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Antimicrobial activity of *Nigella Sativa* and *Lawsonia inermis (Henna)* against Gram negative bacteria isolated from clinical samples

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Abstract: *Multidrug resistant (MDR) Gram-negative bacteria are demonstrating resistance to nearly all currently licensed antibiotics. So, use of medicinal plants which contain the natural bioactive sources for safe and natural therapeutic an alternative to antibiotics is an important point for research because natural remedies should have less toxicity and less costing. The main objective of this study is to find out the antimicrobial activity in Nigella Sativa and Lawsonia inermis (Henna). 62 Gram- negative bacteria samples were collected and identified from patients suffering bacterial infection (33 males and 29 females) .Three different bacterial isolates Klebsiella pneumoniae , Acinetobacter baumannii and Pseudomonas aeruginosa were obtained from 8 different specimens with the following percentage representations: urine (37%), blood (9.67%), sputum (19.35%), Endotracheal aspirate Sample (1.61%), Swab from chest tube (1.61%), wound swabs (27.41%), Central Venous Catheter (1.61%) and pleural fluid (1.61%). The Antibiotics assay were performed by using the disc diffusion methods and using Well diffusion method to measure and record the inhibition zones produced by Nigella Sativa and Lawsonia inermis (Henna) extracts against those types of Gram-negative bacteria isolates obtained. Nigella Sativa recorded antimicrobial activity against Klebsiella pneumoniae , Acinetobacter baumannii and Pseudomonas aeruginosa with inhibition zones 14mm,17mm and 11mm respectively with concentration 50mg% only while Lawsonia inermis (Henna) inhibition zones (16 mm: 32 mm) with all concentrations 10, 20, 25, and 50 mg% with all tested types of bacteria. This study revealed antimicrobial activity of Nigella Sativa and Lawsonia inermis (Henna) extracts against (MDR) Gram-negative bacteria better than antibiotics.*

Keywords: *Multidrug resistant (MDR) Gram-negative, Lawsonia inermis (Henna), Nigella Sativa, Acinetobacter baumannii, Antimicrobial activity.*

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I. Introduction

The spread of multidrug resistant (MDR) bacteria is emerging threatening problem worldwide. It is estimated that about 70% of the bacteria that caused ailments in hospitals, become insensitive to at least one of the drugs currently prescribed for treatment **Purkayastha *et al.*, (2012)**. MDR Gram-negative bacteria infections are concerning because such bacteria are demonstrating resistance to nearly all currently licensed antibiotics **Xu *et al.*, (2013); Huwaitat *et al.*,(2016)** and the discovery of new drugs that could provide clinical efficacy against MDR Gram-negative pathogens remains one of the most successfully overcome the tide of resistance **Bassetti *et al.*, (2011); Huwaitat *et al.*, (2016)**.

Plants were considered as an undeniable source for the discovery of new antibacterial acting directly as bacterial growth inhibitors or as antibiotic modulators **Saleem *et al.*,(2010)** and Use of herbal medicine has amplified dramatically for various diseases because natural remedies should have less toxicity and less cost.

Nigella Sativa is commonly named black seeds has been used for medicinal purposes for centuries both as herbs or pressed it into oil in Asia, The Middle East, and Africa. The antimicrobial activities of black seeds are evaluated for MDR Gram-negative bacteria. Also, *Lawsonia inermis* (Henna) are selected due to their historical importance as medicinal plants to test their inhibitory effect against some types of Gram negative bacteria. To investigate the antimicrobial activity of *Nigella Sativa* and *Lawsonia inermis* (Henna) which may lend more weight to general acceptability of plant products for therapeutic use and that may provide initial information(s) for further development of more potent broad spectrum antibiotics effective against this bacteria, particularly against *Pseudomonas aeruginosa* is a Gram-negative bacteria that inhabits the soil and surfaces in aqueous environments. This bacteria has high antibiotic resistance, broad metabolic versatility and adaptability make it especially hard to treat **Cillóniz, Dominedò and Torres (2019)** also *Klebsiella pneumoniae* and *Acinetobacter baumannii* sensitive only to very few standard antibiotics presently.

