Effect Extract And Nanoparticles of Ginger Root on *Pseudomonas* Bacteria That Isolation From Otitis Infection

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Abstract:

This study was conducted to test the inhibitory activity of alcoholic extracts (using ethanol) of ginger roots, as well as the inhibitory activity of alcoholic and aqueous nanoparticles of ginger roots against (Pseudomonas bacteria) , isolated from cases of ear infection , the natural effective antibacterial chemical compounds contained in the prepared nanoparticles were detected. 100 samples were collected from the patients and patients in Alexandria General Hospital and Morgan Teaching Hospital from October /2022 to April /2023 and included isolates for patient of different ages and genders. The number of bacterial isolates that came from females was 42 and the number of isolates from males was 58. The bacterial isolates calculator tested the directions of 8 antibiotics circulating in health institution. The sensitivity and resistant varied according to nature of the antibiotic and the type of bacterial isolate tested Pseudomonas bacteria, it showed sensitivity against the antibiotic Imipenem, but it had high resistance against the antibiotic Gentamycin. The alcoholic extracts as well as the alcoholic nanoparticles of ginger roots ,showed an effective inhibitory effect on the bacterial isolates , and the nature of the extract and that the inhibitory effectiveness is directly proportional to the increase in concentration , so at a effectiveness of 40 of the extract, the effectiveness was better than the effectiveness of the extract at a concentration of 10 , therefore , the inhibitory activity of alcoholic nanoparticles of ginger root was directly proportional to the increase in concentration , as it was higher at concentration 500 , but at concentration 100 the inhibition was lees than that.

Introduction

Middle ear infection is one of the health problems that exist in many parts of the world, [1-3], and it represents a common health problem among members of Iraqi society and all age groups , and the disease worsens during the years of the siege imposed on us . The high prevalence of the diseases is due to the fact that it is from multiplicity of factors, as chronic rhinitis and sinusitis causes the occurrence of the disease. Some scholars believe that middle ear infections are a complement to viral infections such as cold ,measles and influenza . The use of antibiotics as a topical treatment for the treatment of persistent external ear infections has be the cause cause of disease [4]. It was found that otitis media is anatomically and pathologically related to the upper respiratory tract , and therefore the nasopharynx is a natural reservoir. Many types of bacteria cause this inflammation, including harmful germs and pathogens of middle ear diseases. [5]. Middle ear infection can develop after untreated infection, as well as in cases of improper treatment and resistance shown by bacteria to antibiotics, [6]. To otitis media, which develops rapidly when the upper respiratory tract is infected and extends from the nasopharynx to the middle ear through the Eustachian tube. Perforation of the eardrum leads to an acute inflammation of the middle ear called acute otitis media. [7] Complication of this disease are many include hearing loss, complete deafness, facial paralysis, brain abscess, inner ear infection and mastoiditis [8]. Middle ear infection may be due to bacterial infections, viruses, fungi or allergy.

Nano is a prefix taken from the ancient Gerrk language and means " Nano" in the field of science nano means one of billion (10). The nanometer is used as a unit for measuring the

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lengths of very small particles that can only be seen under an electron microscope. Nanoparticles are small materials. Enough to fall into the Nano scale with one dimension less than a few hundred nanometers and have unique properties such as a high surface – to – volume ratio and unique optical behaviors. otitis media is known as inflammation of the mucous membrane underling the slit of the otitis media and germs are one of the most important causes of its occurrence [9] , And it is considered a serious and common health disease therefore there are tow types of inflammation which are acute otitis media inflammation this type is accompanied by pain and high temperature the second type is known as chronic otitis media inflammation which is accompanied by pus flowing from the hole in the tympanic membrane. The ear consists of three main parts the outer ear (OE) the middle ear (ME) the inner ear (IE) the outer ear of the pinna (pinna) and the external auditory canal (EAC) at the end lies middle ear (ME) is a chamber that contains three bones the malleus the incus ,and the stirrup , the middle ear is connected to the pharynx through the Eustachian tube (TE) the lunate and cochlea [10]. Anatomically the middle ear (ME) is an air cavity surrounded by a mucous membrane located within the bone the temporal bone consists of the squamosin petrous and the membranous (mastoid),(tympanic) and styloid [11] Pseudomonas aeruginosa is known as one of the most widely spread opportunistic human pathogens causing 18 to 63% of infections worldwide [12,13] . It can grow at a temperature of 42 C, this unique character helps to differentiate it from the rest of the Pseudomonas species [14]. Most strains produce water – soluble pigment such as pyocyanin, pyoverdin , pyorubin , and pyomelanin [15,16] . The ability of pseudomonas . aeruginosa to groe in minimum nutritional requirments and to withstand various physical conditional such as disinfectant has assigned this organism to persist both in hospital and community settings [17-18] P. aerugenosa commonly caused a high rate of infection in immunocompromised and cystic fibeosis (CF) patients [19] .[

