G., Voukeng, I.K., Tankeo, S.B., Nkuete, A.H.L. and Kuete, V. (2013) Antibacterial activities of selected edible plants extracts against multidrugresistant gram-negative bacteria. *Biomed Central Complementary and Alternative Medicine*, **13**, 164.
- Ellof, J.N. (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethnopharmacology*, **60**,1–8.
- Ericsson, H.M. and Sherris, J.C. (1971) Antibiotic sensitivity testing . *Report of an International Collaborative Study*.
- Evans, C., Bansot, A. and Samuel, A. (2002) Efficacy of some nape medical plants against *Salmenella*: An *in vitro* study *Journal of Ethnopharmacology*, **80**, 12-24.
- Febles, C.L., Arias, A. and Gil-Rodriguez, M.C. (1995) *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish), *Anuria del Estudios Canarios*, **34**, 181-192.
- Gandhiappan, J. and Rengasamy, R. (2012) Comparative evaluation of antimicrobial activities of the members of Solanaceae. *Pelagia Research Library Der Pharmacia Sinica*, **3**(3), 357-360.
- Greenwood, D. (1983) "Antimicrobial chemotherapy", pp. 28-49, 73-82 Bailliere Tindall London.
- Iwu, M.W., Duncan, A.R. and Okunji, C.O. (1999) New antimicrobials of plant origin. In: "Perspectives on New Crops and New Uses" J. Janick (Ed.), pp. 457-462. ASHS Press, Alexandria, VA.
- Janssen, A.M., Scheffer, J.J.C. and Baerheim Svendsen, A. (1987) Antimicrobial activity of essential oils:a1976–86 literature review. Aspects of the test methods. *Planta Medica*, **53**, 395–398.
- Jahan, F., Lawrence, R., Kumar, V. and Junaid, M. (2011) Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant *Staphylococcus aureus* strains. *Journal of Chemical and Pharmaceutical Research*, **3**(4), 777-789.
- Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H. (2005) Characterization of methicillin resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *Journal of Veterinary Medical Science*, **67**, 107–110.

- Loeraa, J.A., Reyes-Ortizb, C. and Kuoa, Y.F. (2007) Predictors of complementary and alternative medicine use among older Mexican Americans. *Complement Therap. Clin. Prac.* **13**, 224-231.
- Lee, J.H. (2003) Methicillin (Oxacillin)- resistant strains isolated from major food animals and their potential transmission to human. *Applied and Environmental Microbiology*, **69**(11), 6489-6494.
- Lee, K.W., Everts, H. and Beynen, A.C. (2004) Essential oils in Broiler nutrition. *International Journal of Poultry Science*, **3**, 738-752.
- Lima-Filo, J.V.M., Carvola, A.F.U. and Freitas, S.M. (2002) Antibacterial activity of extract of six macro algae from the North eastern Brazilian Coast. *Brazilian Journal of Microbiology*, **33**, 311-313.
- Macedo, J.L.S. and Santos, B. (2005) Bacterial and fungal colonization of burn wounds. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro*, **100**(5), 535-539.
- Mahady, G.B. (2005) Medicinal plants for the prevention and treatment of bacterial infections. *Current Pharmaceutical Design*, **11**, 2405-2427.
- MC-Cutcheon, A.R., Ellis, S.M., Hancock, R.E.W. and Towers, G.H.N. (1992) Antibiotic screening of medicinal plants of the British Columbian native peoples. *Journal of Ethnopharmacology*, **37**, 213-223.
- Middleton, J. R., Fales, W.H., Luby, C.D., Landsay, Oaks, J., Susan Sanchez, Kinyon, J.M., Wu, C.C., Maddox, C.W. and Hartmann, F. (2005) Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. *Journal of Clinical Microbiology*, **43**(6), 2916-2919.
- Motamedi, H., Safary, A., Maleki, S. and Seyyednejad, S.M. (2009) *Ziziphus spina-christi*, a native plant from Khuzestan, Iran, as a potential source for discovery new antimicrobial agents. *Asian Journal of Plant Sciences*, **8**(2), 187-190.
- Nathan, C. (2004) Antibiotics at the crossroads. *Nature*, **21**(431), 899-902.
- NCCLS (1999) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A. Wayne, Pa.
- NCCLS (2002) Supplement M100-S12. Performance standards for antimicrobial susceptibility testing. NCCLS, Wayne, PA.
- Orrett, F.A. and Land, M. (2006) Methicillin-resistant *Staphylococcus aureus* prevalence: current susceptibility patterns in Trinidad. *Bio. Med. Central Infectious Diseases*, **6**(83), 1-6.
- Parameswari, K., Aluru, S. and Kishori, B. (2012) *In vitro* antibacterial activity in the extracts of *Solanum nigrum*. *Indian Streams Research Journal*, **12**, 1-4.
- Perez, G.R.M., Avila, J.G., Zavala, M.A., Perez, G.S. and Perez, G.C. (1990) *In vitro* antibacterial activity of *Loeselia mexicana* and *Croton ehrenbergii*. *Phytomedicine*, **3**, 186.
- Prescott, L.M., Harley, J.P. and Klein, D.E. (1983) "Microbiology", 912 pp. 2nd ed., Wm C brown Publishers. Dubuque, Iowa. Melbourne Australia. Oxford, England.
- Reddy, B.U. (2009) Antimicrobial activity of *Datura stramonium* L. and *Tylophora indica* (Burm. F.) Merr. *Pharmacology online*, **1**, 1293-1300.
- Rodgers, J.D., McCullagh, J.J., McNamee, P.T., Smyth, J.A. and Ball, H.J. (1999) Comparison of *Staphylococcus aureus* recovered from personnel in a poultry hatchery and in broiler parent farms with those isolated from skeletal disease in broilers. *Veterinary Microbiology*, **69**, 189-198.
- Romero, E. (2005) Antibacterial properties of common herbal remedies of the Southwest. *Journal of Ethnopharmacology*, **2**(99), 253-257.
- Roskey, C.T. and Hamdy, M.K. (1972) Bruised poultry tissue as a possible source of staphylococcal infection. *Journal of Applied Microbiology*, **23**, 683-687.
- Samy, A.S., El-Sayed, I.E., Mohammad, R.E., Ahmad, B., Modather, M.E. and Hosam, A.Z. (2003) Methicillin resistance *Staphylococcus aureus*: A problem in the burns unit. *Egyptian Journal of Plastic and Reconstructive Surgery*, **27**(1), 1-10.
- Saranraj, P. and Sivasakthi, S. (2014) Medicinal plants and its antimicrobial properties. A Review, *Global Journal of Pharmacology*, **8**(3), 316-327.
- Sastry and Rao (1994) Antibacterial substance from marine algae: Successive extraction using benzene, chloroform and methanol, *Botanica Marina*, **37**, 357-360.
- Scherrer, R. and Gerhardt, P. (1971) The exclusion limit for *Bacillus megaterium* cell wall was reported as 100,000 daltons. *Journal of Bacteriology*, **107**, 718.