II. Material And Methods

A total of 62 samples were collected from many different patients suffering from bacterial infections in Kasr Al-einiy hospital in Cairo, Egypt, during the period of April 2017 to May 2018 were collected. Studied samples include urine, wound swabs, Swab from chest tube, sputum, pleural fluid, Endotracheal aspirate Sample, Central Venous Catheter and blood samples table (1).Samples were inoculated on petri plates including blood agar, MacConkey and nutrient agar. The plates incubated aerobically at 37º C for 24 – 48 hour. Then Morphological and biochemical identification of bacterial isolates were done by standard methods according to **Collee *et al.*, (1996); Murray *et al.*, (1999); Johnson and Case (2003).** Antibiotic sensitivity testing was done on Muller Hinton agar with disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations **Weinstein *et al.*, (2018).** The diameter of inhibition zone was measured and interpreted as susceptible, intermediate or resistant as showed in table (2).

Table (1): Collection of clinical bacterial specimens

No. of patient	Source of specimen	Age	Gender	Diagnosis
1	Sputum	45	M	Respiratory infection
2	Urine	42	M	Urinary tract infection
3	Urine	55	F	Urinary tract infection
4	Urine	50	F	Infective Endocarditis
5	Urine	55	M	Urinary tract infection
6	Central Venous Catheter	40	M	Central-related bloodstream infection
7	Wound	56	M	Septic wound infection
8	Sputum	56	M	Respiratory infection
9	Sputum	8 D	F	Pneumonia
10	Wound	43	M	Septic wound infection
11	Urine	35	F	Urinary tract infection
12	Sputum	8 D	F	Respiratory infection
13	Urine	20	M	Urinary tract infection
14	Pleural Fluid	8 D	F	Pleural effusion
15	Wound	55	M	Septic wound infection
16	Urine	38	F	Urinary tract infection
17	Wound	40	M	Osteomyelitis
18	Sputum	10 D	M	Pneumonia
19	Urine	60	M	Urinary tract infection
20	Wound	50	F	Septic wound infection
21	Urine	52	M	Urinary tract infection
22	Urine	40	M	Urinary tract infection
23	Blood	6 D	F	Sepsis
24	Blood	36	F	Sepsis
25	Sputum	6 D	F	Respiratory infection
26	Wound	33	M	Septic wound infection
27	Blood	57	F	Sepsis
28	Swap from chest tube	7 D	F	Respiratory infection
29	Sputum	41	M	Respiratory infection
30	Wound	46	F	Septic foot
31	Wound	35	M	Septic wound infection
32	Endotracheal aspirate Sample	70	F	Respiratory infection
33	Urine	55	F	Urinary tract infection
34	Blood	3 D	F	Sepsis
35	Wound	37	M	Septic wound infection
36	Sputum	7 D	F	Pneumonia
37	Sputum	49	M	Pneumonia
38	Urine	61	M	Urinary tract infection
39	Wound	27	M	Burn wound infection
40	Wound	50	M	Septic wound infection
41	Sputum	44	F	Respiratory infection
42	Urine	56	M	Urinary tract infection
43	Urine	25	F	Urinary tract infection
44	Urine	55	F	Urinary tract infection
45	Wound	30	M	Wound infection
46	Urine	30	F	Urinary tract infection
47	Wound	17	M	Wound infection
48	Wound	55	M	Wound infection
49	Wound	49	M	Wound infection
50	Sputum	44	M	Respiratory infection
51	Wound	38	M	Wound infection
52	Blood	60	F	Sepsis
53	Sputum	28	F	Respiratory infection
54	Urine	69	F	Urinary tract infection
55	Urine	34	M	Urinary tract infection
56	Urine	50	F	Urinary tract infection
57	Urine	77	M	Urinary tract infection
58	Urine	43	F	Urinary tract infection
59	Urine	55	F	Urinary tract infection
60	Urine	71	M	Urinary tract infection
61	Wound	6 M	F	Burn wound infection
62	Wound	49	M	Wound infection

Table (2) : The antibiotics used to test the susceptibility of bacterial isolates

