

## Antibacterial Activity of sonicated Zingiber Officinale Rhizome Extract on Some Bacteria Isolated from Otitis media Patients

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### KEYWORDS

Streptococcus, ethanolic sonicated, Antibacterial, Zingiber officinale Rhizome.

### ABSTRACT

**Background:** Zingiber officinale is an herbaceous plant that belongs to the Zingiber officinale family Zingiraceae it is a abundant in the countries of east India, China and Sri Lanka as well as in some Arab countries.

**Materials and Procedures:** The purpose of this study was to evaluate the ethanolic sonicated of zingiber officinale Rhizome against the pathogenic bacteria (Streptococcus) isolated from patient of ear infection. 112 clinical specimens were collected from patient from Babylon hospital of both genders and different ages. From 1/October /2022 to 1/April /2023. The antibiotic susceptibility test was performed. The antimicrobial activity of sonicated ethanolic of zingiber officinale Rhizomes, the (MIC) the (MBC) of sonicated ethanolic of zingiber officinale of all isolates were determined.

**Result:** The results of isolation 100(899.3%) specimens gave positive growth from 42 females and 58 from males, The isolates of Streptococcus isolates showed sensitivity to [Chloramphenicol, Vancomycin, but show resistance to Gentamycin, Clindamycin, Tetracyclines, Azithromycin, Erythromycin], The antimicrobial activity of sonicated ethanolic They showed (27,40,15) mm for sonicated ethanolic, sonicated aqueous, and (27,40,15) mm for sonicated ethanolic. The antimicrobial agents of sonicated aqueous and ethanolic extract by three different methods first (FESEM) was showed these range of sonicated particles between (10.20-27.28)  $\mu$ m (XRD) that showed (23.601-44.015), third (FTIR) was used in range 400-4000cm<sup>-1</sup> the results were showed 64 Mg/l MIC, 128mg/l MBC for Streptococcus

**Conclusion:** In this study, it was found that the highest infection rate at the (1-15) years category and the number of meals with otitis infections is more than females. It was showed that the otitis infection patients in the Urban regions with highest percentage with the lowest percentage observed in rural regions and rate of chronic otitis infections as more that the rate of acute otitis infections.

## 1. Introduction

Otitis media is known as inflammation of the mucous membrane lining the cleft of the middle ear, and germs are among the most important causes of its occurrence [1]. And it is considered a serious and common health disease therefore there are three types of inflammation which are acute otitis media inflammation this type is accompanied by pain and high temperature, as for the second form, it is called otitis media accompanied by fluid accumulation, and it is symbolized by (OME), and here the serous fluid exudation is mucous or purulent, the third form is chronic suppurative otitis media, denoted by (CSOM), which lasts for several weeks and is accompanied by purulent otitis media through the hole in the eardrum [2]. Otitis media arises as a result of infection with some types of Bacteria that are Gram-positive and Gram-negative whose source resides in the hollow of the nose. Otitis medium (OM) is most frequently caused by bacterial infections when the species (streptococcus), (Moraxella catarrhalis), (Haemophilus influenza) and (Streptococcus pneumonia) [3]. While [4] indicate that the causes that lead to the occurrence of chronic otitis media (CSOM) are type (staphylococcus aureus pseudomonas aeruginosa) by observing the most prevalent bacteria found in isolates from patients this infection.

Medicine Plants have been used for a long time as a preferred source of natural remedies for the continuation of human health. Recently, extensive studies have been conducted on these plants, and according to the reports from the World Health Organization, which confirmed that The greatest source is medicinal herbs. for obtaining many medicines [5]. In wealthy countries, about 80% of people utilize herbs. The components of traditional medicine, which are obtained from medicinal plants, requires more research in order to understand a deeper understanding of its properties and therapeutic effects [6]. Medicinal plants and herbs contain chemicals of obvious benefits such as volatile oils and capcodes that aid in the treatment of illnesses affecting both humans and animals.

