Study of antimicrobial activity of zinc oxide nanoparticles and Phenol extract from *Nigella Sativa* L.

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Abstract

Background: This study investigate the antibacterial activity of phenol extract from *Nigella Sativa* L and Zno nanoparticles toward *staphylococcuse aureas* and *Escherichia coli*. **Objective:** Detecting the phytochemical of plant by using reagent, separation phenol compound from alcohol extract and studding antibacterial of phenol extract and Znonanoparticl. **Patients and methods:** the antibacterial test detected by disc diffusion methods at concentration (25,50,75 and 100 mg/ml) for phenol extract , while Zno nanoparticles at concentration (0.2 , 0.3, 0.4 and 0.5 mg/ml). **Results:** the inhibition zone of phenol extract in concentration 25 mg/ml was 16.11 mm, 15.11 mm for S. aureus, E .coli respectively, while concentration 50 mg/ml in S. aureus, E.coli 17.44mm, 16.12mm respectively, while concentration 75mg/ml in S. aureus 19.22mm, E.coli 18.34 mm , concentration 100 mg/ml in S. aureus 21.12mm, E.coli 20.12 mm. While the inhibition zone of Zno nanoparticles was 1.23 mm, 2.23 mm against S. aureus ,E .coli respectively with concentration0.2 mg/ml, while concentration 0.3mg/ml in S. aureus 1.99mm, E.coli 3.54mm, while concentration 0.4mg/ml in S. aureus 2.76mm, E.coli 4.11 mm , concentration 0.5 mg/ml in S. aureus 3.65, E.coli 4.11 mm. **Conclusion:** This effect due to the ability of ZnO nanocomposite to disrupt genes for resistance to oxidative stress, and increase the ROS, which contributes to eradication of bacteria by stimulating macrophages and production of cytokines. Also disrupt the bacterial cell wall (positive, negative) by affecting the osmosis of the cell wall, so the cells treated with this compound appear wrinkled and irregular in shape.

Keywords: Nigella Sativa L , Phenol, Zno nanoparticl. S. aureus, E.coli.

Introduction

Nanotechnology (NPs) is a different core area of information technology because of its greatly effect in many fields ex. medicine, industrial and agricultures *etc* **(1).**Nanotechnology dealing with nanoscale sizes materials, (also called nanoparticles, with sizes of one billionth of a meter. Nanoparticles was use to express one or more components that have 1-100 nm **(2)** range was ZnO nanoparticles are an important field for biologists, due to the distinctive antimicrobial properties they possess Nanoparticles and their outstanding activity that broke new frontiers in biology of science **(3)** especially In its

nanoscale form, it is highly toxic to many microorganisms including bacteria and fungi **(4) .** It was reported that ZnO and Cuo because of nanomaterials whose antimicrobial properties are integrated into a variety of Medicines and skin ointments, and ZnO nanoparticles are used in wall coatings and on hospital floors as antifouling materials for microbes **(5)** Herbal medicines has speedily developments and gain large reception. the majority of herbal drugs was record to has antioxidative activity **(6)** between these natural substances Ns are having multi-use medicinal plant in medicine **(7).**

Nigella sativa (black seed) is consider as a biological modifier. the bioactive and the majority abundant ingredient of the volatile oil was Thymoquinone which has been exposed to have therapeutic effects, including antimicrobial , anticancer, antihypertensive , antidiabetic , and anti-oxidants agent **(8).** The secondary metabolites found in the various part of the plants that could be pharmacologically utilized. Phytochemical efficacy serves as source food supplement, folk medicine and also as chemical precursor for synthetic drugs **(11)** ordinary phenolic compounds are well-known in the plant kingdom **(12).**They are categorized into (A) phenolic acids (B) flavonoid polyphenolics (c) non-flavonoid polyphenolics **(13)**.

