Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon –College of Science
Biology Department

Antibacterial Activity of *Zingiber Officinale* Rhizome Extract on Some Bacteria Isolated from Otitis Media Patients

A Thesis

Submitted to the Council of the College of Science, University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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B. Sc. University of Babylon college of science (2019)

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2024 A.D 1445 A.H

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيم

وَقُلْ رَبِّ زِدْنِي عِلْماً

صدق الله العلي العظيم

سورة طه114

The Supervisor's Certification

I certify that this thesis entitled" Effect Sonicated of Zingiber officinale Rhizome on Bacteria That Isolation From Otitis infection" was prepared by " Duha Abd Al- Ameer Mohammed Shanwer" under my supervision in the University of Babylon / College of Science /Deparmant of Biology as a partial fulfilument of the requirement for the degree of Master of Science in Biology Microbiology.

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/ / 2023

In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

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/ /2023

Dedication

To the one who taught me that the world is a struggle …..... And her weapon is knowledge and knowledge

To the one who did not skimp on anything ……. To the one who sought for my comfort and my success ……. To those who supported me and encouraged me in my academic career……. To the greatest and dearest man in the universe. My dear father.

To the one who supported me in her payers and supplications……. To the one who stayed awake at night to illuminate my path ……. To the one who shared my joys and sorrows……. To the fountain of kindness and tenderness ……. To the most wonderful women in existence. My dear mother.

To those who are support, my pride, my strength. My dears brothers

To the best that supported me and helped me to reach my dreams. My dear's sister

To every knowledge student

I dedicate this study

Duha

Acknowledgements

Initially, I thank Almighty God for giving me the strength and the will to complete this work for the required degree

My deepest thanks to university of Babylon and I would like to thank the staff of College of Science / Department of Biology, for their kind assistance

It is pleasure to express my deep thanks and gratitude to my supervisor professor Dr. Wejdan Ridha Taj – Aldeen who has provided me with valuable scientific guidance and continuous support through the period of preparation, and has devoted a large part of her time for the purpose of writing this dissertation.

I Thank Al- Exandria Hospital for providing the necessary support during my work period and for facilitating the collection of specimens.

I extend my sincere thanks to the patients, without whom my message would not have been enjoyable.

I would like to thank my family for their support in all times, and special thanks to my brothers (Fatima and Ali) for helping me

I thank my friends those who helped me and were with me and accompanied me.

Finally, Thanks and appreciation are to my colleagues and to all those who helped me in completing this work

Duha

Summary

This study was conducted to test the non-sonicated aqueous and ethanolic extract and sonicated aqueous and ethanolic of *zingiber officinale Rhizome* against the types of pathogenic bacteria (*Staphylococcus, Streptococcus* and *Pseudomonas*) isolated from patients of ear infection.

112 clinical specimens were collected from patient from Alexandria General Hospital and Teaching Merjan Babylon of both genders and different ages. From 1/October /2022 to 1/April /2023. The results of isolation100(89.3%) specimens gave positive growth,12(10.7%) specimens gave negative growth, from 42 females and 58 males

100 bacterial were identified according morphology to cultural, microscopical properties and biochemical test and were confirmed by using the Vitek2 compact system to 54 isolated belong to *Staphylococcus aureus*, 25 isolates belong to *Streptococcus pneumonia*, 21 isolates belonged to *Pseudomonas aeruginosa*.

The antibiotic susceptibility test was performed against 18 antibiotics types using the disc diffusion method

The isolates of *Staphylococcus* bacteria, they showed sensitivity to the [Nitrofurantoin (96.29%), Amikacin (98.14%), vancomycin (96.29%), gentamicin (92.59%), chloramphenicol (92.59%), clindamycin (92.7), but ciprofloxacin (68.5%), and to Trimethoprim (92.59%)].

[The isolates of *Pseudomonas* showed resistance to Imipinim (85.71%), pipercillin (100%), meropenem (42%), Gentamycin (71.4%), ciprofloxacin (47.61%), and to Levofloxacin (42.8%)].

The isolates of *Streptococcus* isolates showed sensitivity to [Chloramphenicol (88%), Vancomycin (80%), but show resistance to Gentamycin (100%), Clindamycin (92%), Tetracyclines (76%), Azithromycin (100%), Erythromycin (88%)],

The antimicrobial activity of non-sonicated aqueous and ethanolic, of *zingiber officinale Rhizomes*, was tested on the bacterial (*Staphylococcus, Streptococcus, Pseudomonas*) isolated of three concentration 10mg/ml, 20mg/ml ,40mg/ml and it showed that inhibition zone diameter for

Staphylococcus, Streptococcus, Pseudomonas bacteria respectively (26, 20,15) mm for non sonicated aqueous extract, and (27,16,11) mm for non sonicated ethanolic extract.

The antimicrobial activity of sonicated Aqueous and ethanolic extract, of *zingiber officinale Rhizomes*, was tested on the bacterial (*Staphylococcus, Streptococcus, Pseudomonas)* isolated of three concentration 100µg/ml ,300µg/ml ,500µg/ml and it was showed that inhibition zone diameter for *Staphylococcus, Streptococcus, Pseudomonas* bacteria respectively (25, 20,21) mm for sonicated aqueous extract, and (27,40,15) mm for sonicated ethanolic extract.

The antimicrobial activity of sonicated aqueous and ethanolic extract were analyzed by three different methods first analysis by Filed emission scanning electron microscope was showed these range of sonicated particles between (10.20-27.28)µm second x-ray diffraction analysis that showed (23.601-44.015), third infrared Spectro microscopy (FTIR) was used in range 400-4000cm^{-1}, the result were showed different type (hydroxyl (OH) group, Alkane(C-H),carboxyl, methyl(C-H),fatty acid , Amines NH, Aliphatic organ ,

the minimum inhibitory concentration (MIC) the minimum bactericidal concentration (MBC) of sonicated aqueous and ethanolic of *zingiber officinale* of all isolates were determined and the results were showed 64 Mg/l MIC,128mg/l MBC for *Staphylococcus* and *Streptococcus*. And 32mg /l MIC,64mg/l MBC for *Pseudomonas.*

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 as compared to the lowest percentage observed in rural regions and rate of chronic otitis infections is more than the rate of acute otitis infections.

List of content

List of Figures

List of Table

Lest of Abbreviations

Abbreviations	Word
OM	Otitis media
MD	Middle ear
COM	Chronic otitis media
AOM	Acute otitis media
FESEM	Field Emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
XRD	x-ray diffraction
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
AMR	Antibacterial resistance
CSOM	Chronic supportive otitis media

Chapter One

Introduction

Introduction

Otitis media infection is one of the health problems that exist in many parts of the world, (Bluestone & Klein ,2007), 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 been the cause of disease (Sogebi,2022), 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. (Coleman, *et al*., 2018). Middle ear infection can develop after untreated infection, as well as in cases of improper treatment and resistance shown by bacteria to antibiotics, 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 (Kombade et al., 2021).

Among the types of bacteria that cause this (*Pseudomonas, Staphylococcus aureus, Klebscilla spp Proteus spp. aeruginosa*) and among the viruses that have an effect, they include the following viruses, in order of importance Respiratory virus (respiration) (sonatial virus) rhinoviruses and viruses ardenoviruses ,coronaviruses and

influenzas viruses (Fidan 2020 Nosko- Kovsto *et al* 2015).Recent studies directed to exploit the synergistic effect in the use of antibiotics to treat various diseases. Hence the idea of research came for the purpose of the bacteria causes this disease and studying their resistance to antibiotics and studying the synergistic action of antibiotic on these germs in trying to treat a disease in an optimal way, because of the serious complications that are difficult to treat due to neglect of treatment.

Bacterial resistance means the capability of bacterial cells to prevent antibiotic bacteriostatic or bactericidal effects (Ramamurthy et al.,2022). Resistance of the antibiotic is one of the most serious global public health issues: it has the capacity to kill approximately 700,000 people and may rise to ten million in 2050(Mancuso *et al.,* 2021). In 2019, due to its impact on human health, the World Health Organization included antimicrobial resistance (AMR) is one of the top ten threats to global health (WHO, 2019).

Zingiber officinale was a native plant to Southeast Asia. It belonged to the Zingiberoside family (Vasala, 2001). *Zingiber officinale* had been used in traditional Indian and Chinese medicine for centuries to strengthen gastric and to treat a wide range of gastro intestinal (GI) disorders such as dyspepsia, to cure upper intestine ulcers including gastritis, and peptic ulcer disease (PUD), and therefore, to relieve stomach pain due to bacterial infection (Vasala, 2001; MDidea.com., 2009). Further, *zingiber officinale* was also well-regarded for its ability to fight inflammation, to cleanse colon, to reduce spasms and cramps, and to stimulate circulation. So, it was well justified for the India's Ayurvedic and the ancient Chinese herbalists that had used *zingiber officinale* for 5,000 years as a medical panacea for curing various illnesses (Ghaly et al., 2009; MDidea.com., 2009; Silver, 2007). Further study show that the

zingiber officinale constituents acted as strong antioxidants and effective antimicrobial agents that could heal sores and wounds of internal organs such as stomach and liver. In this relation, Mahady et al. (2005) pointed out that the primary factor associated with gastritis and peptic ulcer disease was the gram-negative bacterium -- Helicobacter pylori (HP). These HP infections were associated with chronic gastritis, gastric carcinoma and primary gastric B-cell lymphoma. (Nanjundaiah et al. (2009) confirmed these findings, after experimenting with albino rats that were previously ulcer induced, infected with H. pylori and then treated with aqueous *zingiber officinale* non sonicated extract orally. Based upon the above evidence, (Nanjundaiah et al. (2009) concluded that the aqueous *zingiber officinale* non sonicated extract was able to protect the gastric mucosa from stress induced *Zingiber officinale* non sonicated extract and its pungent compounds demonstrated greater antibacterial activity against a variety of bacteria species including H. pylori, *Staphylococcus aureus, Pseudomonas aeruginosa* and E. coli. Antibiotic resistance had become a global concern (Westh et al., 2004). Since bacterial resistance to antibiotics was at an increasing rate, interest in discovering new natural antimicrobials was rising. Finding alternative antimicrobials to cure bacterial infections including mastitis in dairy cows was urgently needed. In this relation, finding plant products with antimicrobial properties for a possible application in food production as well as in human and animal health care to prevent the bacterial and fungal growth was emphasized (Lee et al., 2004; De Souza et al., 2005; Gur et al., 2006; Jasmine et al., 2007; Parekh and Chanda, 2007; Eyob et al., 2008; Kumaraswami et al., 2008; Oskay et al., 2008; Adekunle and Adekunle, 2009; Okigbo et al., 2009).