Antibiotics	Disc code	Disc contents (µg)	Resistant (R)	Intermediate (I)	Susceptible (S)
Amikacin	AK	30	≤ 14	15 – 16	≥ 17
Cefazolin	KZ	30	≤ 14	15 – 17	≥ 18
Ciprofloxacin	CIP	5	≤ 15	16 – 20	≥ 21
Ampicillin/Sulbactam	SAM	20	≤ 11	12 – 14	≥ 15
Cefepime	FEP	30	≤ 14	15 – 17	≥ 18
Meropenem	MEM	10	≤ 11	12 – 13	≥ 14
Levofloxacin	LEV	5	≤ 13	14 – 16	≥ 17
Gentamycin	Gm	120	≤ 6	7 – 9	≥ 10
Ceftriaxone	CRO	30	≤ 19	20 – 22	≥ 23
Cefoxitin	FOX	30	≤ 23	24 – 27	≥ 28
Ceftazidime	Caz	30	≤17	18 – 20	≥ 21
Trimethoprim/Sulfamethoxazole	SXT	1.25/23.75	≤10	11 – 15	≥ 16

Plant material and extraction:

Plants were collected from **Horticulture Research Institute (HRI) in Dokki Cairo, Egypt**. Plants were grinded to make fine powder. Fine powder of *Nigella Sativa* and *Lawsonia inermis* (Henna) was taken 30gram of plant powder was mixed with 100 ml of ethanol. Extracts were prepared with the help of Soxhlet apparatus **Liaqat *et al.*, (2018)**. This step was done at the **Central Lab for Soil, Foods and Feed stuff (CLSFF)** at **Zagazig University.**

Antimicrobial assay of plant extracts by well diffusion method:

Inhibitory activity of Nigella Sativa and Lawsonia inermis (Henna) was investigated by well diffusion method. An overnight culture of isolated Gram-negative bacteria was prepared .These bacteria (1.5 × 108 CFU/ml) were inoculated by streaking the swab over the entire Muller-Hinton agar (MHA) surface. Wells sized (6 mm) were cut into the agar plate and 50 µl of each Nigella Sativa and Lawsonia inermis (Henna) with different concentrations were placed into each well separately. The plates were incubated for 24 h at 37 °C and inhibition of growth was examined by clear zone surrounding each well that interpreted as less active, moderately active and highly active with ≤ 10 mm, 11-14 mm and ≥15 mm respectively.

III. Results And Discussion

Isolation of all Gram Negative pathogenic bacteria

Table (3): Percentage of all isolated bacteria

Bacteria type	site of collection								Samples (%)
	Urine	Wound	Sputum	Blood	Pleural fuid	Endotrecheal aspirate	central venous cathetar	swab from chest tube	
Klebsiella pneumoniae	13	6	8	5	1	1	-	-	54.8
***Acinetobacter baumannii*.**	3	2	3	-		-	1	1	16.1
Pseudomonas aeruginosa	7	9	1	1		-	-	-	29

This table showed the percent of all Gram Negative bacteria isolated from 62 sample as well as *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* was 54.8%, 16.1 %and 29 %respectively.

Table (4): Identification of pathogenic bacteria and biochemical tests

Bacteria type	Gram stain	Urease	Citrate	Oxidase	TIS	Motility
Klebsiella pneumoniae	G-Negative	(+ve)	(+ve)	(-ve)	A/A	Non Motile
Acinetobacter baumannii	G-Negative	(-ve)	(+ve)	(-ve)	K/K	NonMotile
Pseudomonas aeruginosa	G-Negative	(-ve)	(+ve)	(+ve)	K/K	Motile

+ve = positive, -ve = negative, A = Acid butt or slant, K= alkaline slant or butt
* TISA= (Triple Sugar Iron Agar)
This table explained the results of biochemical tests for identified isolates after growing on three plates, were Nutrient agar, Mac Conkey's agar and Blood agar.

Antibiotics susceptibility Test:

Table (5): Antibiotic sensitivity of the bacterial isolates

Mode of action	Cell wall synthesis inhibitors							Protein synthesis inhibitors		Nucleic acid inhibitors		Anti-metabolite Activity	% of R
Isolate No.	SAM	MEM	KZ	FOX	FEP	CRO	Caz	GM	AK	LEV	CIP	SXT	
1	R	R	R	R	R	R	R	R	R	R	R	R	100
2	R	S	R	S	R	R	R	R	S	R	R	R	75
3	R	S	R	S	R	R	R	S	S	S	S	R	50
4	R	R	R	R	R	R	R	R	S	R	R	R	91.7
5	R	R	R	R	R	R	R	R	S	R	R	R	91.7
6	R	R	R	R	R	R	R	S	R	R	R	R	91.7
7	R	R	R	R	R	R	R	R	S	R	R	R	91.7
8	R	R	R	R	R	R	R	R	R	R	R	R	100
9	R	R	R	R	R	R	R	R	R	R	R	R	100
10	S	S	R	S	S	S	R	R	R	R	R	R	58.3
11	R	R	R	S	R	R	R	R	S	R	R	S	75
12	R	I	R	R	R	R	R	R	S	S	I	R	66.6
13	R	S	R	R	S	R	R	R	S	S	S	R	58.3
14	R	R	R	R	R	R	R	R	S	R	R	R	91.7
15	R	S	R	R	S	R	R	S	S	S	S	R	50
16	R	R	R	R	R	R	R	S	R	R	R	R	91.7
17	R	R	R	R	R	R	R	R	R	R	R	R	100
18	R	S	R	R	R	R	R	R	R	R	R	R	91.7
19	R	R	R	R	R	R	R	R	S	R	R	I	83.3
20	R	R	R	R	R	R	R	R	S	R	R	R	91.7
21	R	S	R	R	R	R	R	R	S	R	R	R	83.3
22	R	R	R	R	R	R	R	R	R	R	R	R	100
23	R	R	R	R	R	R	R	R	S	R	R	R	91.7
24	R	S	R	R	S	R	R	R	S	I	R	R	66.6
25	R	R	R	R	R	R	R	R	R	R	R	R	100
26	R	S	R	R	S	R	R	S	R	R	R	R	75
27	R	R	R	R	R	R	R	R	R	R	R	R	100
28	R	R	R	R	R	R	R	R	S	R	R	R	91.7
29	R	R	R	R	R	R	R	R	S	S	S	S	66.6
30	R	S	R	R	S	R	R	I	S	R	R	R	66.6
31	R	R	R	R	R	R	R	S	S	S	S	S	58.3
32	R	R	R	R	R	R	R	R	R	R	R	R	100
33	R	I	R	R	R	R	R	R	S	R	R	R	83.3
34	R	R	R	R	R	R	R	R	R	R	R	R	100
35	R	R	R	R	R	R	R	R	R	R	R	R	100
36	R	R	R	R	R	R	R	R	R	R	R	R	100
37	R	R	R	R	R	R	R	R	R	R	R	S	91.7
38	R	S	R	R	S	R	R	S	S	S	S	R	50
39	R	R	R	R	R	R	R	R	R	R	R	R	100
40	R	R	R	R	R	R	R	R	R	R	R	S	100
41	R	R	R	R	R	R	R	R	R	R	R	R	100
42	R	S	R	R	R	R	R	R	R	R	R	R	91.7
43	R	S	R	R	R	R	R	R	S	S	S	S	58.3
44	R	R	R	R	R	R	R	R	R	R	R	R	100
45	R	S	R	S	S	R	R	S	S	S	S	S	33.3
46	R	R	S	R	S	R	R	R	S	S	R	R	66.6
47	R	R	R	R	R	R	R	R	R	R	R	R	100
48	R	S	R	R	S	R	R	S	S	S	S	R	500
49	R	S	R	S	R	R	R	R	R	S	S	R	66.6
50	R	R	R	R	R	R	R	S	R	R	R	R	91.7
51	R	S	R	S	R	R	R	S	S	S	S	R	50
52	R	R	R	R	R	R	R	R	R	R	R	S	91.7
53	R	S	R	R	S	R	R	R	S	S	S	R	58.3
54	R	R	R	R	R	R	R	R	R	R	R	R	100
55	R	I	R	R	S	R	R	R	R	R	R	R	83.3
56	R	R	R	R	R	R	R	R	R	R	R	R	100
57	R	R	R	R	R	R	R	R	R	R	R	R	100
58	R	R	R	R	R	R	R	R	R	R	R	R	100
59	R	S	R	S	R	R	R	S	S	S	S	R	50
60	R	R	R	R	S	R	R	R	S	R	R	R	83.3
61	R	R	R	R	R	R	R	R	R	R	R	R	100
62	R	R	R	R	R	R	R	R	R	R	R	R	100

The bacterial isolates were tested for their susceptibility to approximate 12 antibiotics disc using a standardized disc diffusion method. The results in table (5) indicate that the Amikacin antibiotic is more effective with susceptibility percentage (46.77 %) followed by Meropenem (35.4%), Levofloxacin, Ciprofloxacin with 25.8%, 22.5 respectively. On the other hand, the results showed that 100% of bacterial isolates were resistant to Cefazolin.