Material and method

1- plant collection

Nigella Sativa L was taken in Karbala province in 2/8/ 2022 after cleaning and removing alien objects. As the seed were washed 3 times with running water and once with D.W. Each dry component underwent electrical grinding. Up to the time of usage, the processed components are stored in the refrigerator in 40C **(17,18).**

2-Alcoholic extracts

Alcoholic extract was made by using 100g of powdered material, 100ml of ethanol alcohol solvent (70%) in a 500-ml flask for extraction by soxhelet apparatus through 24 hours and using a rotary evaporation apparatus **(19, 21).**

3- Separation phenolic compounds

1- Take ethanol alcohole extract acidify with (2M)HCL (PH <3).

2- Put extract in separation funnel and washed by chloroform (CHCL3).

3- Mixing .

4- Forming two layer , Take lower layer .

5- Repeat this steps two or three time.

6- Phenols collection was dried in oven (30 – 20) °C .

7- keeping Phenol at 4˚C till used .The method is used by **(22).**

4- Secondary metabolism screen study :

4-1 :Saponins

The studied samples (2.5 mL) were added to sterile distilled water (10 mL) in a test tube to identify saponins using the foam index method.It was then covered and aggressively shook for around 40 seconds. They were permitted to remain standing for perhaps 30 minutes after that.The presence of saponinsis shown by the honey comb foam **(22).**

4-2: Phenols

Examination to lead acetate, the production of precipitous was seen when 1% lead acetate (0.5 ml) added to 5 mg parsley oil extract.

4-3 :Glycosides

When melting 0.5 mg from parsley oils extract to 1 ml ofcreation of the color yellow.H2O and NaOH were added.

5- Determination of antimicrobial action

It was approved out according to disc diffusion method **(25)** the plate of Muller – Hinton agar media was inoculated with Microorganisms

(*E.coli ,S.aureus*) with sterile swabs . six mm sterile paper discs made from Whattman No. 1 were impregnated with phenol extract with different concentrations (25,50,75 and 100 mg/ml). By serial forceps the discs were position on the inoculated plate and pushed gently into agar . Each plant extract was assayed in triplicate. Sterile paper discs loaded with DMSO were used as negative control .The discs were placed aseptically and distinctively onto the inoculated MHA plates. Agar plates were incubated at 37°C for 16-18 hours . After that, the inhibition zones were measured by ruler (mm).

6- Ethical consent:

The study was sanctioned by the Academic and Ethical Committee of AL-Furat AL-Awsat technical university / AL-Mussaib Technical Institue . All participants agreed to participate in the study after signing an informed written

7- Statistical Analysis:

Mean was used to express the data.One-way analysis of variances was used to assess the statistical significance of differences between the control and other groups (ANOVA).The SPSS for Windows version was used for statistical analysis, and P values of 0.05 or less were considered significant (SPSS, Inc., Chicago, Illinois).

Results

1- Percentageyeild of Alcohol and volatile oils extracted

Table (1) Percentage of Alcohol ,volatile oils phenol extracted:

Extracted	**Phenol**
N. sativa	**3.8/100*100=3.8%**

The results of table (1) showed that Percentage of phenol extracted for*N. sativa* was at its proportion (3.8%).

Table (2) Secondary metabolism compoundsscreen of *N. sativa*.

Reagents	**Alcohol extract**
Alkaloid	+
Phenol	+
Glycoside	+
Flavonoid	+
Saponin	+
Tannin	+

The result of phytochemical screening of alcohol extract of *N.sativa*seed in table (2) showing alcohol extract that high positive reaction with used reagent , Alkaloid , Phenol, , Glycoside, and Flavonoid , Saponinand tannin.

Table (3) shows effect the phenol extract from *N.sativa*and their concentrations in the zone of inhibition on bacteria developmen:

Concentrations mg/ml	***S. aureas***	**E.coli**	**Control**
25	16.11	15.11	0
50	17.44	16.12	0
75	19.22	18.34	0
100	21.12	20.13	0
LSD =	1.82	1.92	

Table (4) shows effect the concentrations Znonanoparticles in the zone of inhibition on bacteria developmen:

Concentrations mg/ml	***S. aureas***	***E.coli***	**Control**
0.2	1.23	2.23	0
0.3	1.99	3.54	0
0.4	2.76	3.98	0
0.5	3.65	4.11	0
LSD =	1.78	1.88	

The disc diffusion method was employed in this investigation to ascertain the antibacterial activity of the phenol extracts of *N.sativa* extract and Znonanoparticls.