P. aeruginosa has great diversity and is capable of causing lif- threatening contagious infections in a multifariouseness patients population [20], causing several diseases such as urinart tract infection (UTIs) [21], respiratory tract infection (RTIs) [22] . Burn wounds , skin and soft tissue infections [23] , bacteria keratitis [24] and swimmer ear infection.

This aim was achieved using the following objectives:

1-Isolation and diagnosis of the aerobic bacteria that cause middle ear infection , their diagnosis , and knowledge of the most common bacteria as a cause of these infections.

2-Studying the resistance of isolated bacteria to antibiotics used in the treatment of these infections

3- study the synergistic effect of antibiotics on resistance bacteria

4-Effect of ginger nanoparticles on bacteria that cause otitis media

5-Effect of plant extracts for ginger on bacteria that cause otitis media

Sample collection

The current study was conducted at Alexandria General Hospital of the province of Babylon for the month of October (2022) to Abril (2023) . 100 Samples were collected from patients with otitis media infections after a diagnosis by otolaryngologists the number of males 65 and number of females was 35 with ages ranging from (5-55) year . The number of patient with otitis residing in the urban (68)

people while patients in rural areas were (32)people.

1-Acute otitis

2-Chronic supportive otitis

3-Otitis external

And followed the method of the Scientist (Inde Dharan and Ashraful 1996) in taking swabs by cleaning the outer ear and removing the pus (Discharge) by means of a device that removes the abscess , then the swab was taken from the remnants of pus present in the external auditory canal the swabs were quickly transfer to the laboratory and cultured on the appropriate culture media [25]

Samples were cultured on immediate media

MacConkey agar,

Nutraint agar ,

Brain hart broth,

Brain hart with Glycerol

Methods of preserving and maintaining bacteria

Preparation of short-term culture

The bacteria were distributed on the media of the nutrient agar , according to the instruction of the producing company , and sterilized by autoclave . the bacteria were incubated at a temperature of 37 ◦C for 24 hours , and kept in the refrigerator until use , [26].

Preparing long – term culture

Brain hart media was prepared according to the instruction of the producing company , and 5% of glycerol was added to it . the medium was poured into small heat-resistant plastic tubes and sterilized in autoclaves for 15 minutes at a pressure of 21◦C . at a temperature of 37 for 24 hour . and kept in the freezer at -20◦C until use [27].[

X-ray diffraction (XRD)

The X-ray diffraction was used for characterization of ginger nanoparticle in university of Babylon , the powder of (ginger nanoparticle) was used for test . The nanoparticle sample was dispersed on a minimum background noise sample holder and analyzed in a Bruker D8 Advance x-Ray diffract meter equipped with a Lynx EYE detector . X ray diffraction analysis was operated at a voltage of 40 kv . with current of 40 mA . With copper radiation of 1.54060 A. The scanning was performed in the 20 range of 10 ◦ to 40◦ at 0.02◦/min with time constant of 1.2s

Antibiotics used in the minimum inhibitory dose determination and antibiotic mixing trials

Vancomycin

,Ceftazidime ,Gentamycine ,Flucloxacillin ,Chloramphenicol ,Amikacin ,Tetracycline ,Ciprofloxacin

Application of Antibiotic Discs

1-The antibiotic discs were placed on to the surface of the inoculated agar plate each disc was pressed gently to ensure complete contact with the agar surface by using flamed

The plats were inverted and placed in an incubator at 37C◦ for 18 hr .2-

After incubation, the diameter of growth inhibition zone was measured in millimeter using ruler 3-

4- The results were compared with the inhibition diameter of the CLST(2019)