Effect of plant extract against (*Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acinetobacter baumannii*) by well diffusion method:

Regarding results recorded in Table (6, 7), which proved the antibacterial effect of the two different plants extract by well diffusion method against *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acinetobacter baumannii*. It was cleared from the obtained results that the effect of *Nigella Sativa* against *Acinetobacter baumannii* showed maximum inhibitory action (17mm) at concentration 50 mg %, medium inhibitory action (14mm) against *Klebsiella pneumonia* at the same concentration and minimum inhibitory action (11mm) against *Pseudomonas aeruginosa*

Table (6) the inhibitory effect alcoholic extract of *Nigella Sativa* against *Pseudomonas aeruginosa, Klebsiella pneumonia and Acinetobacter baumannii*

Test Bacteria	*Nigella Sativa* extract concentration (mg %)			
	10	20	25	50
Klebsiella pneumoniae	-	-	-	14 mm
Acinetobacter baumannii	-	-	-	17 mm
Pseudomonas aeruginosa	-	-	-	11 mm

The inhibitory effect of *Lawsonia inermis Linn* (Henna) extract against *Pseudomonas aeruginosa* showed maximum inhibition zone as showed in table (7) for four different concentrations at 10 mg % (21mm), 20 mg % (23mm), 25 mg % (25mm) and 50 mg % (32mm). The other types of bacteria showed less inhibitory activity compared with *Pseudomonas aeruginosa* as showen in table (5).

Table (7) the inhibitory effect alcoholic extract of *Lawsonia inermis Linn* (Henna) against *Pseudomonas aeruginosa, Klebsiella pneumonia and Acinetobacter baumannii*

Test Bacteria	*Lawsonia inermis Linn* (Henna) extract concentration (mg%)			
	10	20	25	50
Klebsiella pneumoniae	20 mm	21 mm	23 mm	28 mm
Acinetobacter baumannii	16 mm	20 mm	24 mm	30 mm
Pseudomonas aeruginosa	21 mm	23 mm	25 mm	32 mm

In this study ethanol extract of *Nigella Sativa* showed no inhibition zone against all the bacteria tested at lower concentrations (<50 mg%). Generally, the ethanol extract of Nigella sativa exhibited higher antibacterial effect compared with Lawsonia inermis (Henna) extract Gram negative bacteria have effective permeability barrier,consist of the outer membrane, which restricts the penetration of amphiphatic compounds and multidrug resistance pumps that extrude toxins across this barrier.

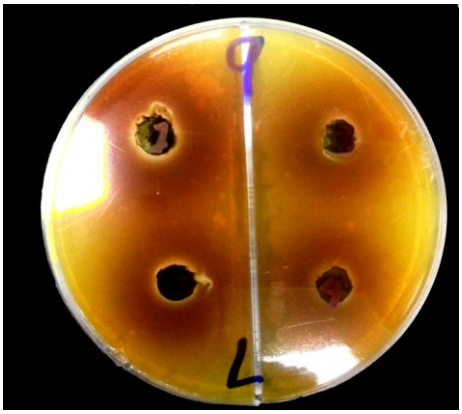

Photo (1) showed inhibitory effect alcoholic extract of *Lawsonia inermis Linn* (Henna) against *Pseudomonas aeruginosa* with four different concentrations.

It is possible that the apparent ineffectiveness of the *Nigella sativa* antimicrobial activity is mostly due to this permeability barrier **Hasan *et al.*, (2013)**. Otherwise, the effect of *Lawsonia inermis* (Henna) ethanol extract against all types of Gram negative bacteria tested gives a maximum inhibitory zone this is may be due to Henna leaves contain up to 5% by weight of the compound (2-hydroxy-1, 4-naphthoquinone) named Quinone, and the Quinones are present in henna they are complex irreversibly with Nucleophilic amino acids in proteins **Tan and Berridge (2008)**, often leading to deactivation of the protein and loss of function. For that reason, the potential range of Quinone antimicrobial effects is big. Portable targets in the microbial cell are surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes **Cowan (1999)**. Quinones may also provide substrates unavailable to the microorganism.

IV. Conclusions

From this study we have concluded that *Lawsonia inermis* (Henna) and *Nigella sativa* extracts possess antibacterial activities against (MDR) Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae and Acinetobacter baumannii*). Thus, they have a great potential as an effective antimicrobial agent for medicinal purposes.

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