The phenolic compounds were evaluated for their ability to inhibit the growth against bacteria *E. coli* and *S. aureus*by the disc diffusion inhibition test contrast to tetracycline which is regarded as standard antibiotic as preliminary test . The results were explained in table (3) showing the effect of different conc. (25 ,50, 75 and 100 mg/ml) of phenolic compounds which increased the inhibition zone against bacteria .

The inhibition zone of phenol was 16.11 mm against *S. aureus*, 15.11 mm against *E .coli* in concentration 25 mg/ml, while concentration 50 mg/ml in *S. aureus* 17.44mm, *E.coli* 16.12mm , while concentration 75mg/ml in *S. aureus* 19.22mm, *E.coli* 18.34 mm ,

concentration 100 mg/ml in *S. aureus* 21.12mm, *E.coli* 20.12 mm.

The Znonanoparticl compound were evaluate ability to inhibit the growth against bacteria *E. coli* and *S. aureus*by the disc diffusion inhibition test. The results were explained in table (4) showing the effect of different concentrations (0.2 ,0.3, 0.4 and 0.5 mg/ml) of Znonanoparticl compounds which increased the inhibition zone against bacteria . The inhibition zone of Znonanoparticl was 1.23 mm against *S. aureus*, 2.23 mm against *E .coli* in concentration0.2 mg/ml, while concentration 0.3mg/ml in *S. aureus* 1.99mm, *E.coli* 3.54mm , while concentration 0.4mg/ml in *S. aureus* 2.76mm, *E.coli* 4.11 mm , concentration 0.5 mg/ml in *S. aureus*3.65, *E.coli*4.11 mm.

Discussion

Present this the study medicinal importance of *N.sativa*plant throughout antimicrobial action of phenolic compound extraction. Some of secondary metabolism compounds involving of single substitute phenolic ring which is in the highest oxidation state. The medicine contain phenols, which is effective antibacterial **(26) .**The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compound, possibly reaction with sulfhydryal groups or through more nonspecific interaction with the proteins **(27)**. carboxylic acids were found to be linked with many antimicrobial and antifungal activities which are found to exist in various plant metabolite molecular structures, which had been reported as a strong antibacterial agent **(28).**These result agree with **(29)** the phenolic extracts at concentration 500 mg/ml gave highest inhibition zone for leaves 25mm, fruits 19mm and barks 21mm against *S. aureus.*

The results showed the ability of ZnO nanoparticles to effect on *S.aureas* and *E.coli* when used in different concentrations and showed the ability of high concentration as compared to little concentration. ability of this Nano material to interact with organic compound of surface wall bacteria and destroy it. That led to destroy the cell wall and death of bacteria **(31)** .ZnO NPs antibacterial movement specifically relates with their focus as announced by a few examinations, in a similar manner, the action is estimate subordinate. In any case, this reliance is additionally impacted by convergence of nanoparticles. Bigger surface region and high fixation were responsible for zinc oxide nanoparticles anti-bacterial action **(32)**.This effect is due to the ability of the ZnOnanocomposite to disrupt the genes for resistance to oxidative stress, as well as increase the ROS, which contributes to the eradication of bacteria by stimulating macrophages and the production of cytokines that contribute to directing the immune action and making it more regulated **(33).** The nanocompositeZnO works to disrupt the bacterial cell wall, whether it is positive or negative by affecting the osmosis of the cell wall, so it turns out that the cells treated with this compound appear wrinkled and irregular in shape **(34)**The inhibitory effect of bacteria for some inorganic minerals such as ZnO, MgO, SiO2 and TiO2 has a selective toxic effect on biological systems, which leads to thinking of using them as therapeutic and diagnostic materials in the future, in devices and surgeries based on their inhibitory effect on invading bacterial cells **(35)**.

Conclusions

1- the *N.sativa* including in there phytochemical (saponin , phenol , glycoside , tannin , alkaloid and flavonoid)

2- phenol extract and ZnO nanoparticles proceed in its antibacterial activity again *S.aureas* and *E. coli*.

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