Alkaloids, lipids, sugars, sugarcane, tannins, saponins, and sterols).

## 2. Methodology

### Specimens Collection

One hundred twelve Specimen were collected from patients with otitis infections after diagnosis by otolaryngologists from both genders with ages ranging from 1-60 years who attended to Alexandria General Hospital and Teaching Merjan Babylon from October 2022 to April 2023 (35 females and 65 males) .

Swabs were taken from the external auditory canal according to method followed by the Scientist in taking specimens by cleaning the outer ear and removing the pus (Discharge), then the specimens was taken from the remnants of pus present in the external auditory canal the specimens were quickly transfer to the laboratory and cultured on the appropriate culture media <sup>[7]</sup>.

### Samples were cultured on immediate media

Mueller Hinton Agar, Blood Agar, Nutrient Agar, and Brain Heart Infusion Broth

### Isolation of Bacteria Isolates

All swabs were transferred to the laboratory and then inoculated on the differential and diagnostic media to isolate differential colonies under anaerobia conditions at 37° for 24-48 hours. All colonies from culturing on media were purified by subculture on nutrient agar for preservation for further tests <sup>[8]</sup>.

### Microscopic examinations

A portion of the bacterial growth was transferred by a loop and placed on a glass slide, fixed and stained with Using gram stain and a light microscope's oil lens, the shape of the Streptococcus bacteria observed as blue cocci arranged in regular groups resembling a twisted chain <sup>[9]</sup>.

The bacterial isolates were diagnosed from the characteristics of culture on different media, by gram stain and from a procedure Biochemical tests base on the <sup>[10]</sup>. Of determinative classifier Bacteriology. The first diagnosis was made based on the characteristics of the cultures on the culture media. Gram-positive bacteria were diagnosed through their growth on blood agar, and pe ofanalyses were observed hemolysis, and then other biochemical tests were performed.

### Identification of Bacterial Isolate with VITEK-2 system

The Vitek2 was employed to validate the biochemical and antibiotic assay, which was carried out in compliance with the guidelines provided by the manufacturer. This gadget is a personal device called a reader incubator, and it is composed of several internal parts, such as card filling process, loading process, card cassette, barcode scanner, card sealer, cassette spiral and incubator. Along with optical transport, electrical control tools and firmware for trash processing. For all common identification tests, the system has an extended identification database, which improves the effectiveness of microbiological diagnosis.that reduce the need for additional testing to improve safety for both the tester and the user. The following steps have been planned according to the manufactures' directions.

I. Making the bacterial suspension: An enough amount of bacteria were transferred using a sterile swab. isolates pure culture colonies (the colony must be 24 hours of age) were suspended separately Fill clear plastic test tubes with 3 milliliters of sterile saline. The turbidity was modified by inserted test tubes into the colony standardization assay system with McFarland's standard solution (1.5x10<sup>8</sup>cells/ ml).

II. Identification card was inoculated with isolated. The isolation suspension test tube was positioned within a designated rack, and the transfer tube was inserted into the matching suspension tube while the card was positioned in the slot next to it. The filled cassette was inserted into a vacuum chamber

station either manually or mechanically. Following application of a vacuum and reintroduction of AI to the microchannels that filled all test wells.

III. Sealing and incubation of the card: The inoculated card is fed into the carousel incubator via a mechanism that cuts the transfer tube and seals the card. The capacity of a carousel incubator is 30 or 60 cards. Online incubation is done for all card kinds at  $35.5 \pm 1.0^\circ\text{C}$ . Every fifteen minutes, each card is removed from the carousel incubator, sent to the optical system for reaction readings, and then put back in the incubator until the next tube reading time. Throughout the incubation period, data were gathered every fifteen minutes.

