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Antimicrobial Activity of Some Plant Extracts and Plant Nanoparticles Against Gram Negative Bacteria Isolated from Clinical Samples

***Atef, M. Amer*[a] ; Yakout, *A. El-senosi* [b] ; *Shaimaa, S. Naaom* [c]*; *Eman, Y.T. Elariny* [d]**

[a] *Organic Chemistry, Faculty of science, Zagazig University, Sharkia, Egypt.*
[b] *Biochemistry, Faculty of Veterinary Medicine, Banha University, Qaliobia, Egypt.*
[c] *Department of Biochemistry, Faculty of science, Zagazig University, Sharkia, Egypt.*
[d] *Botany and Microbiology, Faculty of science, Zagazig University, Sharkia, Egypt.*

Abstract

Sixty-two bacterial isolates were collected from clinical specimens of patients suffering from a bacterial infection. These bacterial isolates were obtained from 8 different specimens: urine, pus, sputum, Blood, Pleural fluid, Endotracheal aspirate, the central venous catheter, and a swab from the chest tube. Morphology and common laboratory biochemical tests carried out on the bacterial samples were grown on different isolation media namely: Blood agar, MacConkey agar, nutrient agar and CLED agar media and the biochemical tests are Coagulase, Catalase, Oxidase, Urease, Citrate utilization, Indole, Lysine Decarboxylase, Ornithine Decarboxylase, H2S production and TSI (Triple sugar iron agar test). The results showed that there are three different types of Gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. The Antibiotics assay was performed by using 12 type of antibiotics using the disc diffusion methods and using the Well diffusion method to measure and record the inhibition zones produced by *Nigella Sativa* and *Lawsonia inermis* (Henna) extracts against the obtained Gram-negative bacterial isolates. The antimicrobial activity of *Nigella Sativa* extract against *Klebsiella pneumonia*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* has inhibition zones respectively are 14,17 and 11mm with concentration 50mg% only however, *Lawsonia inermis* extract inhibition zones are [16 mm: 32 mm] with 10, 20, 25, and 50 mg% concentrations for all tested bacterium. The antimicrobial effect of *Nigella Sativa* nanoparticles was greater than *Nigella Sativa* extract in the case of *Acinetobacter baumannii* inhibition zone is 18± 1 mm followed by the effect against *Klebsiella pneumoniae* with 15± 1 mm diameter and no effect or no clear zone against Pseudomonas aeruginosa strain.

Keywords: Plant nanoparticles; Antimicrobial activity; plant extracts; *Nigella Sativa; Lawsonia inermis(Henna)*

1. Introduction

WHO assesses that about 15% of all hospitalized patients suffer from a nosocomial or hospital-acquired infection. 'Healthcare-associated Infections' (HCAI) occur in a patient under medical care within the hospital or hospital staff, or visitors.**[1]**

80%–87% of HCAIs are caused by almost 12–17 microorganisms for example *S. aureus*, *Enterococcus spp.*, *E. coli*, *Candida species*, *K. pneumoniae and Klebsiella oxytoca*, *P. aeruginosa*, *A. baumannii*, *Enterobacter spp.*, *Proteus spp.*, *Bacteroides spp.* and other pathogens **[2-3-4].** (20%–40%) from Gram-negative microorganisms have a much higher rate of resistance than others.**[2]**

The Antimicrobial agents helped us compact with the diseases. However, the inappropriate use of these antimicrobials has formed new difficulties. Antibiotic resistance is " The capability of bacteria to protect themselves against the effects of an antibiotic". The appropriate and unsuitable use of antibiotics in the

*Corresponding author e-mail: shimaa.salah@science.zu.edu.eg.; (Shaimaa, S. Naaom).
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past years has the drive to increase the appearance and expansion of bacteria that are resistant to antibiotics. Resistance against antibiotics can either be natural or gained**.[5]**

So that, unavoidable starting for plant-derived new drug discovery long for the identification of the accurate eligible plant by applying maxim on conventional medicine, documentation procedure, tribal non-documented information, an exhaustive literature review. All of these confirm that the appearance of plant secondary metabolites and the axial factors for plant-derived- drugs function, these are currently being strived to be focused on their potentiality of changing the production of bioactive compounds via tissue culture and relevant mechanisms.