Collect ginger roots

Buying ginger from the local market , washing it well , it was cleaned from the suspended dust with water and salt for 3 minutes , then washed a second time with distilled water . it

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was spread on a clean cloth and left to dray at room temperature for a period of tow to three weeks . then it was ground by an electric mill into a dry powder and kept in plastic bags in dry place until use. take (10,20,40) g of dry ginger root powder and mix it with (100) ml of ethanol alcohol separately and putting the solution in a device (shaker) for stirring purpose , then leave the solution for 24 hours at room temperature 25◦C . after that , the solution was filtered using layers of medical sterile gauze to get rid of the remnants the vegetable powder , and then kept in the refrigerator until use. The diffusion method was used by etching according to [28] . bacterial isolates were grown at a dilution of 15 according to the McFarland tube on the surface of a nutrient agar . the plate was left for a period at room temperature . holes were made with a diameter of 6 mm. then different concentrations of ginger rot extract , water and alcoholic ginger root extract were added to the etching separately and at different concentration (5,10,15), (10,20,40) mg /ml add it to the positive control hole that contain distilled water . the dishes were left at room temperature for tow hours , then incubated for 24 hours at a temperature 37◦ C . after that , a ruler was used to measure the diameters of the inhibition zones formed around the holes.

Preparation nanoparticles from ginger root

The nano solution was prepared at the industrial Research and Development Authority – chemical Research center and petrochemical industries in the Ministry of industry and Minerals , and a method was adopted [29]. to prepare100gm of nano ginger , ginger powder was added to 100 ml of distilled water , then mix and melt the mixture over low heat to dissolve all sediment and distribute the solution was placed on plates and left to dry for tow days . after tow days , the powder was collected from the plates the weight of 20 gm of dried ginger powder was added to 800 ml of distilled water and the mixture was applied in the vibra-cell ultrasonic liquid device for an hour and a half , as this device operates for 10 minute , stopping for 5 minute , then the mixture was collected and preserved in the refrigerator at 4◦C until use. The diffusion method was used by etching according to . bacterial isolates were grown at a dilution of (1 .5) cell/ ml according to the McFarland tub on the surface of a nutrient agar . the plate was left for a period at room temperature . holes were made with a diameter of 6 mm. then different concentrations of Ginger root nanoparticles , alcoholic ginger root nanoparticles ,were added to the etching separately and at different concentration (100,300,500) mg /ml add it to the positive control hole that contain distilled water . the dishes were left at room temperature for tow hours , then incubated for 24 hours at a temperature 37◦ C . after that , a ruler was used to mesure the diameters of the inhibition zones formed around the holes.

Result and Discussion

One hindered sample were collected for the period from October /2022 to April /203/23. They included samples for patients of different ages and gender. their source was otitis sample collected from ALExandria General hospital and Murjan Teaching Hospital as a show in table 1,2

TABLE 1: Distribution of positive and negative Growth Culture of otitis Sample

Subjects	No.of samples
Positive growth	100
No growth	12
Total Sample	112

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TABLE 2 : Distribution of Gram positive and Gram Negative Bacteria from Otitis sample

Bacterial samples	No. of bacteria
Gram positive	27
Gram negative	73
Total samples	100

This study showed that gram positive sample recovered from otitis samples that gave positive growth culture were 27 (27%), these samples were diagnosed according to morphological , cultural and biochemical test. The reason for negative culture results either may be due to Contam or because Viral or fungal infection. As a show in table 3 .

TABLE 3 Distribution of study groups by gender

	Patients
Males	58
Females	42
Total	100

The results of the current study agreed with the results of the researcher [30]., as the study showed a higher number of people with middle ear infection of the male sex compared to female sex . as a show in table 4.