### Antibiotic Susceptibility Test

The antibiotics susceptibility of one hundred of *Pseudomonas aeruginosa*, *staphylococcus aureus*, and *streptococcus pneumonia* to different antimicrobials determined according to Using Kirby – Bauer Disc Method on Muller Hinton agar (MHA) <sup>[11]</sup>.

One of the most common methods used routinely in diagnostic laboratories and is based on inoculating the bacteria under test on solid culture medium (Muller Hinton agar) in petri dish. After activation of the bacterial isolates using brain-heart infusion broth for twenty-four hours at  $37^\circ\text{C}$ , and by adding sterile normal saline compared with (0.5) a standard McFarland tube ( $1.5 \times 10^8$  CUF/ml), then apply with a sterile cotton swab on Muller Hinton agar (MHA). and leave it to dry, different antibiotics tables were used in different concentrations

With sterile forceps, the selected antimicrobial disks were placed on the surface of the inoculated medium and inoculated at  $37^\circ\text{C}$  for 24h, during the incubation period the antibiotic spread from the disc to the medium. If the organism is selective to antibiotics, zones of lack of growth appear around the disc, and the higher the sensitivity, the larger the diameter of the area inhibition. Antibiotics inhibition zone were noted and measured with a ruler or caliper, the antibiotics names and its inhibition diameter were used according to The Laboratory Standardization Institute (CLSI) 2021 for sensitivity or resistance of the organism to each antibiotic.

### Collect *Zingiber officinale* Rhizomes

Buying *zingiber officinale* from the local market, washing it well, it was cleaned from the suspended dust with water and salt about for 3 minutes, then washed a second time with distilled water. it was spread on a clean cloth and waited for to dry at room temperature a period of two 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.

### Preparation of sonicated extract from *zingiber officinale* rhizome

To prepare 100gm of sonicated *zingiber officinale*, *zingiber officinale* powder was combined with 100 milliliters of distilled water, mixed, and melted over low heat to dissolve all sediment and distribute the solution was placed on plates then given two days to dry. Two days later, the powder weighing 20 grams of dried rice was removed from the plates. *zingiber officinale* powder was added to 800 milliliters of distilled water were combined, and the mixture was used for 1.5 hours in the vibra-cell ultrasonic liquid device., as this device operates for 10 second, stopping for 5 second, then the mixture was collected and preserved in the refrigerator at  $4^\circ\text{C}$  until use.

### Study the Effect of Sonicated on Bacteria

The diffusion method was used by etching according to <sup>[12]</sup>. Bacterial isolates were grown at a dilution of (1.5) cell/ml according to the MacFarland 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 ethanolic sonicated of *Zingiber officinale* Rhizome, were added to the etching separately and at different concentration (100,300,500)  $\mu\text{g/ml}$  add it to the positive control hole that contain distilled water. the dishes were left at room temperature for two hours, then incubated for 24 hours at a temperature  $37^\circ\text{C}$ . after that, a ruler was used to measure the inhibitory zones' diameters

formed around the holes.

### **Characterization of Synthesized *Zingiber officinale* sonicated.**

The physical attributes of *Zingiber officinale* sonicated were evaluated using FTIR, XRD, and SEM.

#### **1- Analysis by Scanning Electron Microscopy (FESEM) in the Field**

Measurements after the synthesis, sonicated were prepared as a powder and studied analyzed under scanning electron microscope with different magnification powers It provides the synthesized sonicated with a clean image. It displays the morphological characteristics. of sonicated and size measurements by scanning electron microscope (Inspect S 50, fei) according <sup>[13]</sup>.

#### **2-X-ray Diffraction (XRD)**

The characterization of was done using X-ray diffraction. *zingiber officinale* sonicated the powder of *zingiber officinale* sonicated specimens was divided up on a specimen holder with a low background noise and examined using a Bruker D8 Advance X-Ray diffractometer fitted with a LynxEYE detector. The parameters for the X-ray diffraction examination were 40 KV of voltage, 40 mA of current, and 1.54060 Å of copper radiation. With a time constant of 1.2 seconds, the scanning was carried out in the  $2\theta$  range of  $10^\circ$  to  $40^\circ$  at  $0.02^\circ/\text{min}$ . <sup>[14]</sup>.