The broad spectrum of therapeutic usage of plant-derived drugs results in change within the production of bioactive compounds through an attractive alternative passage of biotechnological production systems. The advent of molecular drug designing supplied a new hope of higher success for the novel approach of integrated drug discovery from plant-derived sources. **[6]**

Plant-derived chemicals are a large group of chemical compounds that have been found naturally in plants. The wide entity of these compounds has demonstrated valuable features in terms of antioxidant, antibacterial, and antifungal activities. They can return the clinical application of older antibiotics by increasing their potency and consequently avoid the development of resistance. Some of the plants and/or plant elements containing antimicrobial activities and are commercially accessible to customers based on their chemical structures, they will be classified into many major groups that include alkaloids, sulfur-containing compounds, terpenoids, and polyphenols**.[7]**

Nanomaterials are materials that have at least one dimension (1–100 nm) in the nanometer scale range or whose basic unit in the three-dimensional space is in this range **[8].** The development of nanoparticle (NP) technology has accompanied favorable broad-spectrum NP-antimicrobial agents due to their large physiochemical and functionalization features. In fact, nanoparticles are antimicrobial agents that can unlock the restrictions experienced by classical antimicrobial agents**.[9]**

Synthesis of nanoparticles using plants has dragged more attention from workers because it provides one single step in the biosynthesis operation. Plants bid an excellent option for the synthesis of nanoparticles, as in the protocols including plant sources are free from toxicants; moreover, natural covering agents are readily provided by the plants. **[10]**

This study aimed to evaluate the antimicrobial activity of some plant extracts and Plant nanoparticles against Gram negative bacteria isolated from clinical samples for discovering new effective drugs and safe alternatives to antibiotics for controlling nosocomial infection.

2. Experimental

2.1. Collection of samples

Sixty-two samples were collected from many various patients affected by bacterial infections in Kasr Al-einiy hospital in Cairo, Egypt, from April 2017 to May 2018. Studied samples included urine, wound swabs, Swab from chest tube, sputum, pleural fluid, Endotracheal aspirate Sample, Central Venous Catheter and blood samples.

2.2. Morphological and Biochemical *identification*

Samples were inoculated on Petri plates with blood agar, MacConkey, and nutrient agar. The plates incubated aerobically at 37º C for 24 – 48 hrs. Then Morphological and biochemical identification of bacterial isolates were done by standard methods according to **[11-12-13].**

2.3. Antibiotic sensitivity testing

Antibiotic sensitivity testing was done on Muller Hinton agar with disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations **[14].** The diameter of the inhibition zone was measured and interpreted as susceptible, intermediate or resistant as shown in table (1).

2.4. DNA extraction

DNA was extracted from isolates using the QIAamp DNA Mini Kit Catalogue no.51304, Ethanol 96% Applichem. Preparation of PCR Master Mix according to EmeraldAmp GT PCR master mix (Takara) used for cPCR Code No. RR310A. The Oligonucleotide primers used in cPCR as shown in table (2) (Source: Metabion (Germany))

Temperature and time conditions of the primers during PCR are shown in table (3) according to specific authors and Emerald Amp GT PCR master mix (Takara) kit.

Table (1): 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

Table (2): The Oligonucleotide primers used in cPCR

Gene	Primer ID	Primer	Primer Sequence 5'-3'	Amplified product	Reference
16S rRNA	-	F27	AGAGTTTGATC MTGGCTCAG	1485 bp	Lagacé et al., 2004 [15]
		R1492	TACGGYTACCTT GTTACGACTT		

Table (3): Temperature and time conditions of the primers during PCR

Gene	Primer	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
16S rRNA	F27	94°C – 15 min.	94°C – 30 sec.	56°C – 1 min.	72°C – 1 min.	35	72°C – 10 min.
	R1492						

The DNA Molecular weight marker is Gel Pilot 100 bp plus ladder (cat. no. 239045) supplied from QIAGEN (USA) Number of bands: 11 Size range: 100-1500 bp. The ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

2.5. Agarose *gel electrophoresis with modification [16]*

Electrophoresis grade agarose (1 g) was prepared in 100 ml TBE buffer (Tris borate EDTA (TBE) electrophoresis buffer (1x)) **[17]** in a sterile flask, it was heated in microwave to dissolve all granules with agitation and allowed to cool at 70˚C, then 0.5µg/ml ethedium bromide (10 mg / ml) was added and mixed thoroughly.

The warm agarose was poured directly in gel casting apparatus with desired comb in apposition and left at room temperature for polymerization.