Aim of Study

In order to find effective materials instead of antibiotics that are effective and can reduce the spread of bacterial resistance to antibiotics, it was chosen aqueous and ethanolic of *zingiber officinale rhizome* non sonicated extract well as manufacturing nano material from *zingiber officinale* non sonicated.

Objectives

1-.Isolation and identification of bacteria from otitis media patients

2- Study the antibiotic susceptibility for bacteria isolates

3-Preparation and study the sonicated extract *zingiber officinale rhizome* and non-sonicated extract on bacteria isolates

4- Determine effective groups in sonicated extract ((hydroxyl (OH) group, Alkane(C-H), carboxyl, methyl(C-H), fatty acid, Amines NH, Aliphatic organ).

5- Detection MIC and MBC of non-sonicated and sonicated aqueous extract of *zingiber officinale rhizome.*

Chapter Tow

Literature Review

2-1 Definition of Otitis Media

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 (Lieberthal *et al*., 2013). 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 oe 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 (Lee ,1999).

2-2 Types of Otitis Media Infection

2-2-1 Acute Otitis Media Infection

It is an inflammation of the middle ear, accompanied by rapid an appearance of pathological signs, including pain and redness, accompanied by a slight rise in temperature (Morris *et al*.,2013). After Acute otitis media (OM) infection is particularly common infection in children (chonmaitree *et al.*, 2000). It also occurs in adult, and bacteria is the cause of this disease in children and adults, in addition to the presence of other causes. This infection can be treated with antibiotics, but nevertheless, caution should be exercised when prescribing antibiotic. because their excessive use leads to resistance to these antibiotics by bacteria, and this resistance has been seen among some of the pathogens that cause this infection (Laulajainen – Hongisto., 2016). Acute otitis media is often a complication of upper respiratory tract infection, there are many viruses that are the causes inflammation in the upper respiratory tract such as (infenza viruses – entero- corona adeno – rhino),

sometimes otitis media is a combination of viral and bacterial infection, or a coincidence with both. The most common bacterial organism that causes middle ear infections is (*streptococcus pneumoniae, Staphylococcus aureus*) .

The nature of the tympanic membrane and it is change in color is an important sign of Acute otitis media, as it can be distinguished from the ear of a person without acute otitis media through examination, where the eardrum is transparent and pearl- gray in color. As in Figure (2-1), It changing from translucent to semi- transparent or opaque, and in gray to yellow, white to red in the affected ear, with the possibility of swelling in the tympanic membrane as an important sign of acute inflammation (Lieberthal *et al.*, 2013).

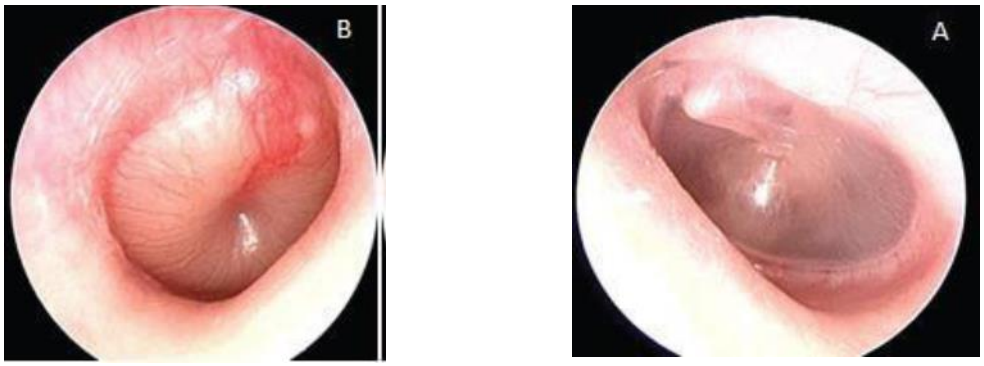

Figure (2-1) show the type of otitis infection (A) The tympanic membrane of a healthy person, (B)Tympanic membrane of a person with acute inflammation (Laulajainen-Hongisto 2016).

2-2-2 Otitis Media with Effusion

This disease may occur as a result of a viral infection of the upper part of the respiratory tract and it may occur before or after acute bacteria otitis media (Rockvill, Perera *et al.*, 2006). Acute otitis media is representing an infection (bacteria or viral), while effusion otitis media is characterized by the presence of a collection of fluids such as secretions, which appear in children in the form of a glue substance or as a corrosive

substance in adults, and the continuation of this gathering leads to hearing loss and loos of elasticity of the eardrum membrane (Parmar.*et al.*, 2019).

One of the most common symptoms of effusion otitis media is hearing loss is caused by poor conduction of sound waves due to an effusion behind the eardrum, persistence for three months or more is considered a chronic condition called otitis media with effusion. Persistent hearing loss may negatively affect speech development, behavior and progress in school (Qureishi *et al*.,2014). Acute otitis media is a bacterial infection of the meddle ear it causes fever and pain and causes severe complication. It manifests itself in the form of a swollen tympanic membrane if the membrane is perforated, there may be a secretion that contains blood, and this inflammation differs from otitis media with effusion, which causing temporary hearing loos as well as bone resorption however, the patient does not show any signs of disease such as pain or fever in general (Ru, De, Grote *et al.*,2018). The collected fluids ear often purulent and watery, so the infected ear is called glue ear (Coleman *et al.*, 2018). This disease affects children in particular. It was found that the percentage of children affected with it is (90%) of those under (2) years old, and (80%) per-schoolers, as for adults, the percentage of their infection is small (Kenna etal., 2005 Zernotti *et al*.,2017).

2-2-3 Chronic Otitis Media

It is a chronic inflammation of the middle ear and the cavity of the papillary protuberance of the temporal bone. It is characterized by the presence of exudation from the eardrum perforation that lasts for more than (2-16) week. It leads to hearing impairment as well as to a delay in speech and learning in children (Lieberthal *et al*., 2013). Chronic otitis media is characterized by with some long – term problem in the middle

ear, such as (a hole in the eardrum that does not get better or continues to return to normal, and in characterized by recurring or persistent ear discharge over (2-6) week, through perforation of the tympanic membrane when the Eustachian tube due to allergies, multiple infections, ear trauma, or swelling of the adenoids (Brunton 2005). It is one of the undisputed forms of one of the most common medical problems. Some studies have indicated that this disease is more common in people who suffer from weakness in the function of the Eustachian tube, and this disease often leads to hearing impairment (Leach, Morris, 2006).

Several rick factors associated with chronic suppurative otitis media are recurrent episodes of acute middle ear infection, upper respiratory tract infection, and trauma (mechanical) to the eardrum (Udden, *et al*.,2018.) Although viruses are the most common causes of otitis media, bacteria often infect those who suffering from chronic supportive otitis media (CSOM). Among these types of bacteria that cause chronic supportive otitis media are (*P. aeruginosa K. pneumonia p. mirabilis. S. aureus*) (Ozcan *et al* 2018).

2-3 Causes of Otitis Media

Bacterial Causes

Otitis media arises as a result of infection with some types of Gram-positive and Gram-negative bacteria whose source is in the nasopharyngeal cavity (Costa *et al.,* 2013). The most common causes of otitis media (OM) are bacterial infection where the species (*streptococcus*), (*Moraxella catarrhalis*), (*Haemophilus influenza*)and(*Streptococcus pneumonia*) (Sierra *et al.,* 2011, Qureishi 2014). While (sattar *et al*.,2012 Aduda *et al*.,2013 Prakash. *et al.,* 2013). Indicate that

the causes that lead to the occurrence of chronic otitis media (CSOM) are type (*staphylococcus aureus pseudomonas aeruginosa*) by observing the most common microbial isolates in patients with this infection.

A study conducted in Iraq showed that the primary cause of otitis media was *P aeruginosa* followed by Proteus (Aldhaher *et al.*,2018). While another study in Iraq recorded the highest percentage of *s aureus P aeruginosa* followed by (Mahmood *et al*.,2019). While the results of studies that took place in a number of East Asian and Arab Gulf countries varied in determining the most common cause of otitis media infection that *p aeruginosa* is the most common pathogen that causes otitis media (CSOM) followed by *S. aureus* (yeo *et al* .,2007 sharma *et al* ., 2010 Dayasena *et al.,* 2011 madana *et al.,* 2011 Afolabi *et al* ., 2012 Asish *et al.,* 2013) in addition to this other studies from Pakistan, Iran , and Saudi Arabia were reported *S aueeus* more common in middle ear pathologies . followed by *p aeruginosa* (Ettehad et al.,2006 Mariam et al Ahmad *et al.,* 2013) the difference in different studies could be due to differences in the number of patients studied and geographical variance (Liu et al., 2011).

Among the most famous of these bacteria that causes otitis media is (*Staphelococcus spp*) where the percentage of infection with this bacterium was (81 .6%) as indicated by (AL Hamadany, 2000). As mentioned by (Kononen *et al.,* 2002). Most infection of the middle ear infection will be caused by Gram – negative bacteria which is due to contamination of the auditory canal, which causes inflammation where it is found (الدليمي ,2002). Found that *p. aeruginosa* is a cause of otitis media in percentage of (35 .5%), and in another study, the percentage of this bacteria was found (52 .8%) (Kuczkowskiet al.,2004). The *pr.*

mirabilis bacteria is one of the bacteriaspecies that occupies the second rank after *pseudomonas aeruginosa* in causing middle ear infection (Madana et al.,2001). Enterobacter spp. It has a role in otitis media, in addition to the fact that some anaerobic bacteria may contribute to the pathogenesis of otitis media. (forbes *et al.*, 2016). Earwax consists mostly of dead skin cell and keratin with a mixture of cerumen, sweat, and oil (cerumen) from the endocrine glands located in the first, third outer part of the ear canal, and it consist mainly of cholesterol, squalene, wax, ester, ceramide, and triglycerides. Earwax shows that some of the antimicrobial properties found in serine can be attributed to the presence of antimicrobial peptide. Other studies have shown that gum directly inhibits the growth of *S. aureus, P. aeruginosa* but its effect on the growth of E. coli is still limited. in the absence of serine, the ear canal is an ideal environment for microbes (Joo Yoon.*et al.*, 2008.)