#### **3- Fourier Transform Infrared Spectroscopy (FITR)**

Using an FT-IR spectrophotometer, the transmittance of the produced formulations was measured at a resolution of  $2\text{ cm}^{-1}$  over a spectral range of  $400\text{--}4000\text{ cm}^{-1}$ . Over 64 scans, the data sets were averaged. <sup>[15]</sup>.

Finding the sonicated rhizome of *zingiber officinale*'s minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).

The MIC is lowest rhizome *zingiber officinale* sonicated concentration that totally stopped the development of bacteria can be found. by using micro-plate method, the turbidity of bacterial suspension was matched and compared to 0.5 MacFarland units of turbidity. A homogenous is roughly correlated with the MacFarland 0.5 standard.  $1.5 \times 10^8$  cells/ml suspended. Each well received a  $100\mu\text{l}$  transfer of BHI material.  $100\mu\text{l}$  of *zingiber officinale* rhizome sonicated, ( $128\mu\text{g}/\text{ml}$ ) was serial dilutions were carried out from column 2 to column 11 in each well of column 2 to determine the final concentrations of NPs., which varied from  $128\mu\text{g}/\text{ml}$  in (2<sup>nd</sup> well) to  $2\mu\text{g}/\text{ml}$  in (11<sup>th</sup> well). A 10 microliter of bacteria in column were added to every well—all wells of (column 1) excluded. Column 12 (NPs free well) has medium in it. and in column and (column 1) contained media only. The microtiter plate incubated at  $37^\circ\text{C}$  for 24hr. OD at 570 nm was recorded spectrophotometrically. The lowest concentration of NPs was identified as the MIC. showing absence of growth as compared with the growth in the rhizome of *zingiber officinale* sonicated— free well <sup>[16]</sup>.

### **Ethical Approval:**

The study was carried out in conformity with the ethical standards set forth in the Helsinki Declaration. Before a sample was taken, it was done with the patient's verbal and analytical consent. According to the document, a local ethics commission evaluated and approved the study protocol, as well as the subject information and permission form number M221001 in October 17, 2022

### **3. Results and discussion**

#### **Demographic distribution**

One hundred twelve specimens were collected for the period from October /2022 to April/2023. They included specimens from otitis patients of different ages and sex. specimens collected from AL-Exandria General hospital and Murjan Teaching Hospital), under the supervision, only gave 100 positive growth culture, 12 otitis specimens were Negative for growth culture, The reason for negative culture results either may be due to Contamination or because Viral or fungal infection as

show in table (1).

Table (1) Distribution of positive and negative Growth Culture of otitis Specimens

| Otitis specimens | No.of specimens | Percentage |
|------------------|-----------------|------------|
| Positive growth  | 100             | 89.3%      |
| No growth        | 12              | 10.7%      |
| Total Specimens  | 112             | 100%       |

Table (2) Distribution of Study Groups According to Age Groups

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

Table (3) shows the study groups' gender distribution.

|         | Patients | Percentage |
|---------|----------|------------|
| Males   | 58       | 58%        |
| Females | 42       | 42%        |
| Total   | 100      | 100%       |

Table (4) Geographical Distribution of Study Specimens

|       | Numbers | Percentage |
|-------|---------|------------|
| Urban | 72      | 72%        |
| Rural | 28      | 28%        |
| Total | 100     | 100%       |

Table (5) Study of the patient with otitis according to the pathological condition

| Pathological | Numbers | Percentage |
|--------------|---------|------------|
| A cute       | 33      | 33%        |
| Chronic      | 67      | 67%        |
| Total        | 100     | 100%       |

### Detection of *streptococcus pneumonia* by biochemical methods

All *Streptococcus pneumonia* isolates has shown a negative result in biochemical test for catalase test, oxidase, urase, voges Proskauer. But positive result to gram stain, hemolysis Alfa hemolysis test, shape diplococci, oxidative fermentation was facultative an aerobe.