- Shah, K., Burange, P. and Singh, S. (2015) Photochemical, antimicrobial and anti-adherence analysis of plant and ayurvedic extracts. *International Journal of Applied Biology and Pharmaceutical Technology*, **6**, 72-79.
- Shittu, A.O. and Lin, J. (2006) Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BioMed. Central Infectious Diseases*, **6**, 125.
- Sibanda, T. and Okoh, A.I. (2007) The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *African Journal of Biotechnology*, **6**(25), 2886-2896.
- Srinivasan, D., Sangeetha Nathan, Suresh, T. and Lakshmanaperumalsamy, P. (2001) Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology*, **74**, 217- 220.
- Swayne, D.E., Senne, D.A. and Beard, C.W. (1998) Avian Influenza in: "A Laboratory Manual for the Isolation and Identification of Avian Pathogens", **4**, 150-155.
- Tahnkiwale, S.S., Roy, S. and Jalgaonkar, S.V. (2002) Methicilin resistance among isolates of *Staphylococcus aureus*; Antibiotic sensitivity pattern and phage typing. *Indian Journal of Medical Sciences*, **56**, 330-334.
- Tenover, F.C. (2006) Mechanisms of antimicrobial resistance in bacteria, *The American Journal of Medicine*, **119**(6), S3-S10.
- Thippeswamy, S., Praveen, P., Mohana, D.C. and Manlunath, K. (2011) Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (JACQ.) Merr. against some human and pathogenic bacteria and fungi. *International Journal of Pharma and Bio Sciences*, **2**, 443-452.
- WHO (2002) Traditional Medicine Growing Needs and Potential - WHO Policy Perspectives on Medicines, No. 002, May, World Health Organization, Geneva, Switzerland.
- York, M.K., Baron, E.J., Clarridge, J.E., Thomson, R.B. and Weinstein, M.P. (2000) Multi laboratory validation of rapid spot tests for identification of *Escherichia coli*. *Journal of Clinical Microbiology*, **38**, 3394-3398.
- Yu, J.Q., Lei, J.C., Yu, H., Cai, X. and Zou, G.L. (2004) Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. *Phytochemistry*, **65**, 881-884.

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كفاءة التصاد الميكروبي لمستخلصات نباتات مصرية على سلالات من البكتريا الحساسة والمقاومة للمضادات الحيوية

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تناولت هذه الدراسة كفاءة التصاد الميكروبي لمستخلصات أوراق ثلاثة نباتات مصرية هي السدر وسم الفأر والداثورة، في حالة البكتريا الحساسة ومتعددة المقاومة للمضادات الحيوية. وتم تجميع عدد 463 من العزلات البكتيرية من الدجاج الحى المصاب في المزارع المصرية ومنها تم تعريف عدد أربعة من الأجناس البكتيرية هي الاشيرشيا كولاي، ستافيلوكوكس أوريوس، والسيدوموناس أرجينوسه والسلمونيلا. وقد تم اختبار حساسية هذه العزلات ضد 11 نوع من المضادات الحيوية هي الأميسيلين والأموكسيسيلين والسيفاكلور والجنتاميسين والأمكاسين والتبراميسين والسيروفلوكساسين والنادكسك أسد والأريزرومابسين والتيتراسيكلين والفانكوميسين وقد أظهرت كل العزلات البكتيرية المعزولة مقاومة شديدة ضد معظم المضادات الحيوية المستخدمة بنسب 68.18%، 59.09%، 54.54%، 54.54% على الترتيب. وقد أظهر مستخلص الكلوروفورم لنبات الداثورة أعلى نشاط للتصاد البكتيري ضد كل عزلات البكتريا بقيم تركيزات ثبت أن جميعها قادرة على تثبيط النمو البكتيري عند تركيزات تتراوح من 75.0 حتى 0.037 ملجم / مل وتبين هذه الدراسة أن مستخلص الكلوروفورم لنبات الداثورة يعد مصدرا محليا واعداد لصناعة العقاقير التي تستخدم في معالجة الأمراض الناجمة عن العدوي بواسطة البكتريا الممرضة والمقاومة للمضادات الحيوية المتداولة.