Fungi can be primary causes or accompanying causes of bacterial infection, or they can be secondary triggers. Fungal infections are mainly characterized by itching, muscle pain, hearing impairment, and tinnitus. various agents have been suggested for fungal ear infection, including immunocompromised hosts, steroid use, swimming, cotton specimenss, use of headgear, use of oils and earplugs, fungal infections elsewhere in the body such as dermatosis, and malnutrition in children (Ray *et al.*, (2015). Among these fungi are (Asprgillus, penicillium, Mucor, Rhizopus, Scopulariopsis Absidiaand and Candida), while (pajor *et al* 2006) indicated the difference in the incidence of chronic otitis media causes by Aspergillus fungus with a rate of (37.1%) and Candida albicans) with a percentage of (22.9%) (Mohamad., *et al* 2017). Indicated that the most common fungus causing this type of inflammation is candida Aspergillies

2-4 Risk Factors of Otitis Media

Infection is the most common causes of acute otitis media. It has been found that the rate of otitis media has increased in the past decades. Ear infections are more likely to occur in the fall and winter. it is possible to develop an ear infection with or without risk factors below. A family history of AOM is a risk factors for developing the disease in children (McCormick *et al.,* 2011).

There are many genetic traits that may compromise with otitis media, including (Ciliary dysfunction, cleft palate, craniofacial anomalies, and dowen syndrome) (Gould, Matz 2010). The more these factors the greater. The risk of infection:

2-4-1 Age

Otitis media, whether of a suppurative or non –purulent type, acute or chronic, affects different ages, and higher cases are found at a younger age (Finkelstein *et al*.,2005). Age is a factor, as three-quarters of children in the world suffer from otitis media. And the risk of ear infection is higher in children because their immune system is incomplete unlike adults (zernotti et *al*.,2017). It is possible that infection with the virus is the direct or indirect cause of most middle ear infection. In addition, to that Eustachin is short in children (Jacoby *et al.,* 2007). In other studies, it was shown that the age factor is one of the most important factors in the spread of the disease, as it is prevalence rate is low in the first months of birth, (Mahmood *et al.,* 2019). While (الصكر,2000) another study recorded the highest incidence of the disease in adults.

2-4-2 Sex

It was found through a number of studies that males are more likely than females to develop otitis media as a result of environmental

conditions in their lives such as swimming pools or in rivers and ponds (Jacoby *et al.*, 2007). This was confirmed in the study (الصكر,2000). The incidence of the disease in males is more than in female and previous studies have shown that males are more susceptible to disease than females due to the interaction between sex hormones, and T helper, and cytokines (Minkoff, 2014).

2-4-3 Day care

It was found that children in schools or in nurseries are more likely to develop otitis media because they are exposed to more infection of the upper respiratory tract, (Asogwns., *et al* 2013). In a study, it was shown that incorrect daily care is one of the indicators that increase exposure to environmental conditions. Many researchers went to the danger of daily care as a factor in otitis media (McCormick *et al.,* 2016 Hatakka *et al*.,2010)

2-4-4 Allergies and Asthma

People who suffer from allergies or asthma are more likely to develop otitis media, (Songu *et al*.,2020). While another study reported an increased incidence in people who suffer from asthma and respiratory tract infection with middle ear infection, (Tagaya *et al*., 2017). There is an association between allergies and otitis media from the age of (6) years and older (Roditi *et al*., 2016). The process of immune response to allergic otitis media is causes by the response that appears in the late stages of respiratory diseases such as asthma and allergic rhinitis (Nguyen *et al*.,2004.).

2-4-5 Other Factors

Children are more susceptible to ear infection if they have colds, pharyngitis, or perhaps some eye infection, although ear infections are not contagious in themselves, however, cold, stomatitis and other respiratory

infection are easily transmitted from one person to another (Labout et al., 2011). The upper respiratory tract (URT) is a region of great importance in the pathogenesis of middle ear infection, as it can serve as a source of pathogens in the middle ear (Folino *et al*., 2020). Another study confirmed the existence of the relationship between genetic factors encoding proteins of innate or adaptive immunity in recurrent ear infection (Esposito *et al*., 2015).

2-5 Sources of Disease Spread

The Eustachian tube, which is a main pathway for inflammation, through which the inflammation reaches the middle ear, and the Eustachian tube has two main functions: maintaining pressure in the ear equal to atmospheric pressure and allowing the secretion of the mucous layer of the respiratory system to penetrate it and reach the nasopharyngeal cavity (Lee,2022). It was noted that acute otitis media occurs in a state of insufficiency in the function of the Eustachian tube (Ridge etal., 2021). And this acute inflammation develops into acute and suppurative otitis media, as it noted that the cavity of the middle ear, the Eustachian tube, and the sinuses are part sinusitis of the upper respiratory system, it is certain that there is a relationship between the appearance of purulent otitis media and sinusitis (Grote ,1985). It was also observed that otitis media is an apparent pathological sgin of chronic sinusitis (Finkelesten *et al*., 2005). Some scientists noted the close link between the presence and absence of chlorine in swimming pool water and disease, as chlorinated water is important factor in the development and persistence of otitis media in children , as well as it recurrence due to irritation of the mucous layer surrounding the opening of the Eustachian tube and the occurrence of edema leading to its blockage (Moffa et al., 2022) .As for infants ,inflammation occurs in them as a result of

contamination of nasopharyngeal orifice of the nasopharynx with vomit or milk (Cebi et al.,2023) . Some researcher considered that the inability of the Eustachian tube to perform its function is a major cause of middle ear infection in children (Maddineni 2022, Skovbjerg 2020). While others believe that any treatment for ear disease should depend on raising the efficiency of the Eustachian tube (Browning, 1997.)

The tympanic membrane represents the second way of infection. When a hole occurs in it, it provides a path for the bacteria present in the external auditory canal to reach the middle ear (Al-zubaidi 2020). Therefore, it is necessary to prevent the entry of water through the tympanic hole while swimming. As for the last way to reach an infection in the middle ear, it is done through bacteremia (Canter, 1997).

2-6 Complication of Otitis

Some researchers indicate that brain abscess is a secondary outcome of otitis media (Ahmad 2022). And other complications include inflammation of the membrane, meningitis, complete deafness, facial paralysis, and inner fistula (Vartianen and Vartianen ,1996). In developed countries, intracranial complications resulting from chronic otitis media are rare and equal to approximately one precents, as it was also noted in a study done with 368 patients that only four cases of complications of the disease occur more and the severity of the disease depends on the virulence of the intruding organism and the defense mechanisms of the infected person (Maksimovic and Rukovanjski, 1993). Some researchers divide the complications of otitis media into two groups. The first includes complications within the skull cavity including subdural abscess, external abscess, meningitis, and brain abscess. The temporal bone, which include facial paralysis, otitis media, and hearing loss (Harold,

1997). Others believe that these complications were common recurrence when vital complications were not available, but the emergence and use of antimicrobials led to a reduction in the recurrence of complications resulting from this disease (Gupta 2020).

3-7 Nanoparticles

Nanomaterials possess unique properties such as the infinitesimal size, the ability to engineer electron exchange, and high interactive capabilities. these particles can easily enter and interact with many components of plant cells and tissues. the engineered nanomaterials contain metals such as different metal oxides (Anjum et al., 2020). And the physical and chemical properties the sonicatedare the main cause of the effects generated, morphology, surface charge, concentration and size distribution

The processes of approaching nanotechnologies are with biological and informatics technologies are accelerating with the aim of bringing about a radical change in the current food and agricultural systems , as a nanotechnology has many promising important biological applications , such as obtaining nanocomposites that enter the human body , monitor disease sites , achieve drugs , order cells to secrete appropriate hormones , and repair tissues he points out that these smart compounds can enter cancer cells and detonate them from the inside . They are called nano-miniaturized bombs they are used in textiles, cosmetics, foodstuffs home appliances, food applications, and the environment. They also work within human systems, including targeted drug delivery, gene therapy, tissue engineering cancer treatment of infectious and genetic diseases, as it is used in solar cells structural materials electrons, and semiconductors (Cardoso et al.,2018).

A number of studies were conducted to evaluate the potential beneficial or toxic effects of these microparticles on plant growth and development, the rate of photosynthesis and metabolism, in addition to their role in the plant defense mechanisms (Cardoso et al., 2018). And it's necessary to understand the mechanism of exposure of sonicatedto living organisms to ensure a safe relationship between nanotechnology and the ecosystem, as the increase in yield, durability and the increase in nutrients are the advantages provided by some nano composites applied to plants similar to herbicides (Kumar et al., 2019

2-8 Medicinal Plants

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 of the world health organization, which confirmed that medicinal plants are the best source for obtaining many medicines (Santos, 1995) (Nascimento, 2000). About 80% of the population of developed consists use herbs. Traditional medicine, which consists of components derived from medicinal plants, requires more research in order to understand a deeper understanding of its properties and therapeutic effects (Ellof1998) . Medicinal plants and herbs contain chemicals of obvious benefit that contribute to the treatment of diseases effecting humans and animals, including volatile oils, capcodes. saponins, tannins, alkaloids, fats, sugars, resins, and sterols) .

Medicinal and aromatic plants and herbs are non-traditional crops, as they are used in medical cases and the treatment of various diseases. their medicinal value lies in the chemicals they contain and their effect on the human body (wiedeman ,1964). Chemistry and pharmacology need to

return to nature and treatment with natural plants and herbs. interest has increased recently about the use of medicinal plants and their non sonicateds in the treatment of many infections (Deiskachary ,1995) research and studies continued to know the benefits of using medicinal plants and their hams in terms of their impact on humans and animals.

A long history of multiple uses and thousands of years ago, as it was used in medical treatments in the various countries it reached. today. its uses have increased after knowing many of its distinctive properties, such as its antimicrobial effect and the role of zingiber officinale.