Table (6) biochemical test of *Streptococcus pneumonia*

| Test                   | Result              |
|------------------------|---------------------|
| Gram stain             | +                   |
| Catalase               | -                   |
| Urase                  | -                   |
| Oxidase                | -                   |
| Voges Proskauer        | -                   |
| Hemolysin test         | Alfa                |
| Shape                  | Diplococci          |
| Oxidative fermentation | Facilitative aerobe |

Table (7) Description resistance and sensitive of antibiotics on bacteria

|                 |    | <i>Streptococcus</i> |       |     |
|-----------------|----|----------------------|-------|-----|
| Tetracycline    | 10 | ≤18                  | 19-22 | ≥23 |
| Gentamycin      | 20 | ≤15                  | 16-18 | ≥19 |
| Chloramphenicol | 21 | ≤12                  | 18-20 | ≥21 |

Table (8): Phenotypic of antibiotic susceptibility of *Streptococcus* Isolation

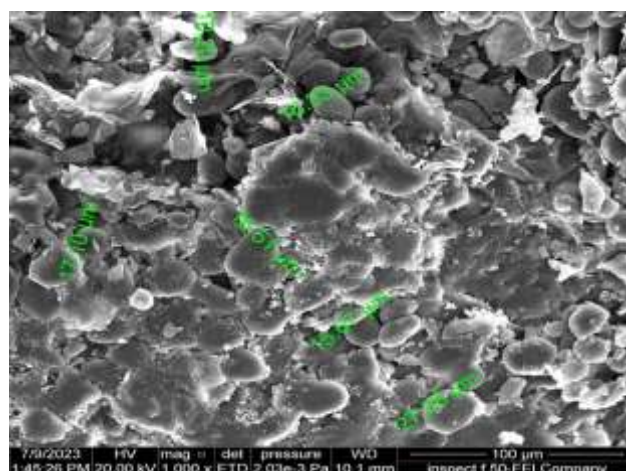


| Isolates | VA | TE | CD | GEN | E | AZM | C |
|----------|----|----|----|-----|---|-----|---|
| 1        | S  | R  | R  | S   | R | R   | S |
| 2        | S  | R  | R  | S   | R | R   | S |
| 3        | S  | S  | R  | S   | R | R   | S |
| 4        | R  | R  | R  | S   | R | R   | S |
| 5        | S  | R  | R  | S   | R | R   | R |
| 6        | S  | S  | R  | S   | S | R   | S |
| 7        | S  | R  | R  | S   | R | R   | S |
| 8        | S  | R  | S  | S   | R | R   | S |
| 9        | S  | S  | R  | S   | R | R   | S |
| 10       | R  | R  | R  | S   | R | R   | S |
| 11       | S  | S  | R  | S   | R | R   | R |
| 12       | S  | R  | R  | S   | R | R   | S |
| 13       | S  | R  | R  | S   | R | R   | S |
| 14       | R  | R  | R  | S   | R | R   | R |
| 15       | S  | S  | R  | S   | S | R   | S |
| 16       | S  | R  | R  | S   | R | R   | S |
| 17       | R  | R  | S  | S   | R | R   | S |
| 18       | S  | R  | R  | S   | S | R   | S |
| 19       | S  | R  | R  | S   | R | R   | S |
| 20       | S  | S  | R  | S   | R | R   | S |
| 21       | S  | R  | R  | S   | R | R   | S |
| 22       | S  | R  | R  | S   | R | R   | S |
| 23       | R  | R  | R  | S   | R | R   | S |
| 24       | S  | R  | R  | S   | R | R   | S |
| 25       | S  | R  | R  | S   | R | R   | S |