The comb was then removed, and the electrophoresis tank was filled with a TBE buffer. Twenty µl of each PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to the UV cabinet. The gel was photographed by a gel documentation system.

2.6. Plant material and extraction:

Thirty grams of fine powder of *Nigella Sativa* and *Lawsonia inermis* (Henna) was mixed with 100 ml of ethanol plants were grouped from the Horticulture Research Institute (HRI) in Dokki Cairo, Egypt. Plants were ground to form a fine powder. Extracts were prepared with the help of the Soxhlet apparatus [18]. This step was done at the Central Lab for Soil, Foods and Feed stuff (CLSFF) at Zagazig University.

2.7. Antimicrobial assay of plant extracts by well diffusion method:

Inhibitory activity of *Nigella Sativa* and *Lawsonia inermis* extracts was examined by a 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 was prepared were placed into each well separately. The plates were incubated for 24 h at 37 °C and inhibition of growth was examined by a clear zone surrounding each well that was interpreted as less active, moderately active and highly active with ≤ 10 mm, 11-14 mm and ≥15 mm, respectively.

2.8. Preparation of Nigella Sativa nanoparticles

5 grams of KOH 97 % Powder weighed and add it to 5 grams of *Nigella Sativa* grinding seeds place the previous mixture in the oven at 500 C for 2 hours. After releasing the mixture from the oven filtrate, it using distilled water. The filtrate now contains nanoparticles of *Nigella Sativa*. This step was done at the Central Lab for Soil, Foods and Feed stuff (CLSFF) at Zagazig University according to [19].

2.9. Characterization *of Nigella Sativa nanoparticles*

2.9.1. Dynamic Light Scattering, Zetasizer Nano ZS (Malvern, UK)

Size Distribution Report by Intensity was done in Egyptian Petroleum Research Institute EPRI, Analysis & Evaluation Department, GC lab, Nanotechnology Centre, DLS Lab

2.9.2. The effect of Nigella Sativa nanoparticles on most resistant bacterial strain:

The antibacterial activities of *Nigella Sativa* nanoparticles were determined by the well diffusion method according to [20]. Inhibitory activity of *Nigella Sativa* nanoparticles was investigated by a well diffusion method. An overnight culture of isolated 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* nanoparticles were placed into each well separately. The plates were incubated for 24 h at 37 °C and inhibition of growth was examined by a clear zone surrounding each well that was interpreted as less active, moderately active and highly active with ≤ 10 mm, 11-14 mm and ≥15 mm, respectively.

3. Result and Discussion

Sixty two samples were collected from many different patients suffering from bacterial infections. The bacterial isolates were collected from urinary tract infections (urine), wound infections (pus), respiratory infections (sputum), Blood infection, Pleural fluid, Endotracheal aspirate, central venous catheter and swab from chest tube as 23, 17, 12, 6, 1,1,1 and 1 isolate with percentages about 37.09 %, 27.41 %, 19.35 %, 9.67 %, 1.61 %, 1.61 %, 1.61 % and 1.61 % of the total isolates; respectively shown in table (4). All isolates are Gram-negative according to Gram's stain reaction.

The result of this study revealed that the most common sites of nosocomial infection were urinary tract, wound infections and respiratory tract infection that relatively with result obtained by **[21]** who detected that the most sites infected were respiratory tract, urinary tract, surgical sites and blood stream.

3.1. Morphological *and Biochemical identification*

The bacterial isolates were grown on different isolation media namely: Blood agar, MacConkey agar, nutrient agar and CLED agar media. Growth motility and Gram staining of the isolates are shown in table (5).

Table (4): Percentage of all isolated bacteria according to sample source of isolation.

Sample Source	No. of isolates	%
Urine (urinary tract infection)	23	37.09
Sputum (respiratory tract infection)	12	19.35
Pus (Wound infection)	17	27.41
Blood infections	6	9.67
Pleural fluid	1	1.61
Endotracheal aspirate	1	1.61
central venous catheter	1	1.61
swab from chest tube	1	1.61
Total	62	100

Table (5): Morphological characteristics of bacterial isolates

Group	Gram stain	Motility	Blood agar	MacConkey agar	Other morphology
I	-ve bacilli	Non motile	Non-hemolytic grayish white mucoid or creamy smooth colonies	large mucoid Pinkish color colonies or transparent and small	Fishy smell grayish white mucoid on Nutrient agar
II	-ve bacilli	motile	β-hemolysis with dark greenish-blue pigmented colonies or non – hemolytic mucoid colonies	Pale color colonies or bright pink mucoid	Large flat spreading colonies with blue-green pigment on Nutrient agar