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TABLE 4 Distribution of study groups according to age groups

Age group (years)	Patients
1-15	35
16-30	26
31-45	15
46-60	24
Total	100

TABLE 5 Distribution of patient groups ,disease state

Pathological	Study group
A cute	33
Chronic	67
Total	100

The results of the current study agreed with the results of the researcher [31]. , which he conducted in Babylon Governorate ,that the incidence of chronic otitis media is higher than acute otitis media . as a show in table 6

.**TABLE 6** Geographical distribution of study samples

	Study Group
Urban	72
Rural	28
Total	100

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The results of the current study agreed with the study of the researcher [32]. that the incidence of middle ear infection in urban areas increased

.**Resistance of bacteria to antibiotics**

A sensitivity test was conducted for Pseudomonas bacteria against different type of antibiotics , where the bacteria showed high sensitivity against (IEL) and showed resistance against the antibiotic Gentamycin .The results of the research do not agree with the finding of another study, where the researcher considered Gentamycin to be one of the preferred drugs in the eradication of this disease] 33 .[Another researcher believes that is the Gentamycin is the most effective in treating this [34]

Despite the predominance of Staphylococcus in acute otitis media and the predominance of pseudomonas bacteria in supportive and chronic otitis media, the bacteriological finding showed that there were no distinct differences and differences between children and adults with regard to the bacteria causing the infection . Pseudomonas bacteria caused cases of otitis media in patients whose ages ranged between (6 and 10) years , as well as cases of acute otitis media for children of different ages . On the other hand , Staphylococcus bacteria caused inflammation in adults , as well as infections in infants as well as adolescents . We conclude that there is no relationship between the microbial content and the age of the patient, and this result is consistent with what some researchers have shown[35] . It does not agree with the findings of another researcher , who confirms that Staphylococcus bacteria were isolated at a higher rates in children and Pseudomonas bacteria where isolated at a higher rates in adults [36]

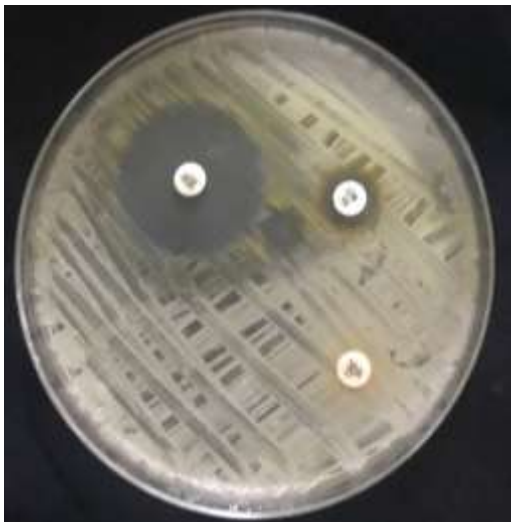

Figure 1 Effect antibiotic on Pseudomonas

X-ray diffraction

The formation of nanoparticles was characterized further by XRD analysis using powder X-Ray Diffractometer The formation of nanoparticles were characterized further by XRD analysis using powder X-Ray Diffract meter .The studies showed a characteristic peak at 20 value of 23.601, 29.896 and 44.015,

D=(K*ƛ)/(β*cosØ(

Where D is the crystal size , K is a constant whose value is approximately 0.9, ƛ is the wavelength of the X-ray , β is the full width at

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half maximum (FWHM) of the peak in radians, and Ø is the Braggs diffraction angle in radians .

Investigation of the effect of aqueous Ginger extracts on pseudomonas bacteria

It was noticed that the aqueous extracts of ginger root had an effect on all types of selected isolates , and with different concentrations of the extracts , except for concentration of the extract except for concentration 10. It was observed that there was no effect on the isolates ,and that the rate of inhibition zones was very few and was not calculated , so it counted as zero in all isolates as for concentration 20,40 they showed different inhibition zones , and according to the types of isolates, the zones of inhibiting ranged according to the following

As for pseudomonas bacteria , it was less affected by the aqueous extract , as a concentration of 20 , there was no effect of the aqueous extract on inhibiting the growth of isolates , while at 40 concentration the inhibition zone was (11)

Investigation of the inhibitory activity of alcoholic extract of ginger roots by diffusion method

the pseudomonas bacteria had the least effect on the alcoholic extract for concentrations 10 and 20, as no inhibition appeared , but at a concentration of 40, the effect of the alcoholic extract was very effective , as the diameter of the inhibition zone was 24 mm These results are consistent with what was indicated by [37] , who obtained an increase in the inhibition area by increasing the concentration of the aqueous and the concentration of the aqueous and alcoholic extract used of ginger roots while the effectiveness was at it is best at a concentration of 40, Figure2 Effect alcoholic extract