Abbreviations: [Vancomycin (VA), Clindamycin (CD), Tetracycline (TE), Gentamicin (GEN), Chloramphenicol, Erythromycin(E), Azithromycin (AZM)]

Table (9) show inhibition zone of ethanolic and aqueous sonicated of *zingiber officinale*

| Concentration ( $\mu\text{m/ml}$ ) | Inhibition zone(mm) of aqueous sonicated | Inhibition zone(mm) of ethanolic sonicated |
|------------------------------------|--|--|
| 100                                |  |  |
| 300                                | 18                                       | 27   |
| 500                                | 20                                       | 40   |



This Figure (1) showed the diameters of sonicated of *zingiber officinale*

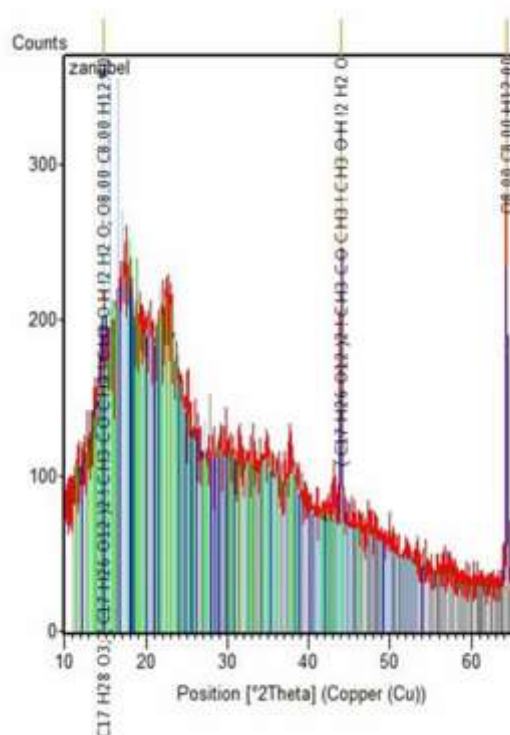


Figure (2) Show XRD pattern of Rhizome Zingiber Officinale Nanoparticles

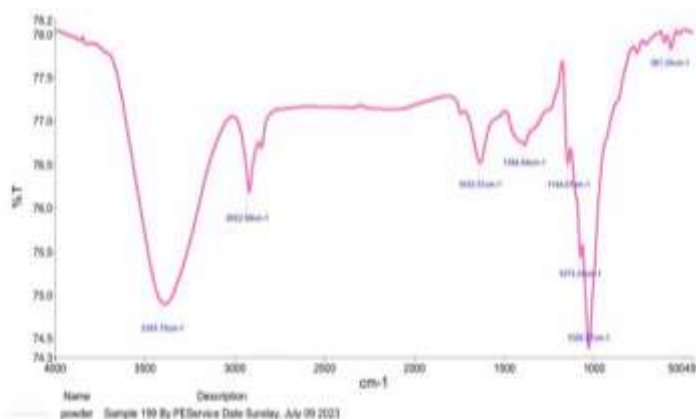


Figure (3) show FTIR spectra of sonicated zingiber officinale

## Discussion

### Identification of bacteria isolates using Vitek-2 small sys

In the small vitek-2 system, the isolates were identified characteristics of all bacterial isolates were done also by VITEK-2 compact system, appendices The VITEK-2 technology is user-friendly, quick to yield results (4–15 hours), and reasonably appropriate for identifying microbiological organisms.

### Susceptibility of bacteria to antibiotic

The sensitivity of the *streptococcus* isolates under study was tested *streptococcus* against 7. has shown the results are that the isolates vary in their sensitivity antibiotics, according to the difference in the isolation area and the area of residence, as well as according to the persons previous use of antibiotics. Residence to antibiotics, and in 1988, the Kirby-Bauer method of disc diffusion was used to test the isolation's susceptibility to drugs. The size of the antibiotic disc-encircling inhibitory zone used to determine the extent of the sensitivity of these isolates to antibiotics.