+ve = positive, -ve = negative, v = variable, (A/A) =Acid slant/acid butt with gas, (K/K) =Alkaline slant/alkaline butt /no gas, TSI= Triple sugar iron agar test

Table (6): Biochemical identification of most resistant bacteria

Biochemical tests	Group I		Group II
Coagulase	-ve	-ve	-ve
Catalase	+ve	+ve	+ve
Oxidase	-ve	-ve	+ve
Motility	-ve	-ve	+ve
Urease	-ve	+ve	-ve
Citrate utilization	+ve	+ve	+ve
Indole	-ve	-ve	-ve
Lysine Decarboxylase	v	+ve	-ve
Ornithine Decarboxylase	-ve	-ve	-ve
H2S production	-ve	-ve	-ve
TSI	K/K	A/A	K/K

The biochemical characteristics of isolated bacteria were divided into 2 groups were, Group I: Non motile, Gram -ve and non-hemolytic bacteria some of them are negative urease, oxidase, indole, Ornithine Decarboxylase and H2S production but positive Catalase, citrate and variable Lysine Decarboxylase and TSI is (K/K)= Alkaline slant/alkaline butt with no gas so they are identified as Acinetobacter species, while some of this bacteria are positive results to Lysine Decarboxylase, Urease and TSI is (A/A) =Acid slant/acid butt with gas they are identified as Klebsiella pneumoniae.

Group II: Motile, Gram –ve bacilli β-hemolysis with dark greenish-blue pigment positive to the following biochemical tests Catalase, Oxidase, Citrate but negative to coagulase, urease, indole, Lysine Decarboxylase, Ornithine Decarboxylase, Ornithine Decarboxylase,H2S and TSI is (K/K)= Alkaline slant/alkaline butt with no gas. This type of bacteria identified as Pseudomonas aeruginosa. Results of biochemical identification illustrated in table (6)

3.2. Antibiotic sensitivity of the bacterial isolates

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 (32.4%), Levofloxacin, Ciprofloxacin with 25.8%, 22.5, respectively. On the other hand, the results showed that 100% of bacterial isolates were resistant to Ceftazidime.

While in the study of **[22]** reported that the results of disk sensitivity testing for *P. aeruginosa* showed 35 (77.7%) of P. aeruginosa isolates were sensitive to Cefotaxime. **[23]** reported results of antibiotic sensitivity showed that *K. pneumonia* mostly sensitive to Amikacin and Imipenem (22.22%). The susceptibility rate of *A. baumannii* isolates was 80% for tigecycline and 53.3% for carbapenem in **[24]** study.

Table (7): Shown the number and percentage of sensitive and resistant isolates against antibiotics used

	SAM	MEM	KZ	FOX	FEP	CRO	Caz	GM	AK	LEV	CIP	SXT
S count	1	22	1	8	13	1	0	13	29	16	14	16
S %	1.61	35.4	1.61	12.9	20.96	1.61	0	20.96	46.77	25.8	22.58	25.8
R count	61	40	61	54	49	61	62	49	33	46	48	46
R %	98.83	66.51	98.83	87.09	79.03	98.83	100	79.03	53.22	74.19	77.41	74.19

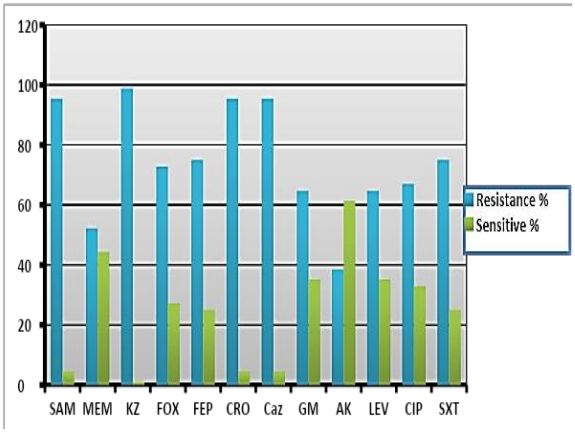

Fig.1 The number and percentage of sensitive and resistant isolates against antibiotics used.