2-9 *Zingiber officinale*

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 (1992 Sarivastava and Mustafa). The medicinal plant that contains the active ingredients that are used in several fields whether medical or in the industry are insecticides (1994 EL- saeid). As the zingiber officinale plant is considered one of the medicinal plants that grow in hot regions, it contains volatile oil that has a strong smell and pungent taste. its Rhizome is also used as a spice in preparing foods and adding a distinctive taste and flavor. *Zingiber officinale* contain many of the main compounds (Geraniol, neral, curcumene Zingiberene, Zingeberol, Linallol, beta-phelanddrine, D-camphor) it also contains a grop Ary alkanes. The most important compound of this group *Zingiber officinale* which contains a compound Gingenol it is the compound that is attributed to the spicy taste it also contain *zingiber officinale* Zingiber officinaledion, which act as natural antioxidants. *Zingiber officinale* also contains other effective compounds Zingibren- shogaol who play an

active role with Zingiber officinaleols in stimulating the immune system as well as raising immunity in the body (2013). it also stimulates the bone marrow to produce white blood cell, as well as secretion of the bile gland, improving bowel movement, and its contribution to reducing the concentration of fats and cholesterol in the blood Zingiber *officinale* may be attributed to the antioxidant effect of its bioactive ingredient Shogalos-6 which can inhibit microglia, *Zingiber officinale* also contains some effective anti-microbial factors such as volatile oils. Picture (2-2) shows the shape of the *zingiber officinale* plant

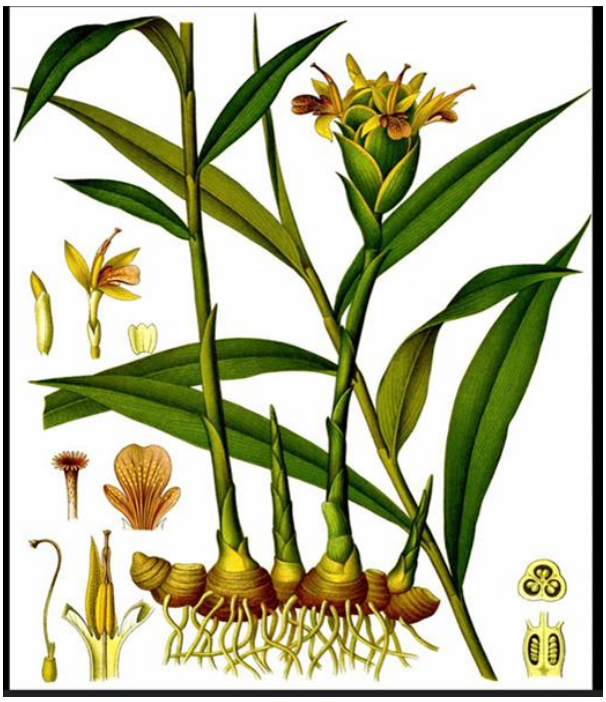

Figure (2-2) show *Zingiber officinale* plant

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2-10 Therapeutic Benefits of *Zingiber officinale*

The therapeutic effect of *Zingiber officinale* is explained below and summarized Figure (2-3)

2-10-1 Antioxidant Effect

Zingiber officinale consumption reduces lipid peroxidation and restores the activities of superoxide dismutase and catalase, glutathione, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase (Ahmed et al., 2008). Before ischemia, supplementation of *zingiber officinale* resulted in a higher total antioxidant capacity that regularized glutathione peroxidase, superoxide dismutase activities, and low total oxidants levels (lower tissue malondialdehyde, protein carbonyl contents, and NO). in comparison to an untreated group of Wistar albino rats. The phytochemistry-rich zingiber officinale contains scavenges free radicals' components, that are produced in biological systems, for energy production generated during the process of oxidation. some free radicals are essential (Ramaa *et al*., 2006). Increases in the production of free radicals show oxidative stress, and that can lead to damage to DNA (Hussein *et al*., 2006) .

2-10-2 Anti-Nausea Effect

Zingiber officinale is commonly utilized for relieving nausea and vomiting. It is also an antiemetic; it is attributed as a carminative effect that helps break up and expel intestinal gas. Researchers compared the effectiveness of Vitamin B6 and *zingiber officinale* and reported that they were equally effective for limit vomiting and reducing nausea episodes during pregnancy (Viljoen *et al* ., 2014 ;Sharifzadeh *et al*., 2018).

2-10-3 Anti-Inflammatory Effects

Zingiber officinale has the capacity to reduce inflammation, discomfort, and swelling. *Zingiber officinale* and its derivatives are used in many countries to boost the immune system. There are many studies that evaluate the effectiveness of *zingiber officinale* in patients suffering from osteoarthritis have controversial results. The study showed the non

sonicated extract of *zingiber officinale* has a significant effect on dropping osteoarthritis symptoms (Maghbooli *et al*., 2014). 6-Shogaol has potent anti-inflammatory and antioxidant effects used as a therapeutic agent in gout and a rheumatic disease of joints (Grzanna et al., 2005). Several researchers were reported that 6-zingiber officinaleol non sonicated extract of dried *zingiber officinale* has exhibit analgesic and potent anti-inflammatory effects (Young *et al*.,2005; Minghetti *et al*., 2007). *Zingiber officinale* is effective for the treatment of patients suffering from hypoalgesia. In addition, *zingiber officinale* has an antimicrobial quality, which helps in the treatment of infectious diseases. It produces free radicals or reactive oxygen species (ROS) during metabolism further than the antioxidant capacity of a biological system resulting in oxidative stress that plays a vital role in neurodegenerative diseases, cardiac diseases, cancer, and the aging process and other (Sharifi-Rad *et al*.,2017). Inflammatory disorders like esophagitis, gastritis, and hepatitis, not only caused by infectious agents such as bacteria, virus, and parasites sometimes affected by physical and chemical agents like acid, heat, cigarette smoke, and foreign bodies, which are recognized as risk factors for human cancer (Nile and Park 2015, Gupta *et al*., 2016).

2-10-4 Cardiovascular Effect

The many of studies show the effect of *zingiber officinale* on blood lipids in both animals and humans. The results show that zingiber officinale decreases plasma cholesterol in animals, but not in patients who are suffering from any heart disease. Research shows zingiber officinale has exhibit antithrombotic activity, in vitro study, its non sonicated extract inhibits platelet aggregation and thromboxane-B2 (TXB2) production. *Zingiber officinale* is used as antiplatelet therapy, and it prevents coronary heart disease (Tabibi *et al*., 2016). Zingiber

officinale has less potent than aspirin, but it has lesser side effects than aspirin. The function of aspirin is inhibiting arachidonic acid-induced platelet release and aggregation and COX activity; zingiber officinale also works as same as the mechanism of action of aspirin. So suggested that the development of effective zingiber officinaleol analogs has been used as a substitute for aspirin therapy to prevent ischemic heart disease (Arzati *et al*.,2017, Koo *et al*., 2001).

2-10-5 Anti- Cancer Effect

Zingiber officinale act as a chemo-preventive spice, there are many researches focused on the *zingiber officinale* and its various bioactive compound have potential cancer therapeutic and cancer preventive application (Ryan., *et al* 2012, Zick *et al*.,2009). *Zingiber officinale* Ingredients like 6-zingiber officinaleol,6- shogaol, 6-paradol, and zerumbone in *zingiber officinale* reveal anti-cancer tumors and anti-inflammatory activities. The *zingiber officinale* effect in preventing or defeating cancer growth has been studied in a variety of cancer types, including lymphoma, colorectal hepatoma, cancer, breast cancer, liver cancer skin cancer, and bladder cancer (Mahomoodally *etal.,* 2019). Non sonicated extract of *zingiber officinale* has been revealed to have anti-inflammatory antioxidant, and anti-tumor effects on cells. The researcher examined the anti-cancer effects of compounds, including asiaticoside (AS) 6-zingiber officinaleol, epigallocatechin gallate (EGCG), and tocotrienol-rich fraction (TRF) vitamin E G+6 triggered apoptosis synergistically and blocked the development of LN18 glioma and cancer cells 1321N1(Rahman *etal.,* 2014). Other researchers (Manju and Nalini ,2005)) investigated the effectiveness of *zingiber officinale* against 1, 2 dimethylhydrazine (DMH)-induced colon cancer. They observed that the supplementation of *zingiber officinale* could activate various enzymes

such as glutathione transferase, glutathione peroxidase, and glutathione reductase that suppress colon carcinogenesis (Kim *etal.*, 2009).

2-10-6 Anti Diabetic Effect

zingiber officinale and other plants have effective both therapeutically and preventively (parveen *etal*., 2019). The University of Sydney found zingiber officinale was effective in glycemic control for people with type 2 diabetes. Other study showed that zingiber officinale non sonicateds could increase the uptake of glucose into muscle cells without using insulin, it may help control high blood sugar levels. Other study of ethanolic non sonicated extract of Zingiber officinale fed orally for 20 days produced a significant anti-hyperglycaemic effect ($P < 0.01$) in diabetic rats. As well, in highfat diets, the ethanolic non sonicated extract of *zingiber officinale* was found to reduce body weights, LDL cholesterol, total cholesterol, triglycerides, free fatty acid glucose, and phospholipids (Nammi *etal.*, 2009) . Overall *zingiber officinale* works on diabetes by increasing insulin release and sensitivity, metabolism enzymes, inhibiting carbohydrate, and improving lipid profiles. *Zingiber officinale* has a very low glycemic index (GI), which means it gradually breaks down to shape glucose and thus does not raise blood sugar levels as high GI foods do. Other studies have proven *zingiber officinale* has a preventive effect against diabetes complications Zingiber officinale can also protect a diabetic's kidneys liver, and central nervous system and reduce the risk of cataracts – a common side-effect of the disease (Shidfar *et al.,* 2015; Arablou *et al* .,2014 , Mozaffari –Khosravi *et al* .,2014).

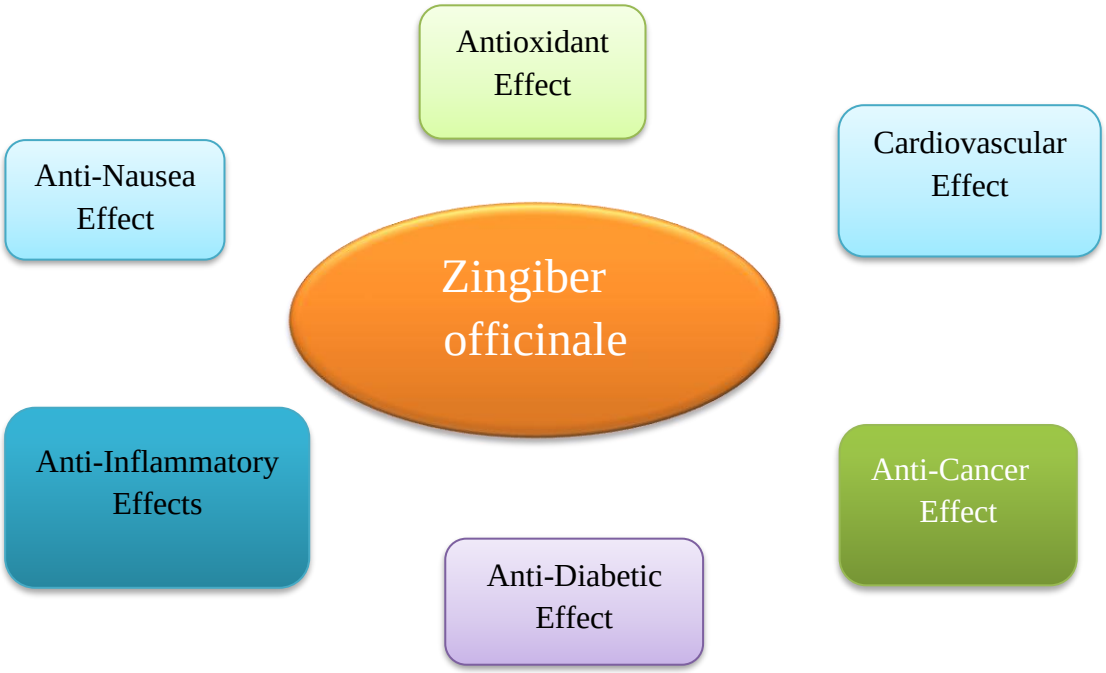


Figure (2-3) Therapeutic properties of gingr

Chapter Three

Materials and Methods

Material and Methods

3-1- Materials

3-1-1 Equipment of Laboratory and Instruments

The laboratory equipment and instrument are used in the present study are listed in Table (3-1).