### Susceptibility of *Streptococcus* bacteria to antibiotics

Twenty-five identification *S. pneumonia*, isolates were evaluated against 8 common antibiotics, as it all isolates showed sensitivity against Gentamycin (100%), and (88%) isolates were sensitive to Chloramphenicol, and (92%) of isolates showed resistance against Clindamycin, the Tetracycline were (76%) isolate resistance, tetracyclines have been extensively utilized to treat a range of infections for many years. Tetracycline is far more effective than tetracycline and can still work on bacteria that have tetracycline resistance. as for Azithromycin all isolates were resistant to it (100%). While the percentage of resistance to erythromycin was (88%), but (80.0%) isolates were sensitive to vancomycin, A glycopeptide antibiotic, vancomycin is commonly used to treat severe infections. It binds to the lipid II dipeptide D-Ala4-D-Ala5, preventing trans glycosylation and transpeptidation that are mediated by PBP2 and PBP2a and opposing peptidoglycan remodeling. <sup>[18]</sup>.

### Investigation of the Aqueous Solution's Inhibitory Activity of Various *Zingiber officinale* Rhizome Sonicated by well Diffusion Method

We made tow particles from the *zingiber officinale rhizome*, aqueous and ethanolic, at three concentrations of (100,300,500) µg/ml to each particle, and we treated them with three types of *Staphylococci*, *Streptococcus*, *Pseudomonas* bacteria

The results of the investigation showed the inhibitory activity of zingiber officinale sonicated synthesized from *zingiber officinale Rhizomes* at a different concentration (100, 300, 500) µg/ml and by using the diffusion method by drilling there is a clear effect of these sonicated on these different bacterial isolates, as in Figure 4: An aqueous solution's impact solution of *zingiber officinale* sonicated at three concentrations on the types of isolates under study.

the *streptococcus* bacteria, no effect of the solution was shown at a 100 µg/ml concentration, as in *staphylococcus* bacteria. at a 300 µg/ml concentration, the diameter of the inhibition zones was 18 mm, and in the concentration of 500µg/ml, the inhibitory zone's diameter was higher, as it was 25mm.

### Investigation of the inhibitory activity of a ethanolic solution of different *zingiber officinale* Rhizome sonicated by well diffusion method

After the inhibitory effectiveness of the ethanolic zingiber officinale solution was investigated at different concentration towards the isolates of *streptococcus* the effect of the various isolates was observed, As for the *streptococcus* bacteria, it was clearly affected by the ethanolic *zingiber officinale* sonicated according to the different concentration, where the inhibition zones ranged at a concentration, of 500µg/ml (40) mm and at a concentration of 300µg/ml to (27) mm, but at a concentration of 100µg/ml, the bacterial don't affect .

The increase in the diameter of inhibition rates for the growth of the studied bacteria by increasing the concentration of nano-solution this result was in agreement with <sup>[19]</sup>. indicated that increasing the concentration of the sonicated- solution increase its effectiveness in inhibiting the growth of microorganisms. It is the effective effect of the ethanolic solution, perhaps due to the ability of the non-sonicated aqueous extract the largest possible amount of the active substances from the plant tissues used including the compounds of tenanted, saponins, flavonoids, and volatile oils as these compounds have an effective effect in inhibiting the expansion of microorganisms, as well as the phenolic substances present in them have an effective role in inhibiting microorganism growth even at low concentration <sup>[20]</sup>.

The manufacture of sonicated 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, <sup>[21]</sup>. The role of the active compounds in plant nano- solutions is also attributed to the



inhibition of microorganisms, but these materials are equal to the interaction with the components of the cell, or perhaps they do not have In order to prevent the action of enzymes, coenzymes, and other compounds, specific receptors on the bacterial cell wall and vectors connected to the transport of their molecules into the cell, effective biology <sup>[22]</sup>.