3.3. PCR among different isolates

3.3.1. Detection of 16S rRNA gene positive by PCR

Detection of 16S rRNA gene by PCR was carried out in three different isolates of the study, all of them showed expression of 16S rRNA gene as shown in Fig.2. From the obtained results of 16S rRNA gene PCR amplification of (1485 bp product) fragment of 16S rRNA of different isolates. Lane(L): DNA ladder (1500 bp.) Lane(1-3): samples Lane (4): positive control , Lane 5: negative control

3.3.2. Effect of plant extract against

The ethanolic plant extracts which derived from *Nigella Sativa* and *Lawsonia inermis* (Henna) are screened for its antibacterial activity against the three identified Gram- negative bacterial pathogens namely *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa.*

It was clear from the obtained results in table (8) 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*.

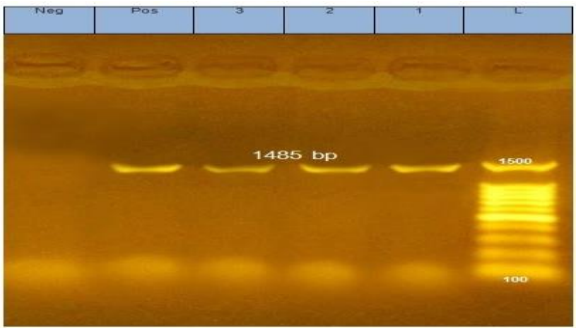

Fig.2 Agarose gel electrophoresis showing amplification of 1500bp. fragment of 16S rRNA gene of different isolates . Lane(L): DNA ladder (1500 bp.) Lane (1-3): samples Lane (4): positive control , Lane 5: negative control

Table (8): The inhibitory effect alcoholic extract of *Nigella Sativa* against *Pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter baumannii*

Bacterial isolates	***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* (Henna) extract against *Pseudomonas Aeruginosa* shows maximum inhibition zone as shown in table (16) for four different concentrations at 10 mg % (21mm), 20 mg % (23mm), 25 mg % (25mm) and 50 mg % (32mm). The other types of bacteria shows less inhibitory activity compared with *Pseudomonas Aeruginosa* as shown in table (9)

Table (9): The inhibitory effect alcoholic extract of Lawsonia Inermis (Henna) against Pseudomonas Aeruginosa, Klebsiella Pneumoniae and Acinetobacter Baumannii

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

Taylor *et al*., proved that Negative results do not indicate the absence of bioactive components; the active compounds may not be enough to show activity with the used concentration **[25].**

Erdman *et al*., show that the various sources of extracts, agro-climatic factor, jugglery of the experiment, and phytochemical components in the extract also share in the variations in gained results **[26].**