Table (3-1) Laboratory Equipment and instruments

Equipment and instrument	Manufacture company	Origin
Autoclave	Labech	Korea
Burner	Amal	Turkey
Centrifuge	Hitachi	Japan
Cotton	Gelson	France
Vitek 2-compact	Biomerieux	France
Disposable syringes	Changzhon	China
Distilled Water	Memmer	Germany
Field. Emission-scanning Electron microscopes (FESEM)	Type-S-1640HITACHI Company	Japan
Fourier Transform Infrared (FT-IR) Spectrophotometer	Perkin-Elmer 1725x	Japan
Hood	Bio lab	Korea
Incubator, oven	Memmert	Germany
Light Microscope	Olympus	Japan
Loop	Himedia	India
Micropipette	Eppendrof	Germany
Millipore filter	Sartorius	Germany
Mixer	Thermolyne	USA
Petri dishes	Sterilin	UK
Pipette Tips	Sterillin	USA
Plain tube	AFCO-DISPO	Germany
Refrigerator	Beko	Korea
Sensitive balance	Denver	Canada
Slid and cover slid	Meheco	China
Stick	Meheco	China
Swaps	ATACO-Brand	Europe
Test Tube	Labcco	Germany

UV Visible spectrophotometer	Shimadzu	Japan
Vibra- cell Ultrasonic Liquid	Wiseclean	Korea
Water Flask	Himediu	India
X- ray diffraction	Bruker	Germany

3-1-2: Biological and Chemical Materials

The following biological and chemical materials were used in the present study illustrated in table (3-2)

Table (3-2) Biological and chemical materials

Type of Biological and chemical	Company	Origin
Catalase reagent	BDH	England
Oxidase reagent	BDH	England
Ethanol	Fisher	England
Glycerol(C3H8O3)	Merck	UK
Mcfarland standard solution	Biomerieux	France
Gram stain solution	Himedia	India
Normal saline solution	S.D. I	Iraq
Kovacs reagent	HIMEDIA	India
Urea solution	SD-Fine	India
Methyl red	Merck	England
Motility	BDH	England
Kovacs	Biomerieux	France
Urea solution	Biomerieux	France
Voges Proskauer reagent	BDH	England

3-1-3: Culture Media

The media that used in this study and the manufacture company

Table (3-3) cultures media used in this study

Culture media	Purpose of Use
Blood agar	To isolate the bacteria that are Gram positive
MacConkey agar	To isolate the bacteria that are Gram negative
Nutrient agar	To growth bacteria
Brain heart	To activation the isolates and for short-term

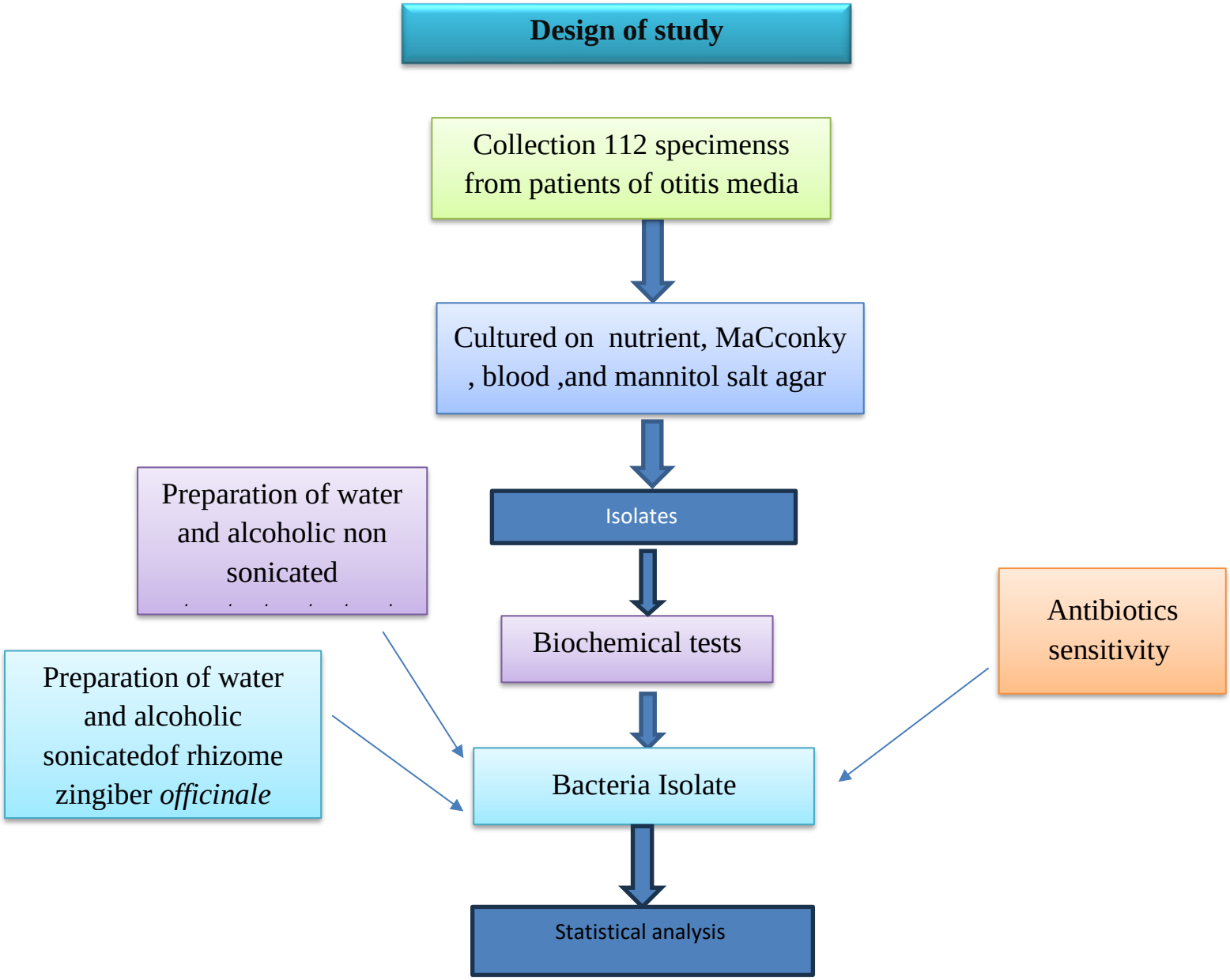
	preservation
Brain heart Infusion broth	Use for preserving bacterial isolates by adding 15% glycerol to85% of the liquid medium after sterilization and stored at (-20°C)for 2-6 months and activation the isolates after preservation
Mannitol salt agar	Isolation of *staphylococcus* and differentiation of *Staphylococcus aureus*
Mueller Hinton Agar	Use this medium to test for antibiotics susceptibility
Sugar fermentation medium	Detection of the ability of bacteria to ferment sugars
Simmons citrate	Detection of the ability of bacteria to use citrate as a nitrogen source

3-1-4 Antibiotics Disks:

Table (3-4) Shown the disk of antibiotics

Antibiotics	Antibiotics classes	Symbol	diskμ /	Inhibition zone /diameter Mm			Company /origin
				S	IN	R	
Ciprofloxacin	Fluoroquinolones	CIP	5	21≥	16 - 20	≤15	Condalab /Spain
Clindamycin	Lincosamides	CD	2	21≥	15 - 20	≤14	Roseto/ Italy
Vancomycin	Glycopeptides	VA	30	≥15	18 - 20	≤10	Bioanalys/Tur
Nitrofurantion	Fluoroquinolones	F	300	≥15	15 - 16	≤14	Roseto/ Italy
Gentamycin	Aminoglycosides	CN	10	≥15	13 - 14	≤12	Condalab /spain
Ceftazidime	Cephalosporin	GAZ	5	≥23	20 - 22	≤19	Roseto/ Italy
Erythromycin	Microlides	E	15	≥23	14 - 22	≤13	MAST/ U. K
Trimethoprim	Folate pathway antagonists	TM	10	≥16	11 -	≤10	Bioanalyse/Tur

					15		
Amikacin	Aminoglycoside	AK	15	≥14	15 - 16	≤17	Roseto/Italy
Gentamycin	Aminoglycoside	GEN	5	≥12	13 14	≤15	Condalab /spain
Imipenem	Carbapenem	IPM	10	≥15	16 - 18	≤19	Roesteto/ Italy
Azthromycin	Macrolide	AZTH	5	≥15	16 - 21	≤22	Roesto/Italy
Cloramphenicol	Antimicrobials	C	30	≥18	13 - 17	≤12	Bioanalyse/Tur
Meropenem	Bioanalyse	MEM	30	≥15	10 - 18	≤19	Turkey
Levofloxacin	Fluoroquinolones	NX	10	≥19	16 - 18	≤15	Himedia India
Ciprofloxacin	Cephem	CIP	30	≥21	16 - 20	≤15	Roesto/Italy
Piperacillin	Penicillins	PRL	100	≥12	13 - 15	≤16	Roesto/Italy


Design of study
Collection 112 specimenss from patients of otitis media
Cultured on nutrient, MaCconky , blood ,and mannitol salt agar
Isolates
Preparation of water and alcoholic non sonicated
Biochemical tests
Antibiotics sensitivity
Preparation of water and alcoholic sonicatedof rhizome zingiber *officinale*
Bacteria Isolate
Statistical analysis

3-2 Methods

3-2-1 Reagents and solutions

3-2-1-1Catalase Test

It was prepared by adding 3% of H2O2 to 100 ml of distilled water and kept in a suitable container, the test used to identify the capability of bacteria to production Catalase enzyme (Harley and Prescott,2002) .

3-2-1-2 Oxidase Test

It was prepared freshly by adding 0.1g of tetramethyl P-Phenyl diamine –dihydrochloride in 10 ml of distilled water the purpose of this reagent is to identify the capability of bacteria to produce Oxidase (Forbes et al., 2007) .