### Characterization of *Zingiber officinale* sonicated

#### Analysis by field Emission electron scanning microscopy (FESEM)

FESEM was employed to verify the size and shape of the *zingiber officinale* sonicated, shape *zingiber officinale* sonicated with size range between (10.20µm – 27.28µm) were reported. One kind of electron microscope is the SEM, which uses a raster scan pattern to scan high-energy electron packages to create an image of the specimens. When an electron interacts with atoms that potentially comprise a specimen, signals are produced that carry details on the specimen's synthesis, surface topography, and other characteristics like electrical conductivity <sup>[23-30]</sup>. Changes in the bio-reeducation circumstances, such as the kind of culture and organism, the nature of the medium, and the length of the incubation period, can regulate the sizes and forms of biogenic sonicated <sup>[31-37]</sup>.

(10.20 µm, 15.67µm ,16.33µm, 16.51µm,17.10µm ,27.28µm) indicated the diameters of sonicated were appropriate and accurate as sonicated of *zingiber officinale* FESEM was used to determine the size, shape and location of sonicated of *zingiber officinale* This image showed that *Zingiber officinale* sonicated are spherical clusters (hexonal) in shape, and their sizes are less than70 nm this result was compatible with the result of <sup>[38-45]</sup>.

#### X-ray Diffraction (XRD).

The formation of sonicated was characterized further by XRD analysis using powder X-Ray Diffract meter. The studies showed a characteristic peak at 20 values of 20,1456, 28,6191 and 35,9395

$x=(k*\lambda)/(\beta*\cos\theta)$  where  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width at half maximum (FWHM) of the peak in radians,  $\theta$  is the Braggs diffraction angle in radians, D is the crystal size, and K is a constant with a value of roughly 0.9..

#### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis it was employed to gather data regarding the chemical substances involved in the stabilization and decrease of *Zingiber officinale* nanoparticles. FTIR spectra of *Zingiber officinale* sonicated (powder and with ethanol) showed the present of peak at 3393.57 cm<sup>-1</sup> can be assigned to hydroxyl (OH) group. 2922.58 cm<sup>-1</sup> was assigned to Alkane (C-H stretching). 1633.51 cm<sup>-1</sup> present of (C=C stretch binding carboxyl stretch protein). 1364.64 cm<sup>-1</sup>, 1144.07cm<sup>-1</sup> assigned to methyl (C-H). 1073.34 cm<sup>-1</sup> was assigned to (C-O stretch). 567.25 associated with Aliphatic organ halogen compound (C-I stretch). Figure (4-7) showed FTIR spectra powder of Rhizome *zingiber officinale* Figure (4-8) showed FTIR spectra with ethanol Ethanolic of Rhizome *zingiber officinale*.

#### Detecting Minimum bactericidal concentration (MIC) and Minimum inhibitory concentration (MIC) of sonicated

The MIC was detected by microtiter plate method. Different concentration of Rhizome of *zingiber officinale* sonicated ranging from (4µg/ml-128µg/ml) were used. The minimum inhibitory concentration of sonicated (MIC) was calculated after the incubation of the microtiter plate for 24 hours at 37°C (OD570nm). A concentration which prevents growth of bacteria is considered the lowest concentration (MIC). MIC, MBC for *Streptococcus* bacteria were (64µg/ml) and (128µg/ml) respectively,

### 4. Conclusion and future scope

*Streptococcus pneumonia* that also isolated from otitis infection patients was the most influenced to sonicated ethanolic extract of *Zingiber officinale* rhizome at low concentration. *Zingiber officinale*

rhizome have antibacterial activity against pathogenic bacteria with different chemical structure when analyzed under Infrared spectroscopy

## Recommendations

Study the effect Zingiber officinale rhizome extract against different pathogenic microorganisms (including multi resistant species)

Study the Crude extract's cytotoxic effects on cancerous and normal cells

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