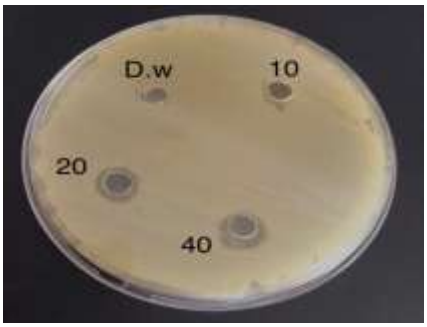

Figure 2 Effect alcoholic extract on Pseudomonas bacteria

Figure 3 Effect aqueous extract on Pseudomonas bacteria

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. Investigation of the inhibitory activity of a aqueous solution of different ginger root nanoparticles by diffusion method

The results of the investigation showed the inhibitory activity of ginger nanoparticles synthesized from ginger roots at a different concentration (100, 300, 500 micro) and by using the diffusion method by drilling there is a clear effect of these nanoparticles on these bacterial sample for the pseudomonas bacteria , it had a weak effect in front of the aqueous solution of nano- ginger , where at a concentration of 100 and a concentration of 300 , no inhibition appeared in the growth of bacterial isolates , but at a concentration of 500 inhibition of bacterial growth appeared , and the diameter of the inhibition n zones was 20 mm.

Investigation of the inhibitory activity of a alcoholic solution of different ginger root nanoparticles by diffusion method

for the pseudomonas bacteria , there was also a clear effect of the ginger nanoalcohol solution , where different inhibition zones appeared , and according to the concentration , at a concentration of 500 the stabilizeation zones was 35 mm and at a concentration of 300 only , it was less than that , reaching 30 , and at a concentration of 100 it reached 24mm It is the effective effect of the alcoholic solution , perhaps due to the ability of the alcohol to extract the largest possible amount of the active substances from the plant tissues used , including the compounds of tenanted, saponines , flavonoids , and volatile oils, as these compounds have an effective effect in inhibiting the growth microorganisms [38] , and the phenolic compounds present in them have an effective role in inhibiting microorganism growth even at low concentration[39] (

The manufacture of nanoparticles from nature components contributes to increasing their efficiency , in addition to being an environmentally friendly method , as it is inexpensive on the economic level . According to the results , it was observed that the efficiency increased by inhibiting and killing bacterial cells ,[40-41] .

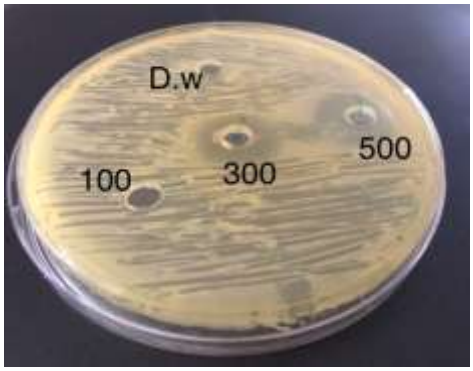

Figure 4 Effect alcoholic nanoparticles of ginger root on Pseudomonas bacteria

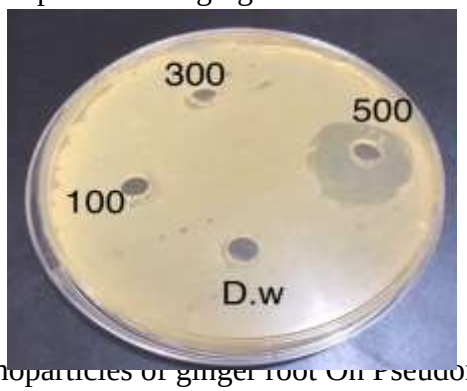

Figure 5 Effect aqueous nanoparticles of ginger root on Pseudomonas bacteria

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Conclusion

- High incidence of otitis media males compared to females.1-

The age group (1-15) years were more susceptible to otitis media .2-

High incidence of chronic otitis media compared to acute otitis media .3-

4-High incidence of ear infection in urban areas compared to rural areas

-55- All Pseudomonas sample are sensitive to the antibiotics (IEL) All staphylococcus sample were resistant to the antibiotic(Gentamycin) .

-66-Increasing the effect of the inhibitory activity of aqueous and alcoholic extracts, as well as aqueous and alcoholic solutions of ginger roots by increasing the concentration

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