3-2-1-3Coagulase Test

This test is used to distinguish isolates of Staphylococcus spp. that produce the enzyme coagulase from bacteria that do not produce it. The slide method was used to conduct this test, according to (Forbes et al. , 2007). A drop of sterile normal saline was placed on one slide of the glass slide , and a drop of rabbit blood plasma was placed on the other slide.1-2 of the bacterial colonies growing on the nutrient agar medium were taken for 24 hours at a temperature of 37◦C, and the same amount was added to the drop of saline solution .To a drop of rabbit blood plasma , the result is considered positive when a clot is observed within 5-10 seconds with the plasma, while the mixture remains homogenous and free of lumps from the brine side.

3-2-1-4 Urase test

Urea agar slant was inoculated with tested bacterial culture by sterile loop, and then incubated at 37C◦ for 24hours; existence of pink color indicates a positive result. This test was used to detect the bacterial

capacity to produce urase enzyme which hydrolyzes urea to ammonia and carbon dioxide (Cappuccino and Welsh,2018).

3-2-1-5 Voges-Proskauer reagent

This substance consisted of two solutions: α-naphthol solution made by dissolving 5 gm of -naphthol in 100 ml of (95%) ethanol, storing the solution in a dark bottle, and mixing it prior to use.40 percent potassium hydroxide solution made by dissolving 40 grams of KOH in 100 milliliters of deionized water and mixing the solution prior to use (Macfaddine,2000).

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3-2-1-6 Motility test

Semisolid mannitol media were stabbed in the center with an inoculated needle and incubated at 37°C for (24) hours. Spread out growth from the line of inoculation indicates the existence of motile bacteria (Macfaddin ,2000) .

3-2-1-7 Kovacs reagent

Ten grams of dimethyl-amino benzaldehyde were dissolved in 150 milliliters of isoamyl ethanol by heating in a water bath at 50 degrees Celsius, followed by the addition of 50 milliliters of concentrated HCL. Small quantities of the reagent were made and stored in the refrigerator (Macfadden,2000) .

3-2-1-8 Gram Stain Solution

Gram stain solution was provided from Himedia company. These solutions included: Four solutions (Crystal violate, Iodine, ethanol, and safranin stain. It has been used to study the morphology and arrangement

of cells, to differentiate between Gram – negative bacteria and Gram – positive bacteria (Forbes et al., 2007.(

3-2-1-9 Normal Saline

It was prepared by dissolving 0.85 g of NaCl to 90 ml of distilled .water and completed by distilled water to 100ml (Colle et al., 1996)

3-2-1-10 McFarland Solution

Solution A: prepare by dissolving 0.175 of Barium chloride (Bacl) in 100ml of distilled water .

Solution B: prepare by dissolving 1 ml of concentrated Sulfuric acid (H2SO4) in 100 ml of distilled water .

When using 0.05 ml of solution A is added to 99.5 ml of solution B. Use the solution for comparison to give on approximate number of bacteria cells (1.5× 10) cell/ ml the purpose of conducting a sensitivity test against antibiotics .

3-2-1-11 Methyl red indicator

This solution was prepared by dissolving 0.2 gm of methyl red in 300 ml of (95%) ethanol, and then the volume was completed to 500 ml by D.W (Macfaddden ,2000) .

3-2-1-12 Urea solution

It was prepared by dissolving (20) g of urea powder in a volume of distilled water, then adding 100 ml of also distilled water, the final concentration of which was 20%. Then the solution was sterilized using membrane filters with a diameter of 0.45µg (Macfadden ,2000).

3-3 Culturing of Specimens

Specimens were cultured on immediate media:

3-3-1 Blood Agar

First, blood agar base medium was prepared pursuant to Protocol of manufactures instruction and sterilized by autoclave, and it was cooled to m approximately 45◦C, then added to this medium 5% of blood and poured into sterile petri dishes blood agar, this medium used to diagnosis gram positive bacteria, and is appropriate for the isolation and culturing of bacteria and for the detection type of hemolysis (Collee *et al*.,1996).

3-3-2 MacConkey Agar

This medium was prepared by dissolving 40g of MacConkey agar in 1000 ml of distilled water then sterilized by autoclave at 121◦C for 15 minutes, then put in plastic petri dish until used this medium used to diagnosis gram negative bacteria (British Pharmacopoeia,2011).

3-3-3 Brain Heart infusion

This medium is prepared pursuant to the instruction of the supplied company, by dissolving 37g of brain hart agar in1000ml of distil water, and sterilized by autoclave at 121 c for 15 minutes. It was used for developing and activating bacteria.

3-3-4 Brain Heart infusion broth

After preparing brain heart, we destroy 15% of Glycerol to 85% of the liquid medium after sterilization and stored at (-20C◦) for 2-6 months, it is used to preserve bacterial isolates for long periods (Forbes *et al*., 2007).

3-3-5 Nutrient Agar

This medium is prepared according to the manufactures instruction by dissolving 38 g of nutrient agar in 1000 ml of distill water and sterilizer by autoclave 121c for 15 minutes. It has been used to developing and diagnosis bacterial specimens (Macfaddin, 2000).

3-3-6 Mannitol Salt Agar

This medium is prepared according to the instructions of the supplied company, by dissolving 54 g of mannitol agar in 500 ml of distill water, and sterilizing it by autoclave at 121c for 15 minutes. This medium was used as a selective media for the isolation and differentiation of *staphylococcus aureus* (Macfaddin, 2000).

3-3-7 Mueller Hinton Agar

This medium is prepared according to the instructions of the supplied company, by dissolving 38g of the medium in a liter of distilled water. Sterilize the medium using an autoclave at 121c for 15 min, and after completing the sterilization process, cool it to (45-50) C◦ and pour it into sterilized petri dishes. The medium was used to grow bacterial isolates for antimicrobial susceptibility testing (Forbes *et al* .,2007).

3-3-8 Simmons citrate agar

This medium was prepared according to the manufacture instruction and distributed in glass tubes at rate of 5 ml per tube. The tubes were sterilized in an incubator and left to cool at an angle this medium was used to detect the ability of bacterial isolates to citrate as carbon source (Macfaddin,2000).

3-3-9 Sugar fermentation medium

This medium was prepared by adding (10)g of peptone and (5) g of sodium chloride (NACL) to an amount of distilled water. The volume was completed to (1) liter and (0.002) of phenol red reagent was added to it. At a concentration of (2%) and the PH was adjusted to (7.2) the medium was distributed in glass tubes at rate of (4) ml per tube. These tubes were sterilized in an autoclave and cooled, then (1) ml of sugar solution was added to them, each individually sterilized by filtration. Using membrane filters (0.45) µg which were prepared by adding (0.5) g of the sugar to be tested to (10) ml of distilled water (Macfaddin,2000).

3-4 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 (Mims *et al*., 1993, Inde Dharan and Ashraful ,1996).

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Laboratory Diagnosis

3-5 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, (Brown *et al*., 2005).

3-6 Culture and characteristics

Staphylococcus ,streptococcus ,pseudomonas grow easily in most routine media under aerobic conditions .It grow rapidly at (37°C) but pseudomonas can grow until on 42°C , Staphylococcus aureus usually form gray to golden yellow colonies ,producing β hemolysis on blood agar plates , Streptococcus pneumonia form gray colonies on blood agar ,producing α-hemolysis under aerobic conditions and producing β-hemolysis under anaerobic conditions ,but Pseudomonas bacteria transparent colonies form on the MacConkey agar .

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3-7 Microscopic examinations

A portion of the bacterial growth was transferred by a loop and placed on a glass slide , fixed and stained with gram stain and examined under the oil lens of light microscope, the shape of the Streptococcus bacteria observed as blue cocci arranged in regular groups resembling a twisted chain , the shape of the Pseudomonas bacteria was observed as red cocci , arranged in irregular group it resembles a penis and has one flagellum the shape of the Staphylococcus bacteria was observed as blue cocci , arranged in irregular clusters (Gillet et al .,2002) .

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 (Bergoys manual, 1994). 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.

3-8 Identification of Bacterial Isolate with VITEK-2 system

The Vitek2 was used to valiate the biochemical and antibiotic assay which was performed according to the manufacturer's instructions. This machine consists of personal device, reader incubator, which is made up of several internal components including; card filling process, loding process, card cassette, barcode scanner, card sealer, cassette spiral and incubator. Along with optical transport, waste processing electronic control tools and firm ware. The system is equipped with an expanded identification database for all routine identification tests that orivide improved microbial diagnostic efficiency 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. Preparation of the bacterial suspension; A sterile swab was used to transfer a sufficient number of bacterial isolates pure culture colonies (the colony must be 24 hours of age) were suspended separately in 3 ml of sterile saline in transparent plastic test tubes. The turbidity was modified by inserted test tubes into the colony standardization assay system with McFarland's standard solution (1.5x108cells/ ml).

II. Identification card was inoculated with isolated. The test tube containing isolates suspension was placed into a special rack and the card

was placed in the adjacent slot while the transfer tube was inserted into the corresponding suspension tube. The filled cassette was placed manually or automatically into a vacuum chamber station. After vacuum was applied and ai was reintroduced to the microchannels that filled all test wells.

III. Card sealing and incubation ;Inculcated card was passed by a mechanism , that cuts the transfer tube and seals the card before being loaded into the carousel incubator .Carousel incubator can accommodate up to 30 or up to 60 cards .All types of cards are incubated online at 35.5+1.0°C.Each card is taken out of the carousel incubator once every 15 minutes ,transmitted to the optical system for reaction readings ,and then returned to the incubator until tube next reading time, data were collected at 15 minute intervals during the entire incubation period.

3-9 Methods of Preserving and Maintaining Bacteria

3-9-1 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 in autoclaves for 15 minutes at a pressure of 21, the bacteria were incubated at a temperature of 37 ◦C for 24 hours, and kept in the refrigerator until use, (Benson, 2002).

3-9-2 Preparing Long – Term Culture

Brain heart media was prepared according to the instruction of the producing company, and sterilized in autoclaves for 15 minutes at a pressure of 21◦C, and 5% of glycerol was added to it. the medium was poured into small heat-resistant plastic tubes, and placed in the incubator

at a temperature of 37 for 24 hours. and kept in the freezer at -20◦C until use (Vandepitte *et al*., 2003).

3- 10 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) (Bauer,1996).

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 at (37°C)for (24) hours , and by adding sterile normal saline compared with (0.5) a standerd Mcfarland tube($1.5x10^8$CUF/ml), then spread on Muller Hinton agar (MHA) using a sterile cotton swab 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°C for24h ,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 slandered inhibition diameter were used according to the clinical and laboratory standard Institute (CLSI)2021) for sensitivity or resistance of the organism to each antibiotic.