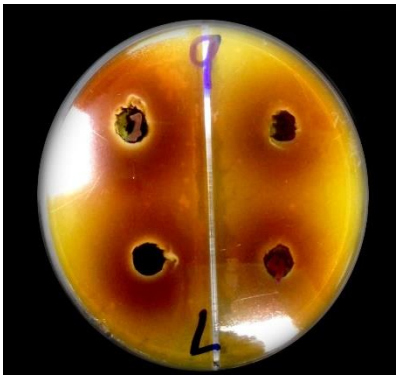

Fig.3 Effect of *Lawsonia inermis* extract on *P.aeruginosa*

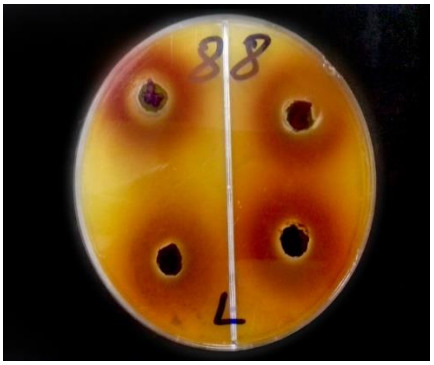

Fig.4 Effect of Lawsonia inermis extract on Acinetobacter baumannii

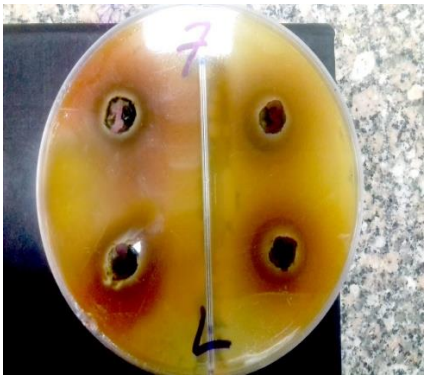

Fig.5 Effect of Lawsonia inermis extract on Klebsiella pneumoniae

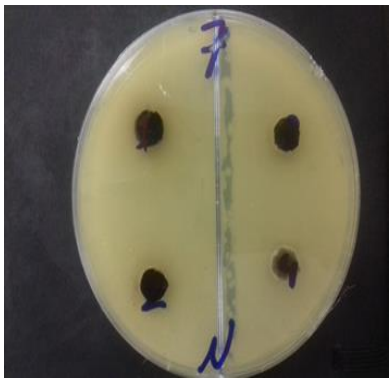

Fig.6 Effect of Nigella Sativa extract on K. pneumonia

Fig.7 Effect of Nigella Sativa extract Acinetobacter Baumannii

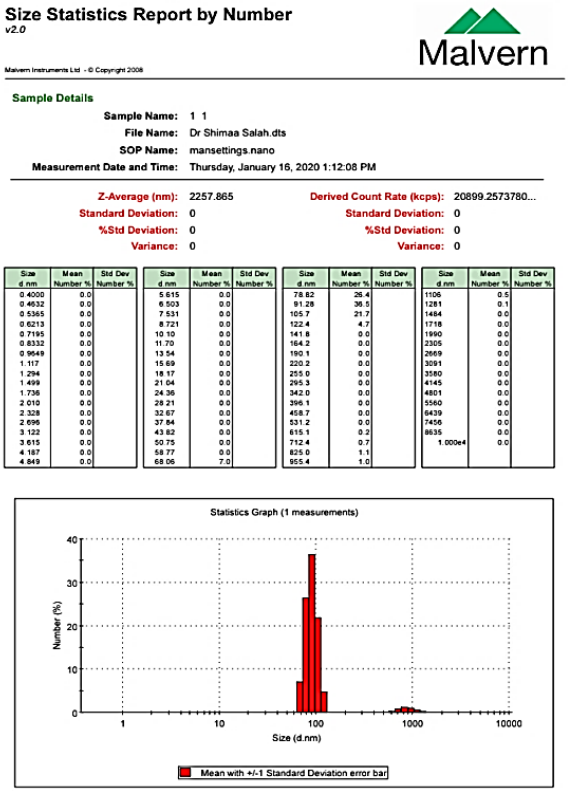

Fig.9 Size Static Report by number for Nigella Sativa nanoparticles

3.4. Characterization of Nigella Sativa nanoparticles

3.4.1. Size Distribution Report by Intensity

Using Dynamic Light Scattering, Zetasizer Nano ZS (Malvern, UK) to measure the size of nanoparticles. The size report shows that there are nanoparticles with size 90.94 nm of *Nigella Sativa* nanoparticles as shown in figure (8) and the average size report is shown in the figure (9).

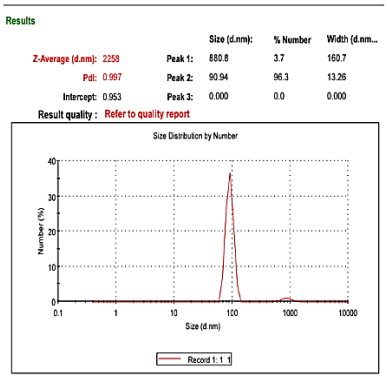

Fig.8 The Second Size Distribution Report by Intensity of Nigella Sativa nanoparticles

3.4.2. The effect of Nigella Sativa nanoparticles on Gram-negative bacterial strain:

In this study *Nigella Sativa* nanoparticles were screened for its antibacterial activity against the multi drug resistant Gram-negative bacteria (MDR), for three identified bacterial pathogens namely *Pseudomonas Aeruginosa*, *Klebsiella Pneumoniae*, and *Acinetobacter Baumannii*. The results in fig.10,11,12 and table (9) show that the highest effect of *Nigella Sativa* nanoparticles against *Acinetobacter Baumannii* with clear zone is 18± 1 mm followed by the effect against *Klebsiella Pneumoniae* with 15± 1 mm diameter and no effect or no clear zone against *Pseudomonas Aeruginosa* strain.