3-11 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 left to dray at room temperature for 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. Picture (3-1) shows zingiber officinale rhizome, Picture (3-2) show *zingiber officinale rhizome* plant after it has been cut

Figure (3-1) Rhizome of *Zingiber officinale* plant

Figure (3-2) *Zingiber officinale Rhizome* plant after it has been cut

3-12 Preparation of *Zingiber officinale Rhizome* non sonicated extract

3-12-1 The Non sonicated aqueous extract

Take (10,20,40) mg/ml of dry *zingiber officinale Rhizome* powder and mix it with (100) ml of distilled water 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 by using three

layers of medical sterile gauze to get rid of the remnants the vegetable powder then centrifuge 3000 pm for 10 minutes, then with filtered by Millipore 0.45 Mm, and then kept in the refrigerator until use (Hernandezi *et al*., 1994).

3-12-2 The Non sonicated ethanolic extract

Take (10,20,40) mg/ml of dry *zingiber officinale Rhizome* powder and mix it with (100) ml of ethanol ethanolic 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 by using three layers of medical sterile gauze to get rid of the remnants the vegetable powder and then with filter paper, and then dry it by put in oven 40°C (Hernandezi *et al* ., 1994)

3-13 Study the effect of *zingiber officinale rhizome* non sonicated extract on bacteria

The well diffusion method was used by etching according to (Zinedine and Faid, 2007). Bacterial isolates were grown at a dilution of 1.5 according to the MacFarland 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 non-sonicated aqueous of *zingiber officinale* Rhizome, and non-sonicated ethanolic extract of *zingiber officinale Rhizome* ,were added to the etching separately and at different concentration (10,20,40) mg /ml add it to the positive control hole that contain distilled water. the dishes were left at room teem premature in order for the non-sonicated extract penetrate into the medium, for two 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

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3-14 Preparation of sonicated extract from *zingiber officinale rhizome*

To prepare100gm of sonicated *zingiber officinale*, *zingiber officinale* 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 two days. after two days, the powder was collected from the plates the weight of 20 gm of dried *zingiber officinale* 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 second, stopping for 5second, then the mixture was collected and preserved in the refrigerator at 4◦C until use. Picture (3-3) show sonicated of *zingiber officinale rhizome* (Mazhir *et al*., 2020).

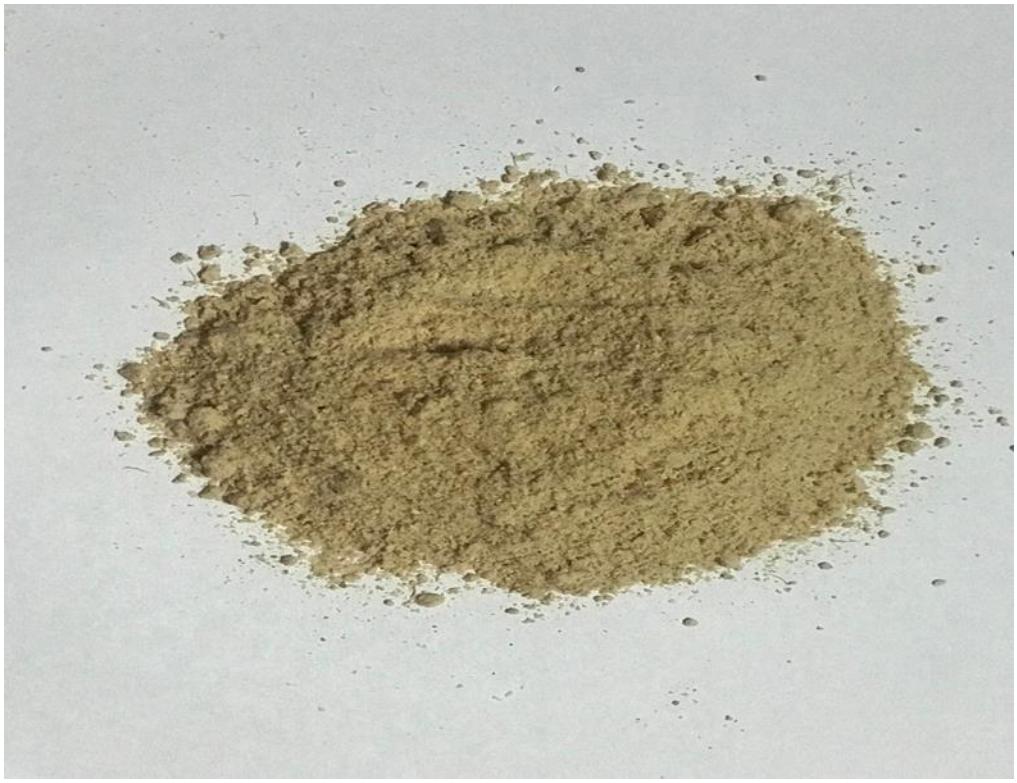

Figure (3-3) sonicated of *zingiber officinale Rhizome*

3-15 Study the Effect of Sonicated on Bacteria

The diffusion method was used by etching according to (Zinedine and Faid, 2007). Bacterial isolates were grown at a dilution of (1 .5) cell/ ml according to the MacFarland 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 *Zingiber officinale Rhizome* sonicated, water and ethanolic *zingiber officinale Rhizome* sonicated, were added to the etching separately and at different concentration (100,300,500) µg /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◦ C. after that, a ruler was used to measure the diameters of the inhibition zones formed around the holes.

3-16 Characterization of Synthesized *Zingiber officinale* sonicated

The physical characteristics of *Zingiber officinale* sonicated were .characterized by SEM, XRD, FTIR

3-16-1 Analysis by Field Emission Scanning Electron Microscopy (FESEM)

Measurements after the synthesis, sonicated were prepared as a powder and studied analyzed under scanning electron microscope with different magnification powers which gives a clear image to the synthesized sonicated It reveals the morphological features of sonicated and size measurements by scanning electron microscope (Inspect S 50, fei) according (Sadhasivam *et al*.,2010).

3-16-2 X-ray Diffraction (XRD)

The X-ray diffraction was used for characterization of *zingiber officinale* sonicated the powder of *zingiber officinale* sonicated specimens was dispersed on a low background noise specimens' holder and analyzed in a Bruker D8 Advance X-Ray diffractometer equipped with a LynxEYE detector. X ray diffraction analysis was operated at a voltage of 40KV, with current of 40 mA, with copper radiation of 1.54060 A. the scanning was performed in the 2ɵ range of 10◦ to 40◦ at 0.02◦/min with timeconstant of 1.2 s. (Gangadoo *et al*., 2017)

3-16-3 Fourier Transform Infrared Spectroscopy (FITR)

The transmittance of the prepared formulations was accomplished by FT-IR spectrophotometer, in a spectral range of 400-4000 cm-1 at 2 cm-1 resolution. The data sets were averaged over 64 scans (Tugraova *et al*., 2018).

3-17 Detection of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of sonicated

The MIC is lowest rhizome *zingiber officinale* sonicated concentration that inhibited completely the bacterial growth can be detected by using micro-plate method , the turbidity of bacterial suspension was compared and matched with the turbidity of 0.5 MacFarland units .The MacFarland 0.5 standard corresponds approximately to a homogeneous .Suspension of 1.5×10^8 cells/ml .An amount of 100µl of BHI media was transferred to each well 100µl of *zingiber officinale* rhizome sonicated,(128 µg /ml) was added in each well of colum2 serial dilutions were performed from column 2 to column 11, to obtain the final NPs concentrations ,which varied from 128 µg/ ml in (2^{nd} well) to 2 µg /ml in (11^{th} well) . A 10 microliter of bacteria in column were added to each well, except all wells of (column 1). NPs free well (column 12) contain medium and in column and (colum1) contained media only. The microtiter plate incubated at 37°C for 24hr. OD at 570 nm was recorded spectrophotometrically. MIC was determined as the lowest NPs concentration showing absence of growth as compared with the growth in the rhizome of *zingiber officinale* sonicated– free well (Kumar *et al*.,2020).

3-18 Statistical Analysis

The statistical program (SPSS) version (12) was used to analyze the results of this study, as the one-way ANOVA test was used, and the least significant difference (LSD) was used to search for the presence of significant differences between the different coefficients at a significant level of 0.05(Morgan *et al*.,2004).

Chapter Four

Results and Discussion

4-1 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 (4-1).

Table (4-1) 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%

4-2 Study Group

4-2-1 Distribution of Study Groups According to Age Groups

Table (4-2) shows the number of infections with middle ear infection and its percentage for the study groups according to the group. the results of the current study showed that the highest infection rate was in the category (1-15) years.

Table (4-2) Distribution of patients with otitis 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%

The results of the current study agreed with a study by (Mahmood *et al*., 2019). The current study agreed with a study conducted by (Jabber 2019). the current study agreed with a study conducted by the researcher (2010) in Najaf governorate with the study. the current study also agreed with a study in Saudi Arabia (Al-Hammar *et al*., 2018). A study conducted by the researcher (Seid *et al*., 2013) showed that the younger age group had the highest incidence of the disease. (Salman,2008) found that the highest prevalence of otitis media was within the age group of (20 - 11). The study conducted by the researcher (Moderate and others (2018) also agreed that the younger age groups are more affected by the disease. the study of the researcher (Osazuwa and other2011) showed that the age group (1-15) years is the most age group with the disease. the current study also agreed with a study conducted by the researcher (Nafa and Al kwaty 2015).

After otitis media is a significant disease and increases vulnerability in younger age groups as a result of exposure to many external conditions such as exposure to smoke, crowding, and living conditions as well as the social group with low economic income , and these case are among the risk factors for otitis media (gb *et al* ., 2010) , the highest rate of infection in the younger age group is due to the incompleteness of the immune system , especially in children , and poor physical structure (DeAntonio *et al*., 2016) this could be caused by lack of hygiene, inserting contaminated tools into the ear , or it occurs as a result of transmission of infection between students .Narrowing of the Eustachian tubes , which makes them more susceptible to an increase in the infection , leads to the transmission of pathogens from the respiratory tract to the ear (Leu *et al*.,2014).

4-2-2 Distribution of the Study Groups According to Sex

The study consisted of (100) patients with otitis media. if in males 58 and females (42). The results of the current study added that the number of males with Otitis inflammation is more than females as shown in Table (4-3)

Table (4-3) Distribution of patient with otitis according sex

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

The results of the current study agreed with the results of the researcher (Jabber 2019), as the study showed a higher number of people with middle ear infection of the male sex compared to female sex. the results of the researcher (Al-Arraqchi *et al*., 2019) showed. higher incidence of middle ear infection in males compared with females. the current results also agreed with the results of the (Alansary study 2016) that the percentage of males infected with the disease was higher than the percentage of females. The current study also showed agreement with results (Almamorys2014) if it showed a high incidence of middle ear infection in males as it reached (58) compared to females (42).