Table (9): Effect of different concentrations of Nigella Sativa nanoparticles on Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii

Bacterial isolates	**Clear zone diameter (mm) *Nigella Sativa* nanoparticles**
Klebsiella pneumoniae	-
Acinetobacter baumannii	15± 1
Pseudomonas aeruginosa	18 ± 1

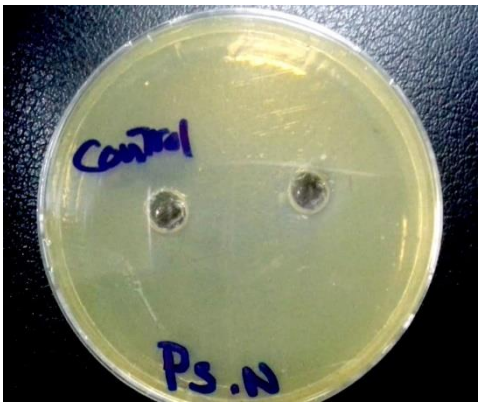

Fig.10 Nigella Sativa nanoparticles on Pseudomonas aeruginosa

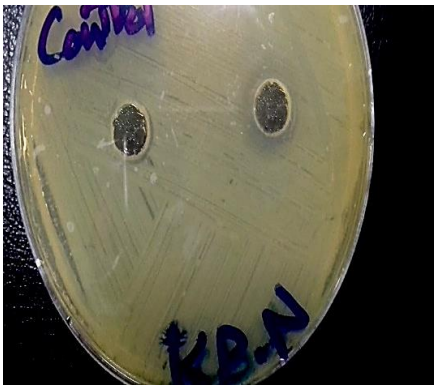

Fig.11 Nigella Sativa nanoparticles on Klebsiella pneumoniae

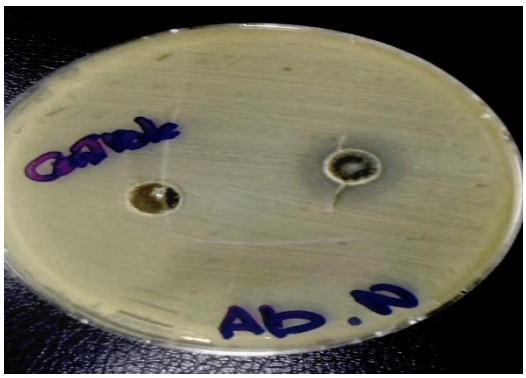

Fig.12: Nigella Sativa nanoparticles on Acinetobacter baumannii

The results show that the highest effect of *Nigella Sativa* nanoparticles against *Acinetobacter Baumannii* with clear zone is 18± 1 mm followed by the effect against *Klebsiella Pneumoniae* with 15± 1 mm diameter and no effect or no clear zone against *Pseudomonas Aeruginosa* strain.

It is similar to the study of **[27]** that the measurement using particle size analyzer DelsaNanoTM, can be complemented that (*Coleus Atropurpureus (L.) Benth*) Miana leaves extract has fulfilled the nanoparticles requirement since it still on range 10-1000 nm amounted 89,77 nm, and this study showed that Nanoparticles using Miana leaves extract testing were obtained to ensure that Miana leaves extract powder as yet has an antibacterial activity after the drying process. However, this testing also obtained to confirm that nanoparticles form more efficiently since the administration of medicine is well sufficient. Previously, it had been stated that Miana leaves extract amounted to 2,5% equal with nanoparticles powder 1,17%. The result be relieved that inhibition diameter for nanoparticles powder larger than the extract in the same concentration and proved that Nanoparticles using Miana leaves extract produce antibacterial activity greater than the extract under the same concentration regarding *S. Aureus* bacteria (14,43 mm / 14,03 mm) and *E. coli* (16,93 mm / 15, 25 mm).

4. Conclusions

Medicinal plants, namely, alcoholic extracts of *Nigella Sativa* seeds and *Lawsonia inermis* leaves, can be used as antimicrobial agents and the formation of *Nigella Sativa* nanoparticles from its alcoholic extract was observed by measure the size of nanoparticles. The size report shows that there are nanoparticles with a size of 90.94 nm. *Nigella Sativa* nanoparticles can inhibit the growth of *Acinetobacter baumannii* and *Klebsiella pneumoniae bacteria,* and the antimicrobial activity of the *Nigella Sativa* nanoparticles is better than the antimicrobial effect of alcoholic extracts of *Nigella Sativa* seeds, while the antimicrobial effect of alcoholic extracts of *Lawsonia inermis* leaves on *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* bacteria is the best compared with

5. Conflicts of interest

"There are no conflicts to declare".

6. References

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