It is believed that males are exposed to different condition at work such as dust and humidity, and may be more involved in outdoor activities, and thus be more vulnerable to a polluted environment, and wearing a headscarf for females can be considered an important factor to reduce infection. (Al Atachi *et al*., 2019). it is also believed that the reason for the higher incidence of disease in males than in females is due to the fact that males are more vulnerable to environmental conditions in their lives such as swimming pools or in rivers and other

ponds have females, and this result is consistent with other results (Kallin *et al*., 2013, morris *et al*.,2005) indicated that both sexes are affected by the disease , and there were no significant differences between infection males with otitis media and infection females with otitis media, as the percentage reached (compared to) the differences are due to sex specific hormonal factors.

4-2-3 Geographical Distribution of Study Specimens

The results of the current study showed that the highest incidence of middle ear infection in the regions urban areas, with a percentage of (72 %), and the lowest percentage was in rural areas (28%), as shown in table (4-4).

Table (4-4) Geographical distribution of study specimenss

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

The results of the current study agreed with the study of the researcher (worku *et al*. ,2017) that the incidence of middle ear infection in urban areas increased by compared to rural areas by. The results also agreed with the results of the study of (Ali,2011), and he mentioned I n his results that the highest incidence of otitis media was urban areas, compared to rural areas. The results of the researcher (Martines *et al*., 2016) showed a high incidence of otitis media in urban areas. compared to rural areas the results of the current study are consistent with the results of the study conducted by (Morris *et al*., 2005), how found that the percentage of people with otitis media reached in rural.

The reason for the increase in people with otitis media in urban areas is more than in patients from rural areas, due to the lack a visitor from

rural areas (Ali 2011., Auinge, 2003) because there are significant differences between parents who live in rural areas compared to urban areas because parent in rural areas is looking for traditional centers and lack of health awareness (shaheen *et al*., 2012).

4-2-4 Study of the patient with otitis according to the pathological condition

The results of the financial study showed the highest percentage of chronic otitis media and the lowest percentage of acute otitis media, and as shown in table (4-5)

Table (4-5) Distribution of patient groups, disease state

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

The results of the current study agreed with the results of the researcher (Hassooni 2018), which he conducted in Babylon Governorate, that the incidence of chronic otitis media is higher than acute otitis media. the results of the (Al-Hamadany 2017) study showed that the incidence of chronic otitis media was higher when compared with acute otitis media. the results of the researcher (Salmman,2008) That the incidence of insured middle ear infection was higher than that of patients suffering from acute otitis media.

Cases of chronic otitis media (COM) are more common than (ACM) and this can be attributed to the fact that the infection (OM) is the most received after respiratory infections as the insured infections are more than (AOM) especially in winter outbreaks (Hamadany 2017) this disparity can be explained in part by a lack of health awareness in the community, inadequate health infrastructure structure, and limited access

to medical care (Roy *et al*.,2007). The reason for the rise in chronic otitis media is due to the ineffective treatment of acute otitis media and its transformation into chronic inflammation (Yaseen Qader 2012), (Jameel *et al*., 2017) due to the large number of Iraqi families living in the crowded regions, as well as lack of hyhiene, malnutrition, and overcrowding are more susceptible to disease, including otitis media (Lasisi *et al*., 2008).

4-3 Detection of *staphylococcus aureus, streptococcus pneumonia pseudomonas aeruginosa* using Traditional methods

A total of 100 isolates of *staphylococcus aureus ,streptococcus pneumonia pseudomonas aeruginosa* among the culture -positive samples, from patients with otitis media infection ,they are cultured for the isolation and diagnosis of *staphylococcus aureus ,streptococcus pneumonia pseudomonas aeruginosa* ,using differential media (blood agar ,nutrient agar, mannitol salt agar ,MacConkey agar , and Muller Hinton agar) ,The Gram staining technique was performed to study the microscopic properties and was observed under an oil immersion lens (100X) .

staphylococcus aureus can be distinguished by producing yellow colonies on mannitol salt agar with surrounding yellow medium (mannitol fermentation, thus changing the color of the medium from red to yellow) , in blood agar , all isolates produce clear β- hemolysis around their colonies .Positive from of the coagulation reaction.

Streptococcus pneumonia can be distinguished Gram negative, the media appears as a greyish, shiny mucus, that is soluble in solution of bile salt, with age, autolysis occurs and the colony collapses. On blood agar α-

hemolysis will appear under aerobic conditions and β-hemolysis will appear under anaerobic conditions.

Pseudomonas aeruginosa can be distinguished by transparent colonies form on the MacConkey medium and have an unpleasant odor resembling that of grapes

4-4 Detection of *staphylococcus aureus, streptococcus pneumonia pseudomonas aeruginosa* by biochemical methods

The gram stain of these isolating cultures revealed single or bi arrangement. All *Staphylococcus aureus* isolates have shown appositive result in biochemical test for catalase test coagulase, hemolysis test and mannitol fermentation while negative results to oxidase test as shown in figure (4-6).

Table(4-6) show biochemical test of *Staphylococcus aureus*

Test	Results
Catalase	+
Oxidase	-
Coagulase	+
Hemolysis test	Alfa, Beta
Mannitol fermentation	+
Shape	Diplococci
Voges Proskauer	
Motility	
Methyl red	
Oxidative fermentation	Facilitative aerobe

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 as shown in table (4-7).

Table(7-4) show biochemical test of *Streptococcus pneumonia*

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

All *Pseudomonas aeruginosa* isolates have shown appositive result in biochemical test for catalase test oxidase, Simmons citrate, motility test, H2o production, and growth at 42°C. But negative to Gram stain test, methyl red, urase, Voges Proskauer, indole test, to oxidative fermentation obligate aerobes, as shown in table (4-8).

Table (4-8) show biochemical test of *Pseudomonas aeruginosa*

Test	Result
Gram stain	-
Catalase	+
Oxidase	+
Simmons citrate	+
Motility test	+

H2O production	+
Methyl red	-
Urase test	-
Indole test	-
Voges prosKular	-
Oxidative fermentation	Obligate aerobic

Table (4-9) Distribution of Gram positive and Gram-Negative Bacteria from Otitis specimens

specimens specimens	No. of bacteria	Percentage
Gram positive	79	79%
Gram negative	21	21%
Total specimens	100	100%

Table (4-10) Shows three types isolate of bacteria

Isolates bacteria	Number of isolates	Percentage
Staphylococcuss pp.	54	54%
Streptococcus spp.	25	25%
Pseudomonassp p.	21	21%

This study showed that Gram positive specimens recovered from otitis specimens that was 79 (79%), and gram negative was 21 (21%), which is relatively similar to the result obtained by (Moges et al., 2002).

All these specimens were diagnosed according to morphological, cultural and biochemical test

4-5 Identification of bacteria isolates using Vitek-2 compact system

In vitek-2 compact system, the isolates were identified as *Staphylococcus aureus* of (54) %, *Streptococcus pneumonia* of (25) %, *Pseudomonas aeruginosa* of (21) %. biochemical characteristics of all bacterial isolates were done also by VITEK-2 compact system, appendices (1,2,3), VITEK-2 system is an easy to handle, provides a rapid result (during 4-15h) and reasonably suitable for the identification of microbial species (Garcia-Garrote et al.,2000).

4-6 Susceptibility of bacteria to antibiotic

The sensitivity of the *staphylococcus, streptococcus, pseudomonas* isolates under study was tested *staphylococcus* against 8, *streptococcus* against 7, and *pseudomonas* against 3 types of antibiotics. 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 the sensitivity of the isolation to antibiotics was investigated by the Kirby –Bauer method of disc diffusion in 1988. The diameter of the inhibition zone around the antibiotic discs used to determine the extent of the sensitivity of these isolates to antibiotics

4-6-1susceptibility of *Pseudomonas* bacteria to antibiotics

Twenty one identification *P.aeruginosa* isolates were evaluated against 6 common antibiotics ,as shown in appendix table (2)

The study revealed that *P.aeruginosa* is 100% resistant to piperacillin in specimens according to (Vitkauskien et al.,2010), and (Hussein et

al.,2018),who reported rates of 37.0% ,59.61% respectively , this result is completely inconsistent, Although Al- Marzoqi (2013) and Corehtash (2015), reported resistant rates of 100% and 85.4% respectively ,this result is comparable to or near to those results .Beta lactam -beta-lactmase inhibitors combination antibiotics also showed resistance to pipercillion -tazobactm (26%) .

A sensitivity test was conducted for *Pseudomonas* bacteria against different type of antibiotics, this bacteria resistance against carbapenem (Imipenem and meropenem) ,where (85.71%,42%)isolates were resistance to it .This result disagreed with(Begum et al.,2013) who reported sensitivity rate, toward Imipenem (93.3%) and the curet study agreed with an earlier report by (Amutha et al .,2009) who reported resistance rate of *Aeruginosa* strain against Imipenem (5%), these antibiotics are members of β-lactams family, mainly used to treat Aeruginosa infections. The result of this study showed that Carbapenems antibiotics and this could be due to its proper and infrequent use in the treatment so can be considered as the drug of choice for treatment *P.aeruginosa* infection .The current study show more isolates were resistant to Ceftazidime where was his resistance rate (90.47). But some isolates was resistance to Gentamycin where (71.4%) isolates were resistant, this result was close with (Negi *et al*., 2015) who reported (45.5%) of isolates resistance to gentamycin. And the current study agrees with the finding of another study, where the researcher considered Gentamycin to be one of the preferred drugs in the) eradication of this disease 1998 Al- ameer .(Another researcher believes that is the Gentamycin is the most effective in treating this disease Alkhalil,1980),Resistance to flouroquinolones showed (47.61%),(42.8%) to ciprofloxacin and levofloxacin, respectively , for ciprofloxacin this result is compatible with the data reported by (Alderzi 2012),who record

that (23.9%) of isolated were resistance to ciprofloxacine, but disagree with that reported by (Othman et al., 2014) ,who recorded (61.3%) resistance .For levofloxacin(42%) this rate is near to the result of (Yayan et al.,2015) (30.6%) and (Lila et al.,2017)(36.1%) . Flouroquinolone The results showed high resistance to beta lactam's mainly piperacillin and this is mediated by beta lactamase due to that when use piperacillin the resistance was dropped from 100% to 26%.

4-6-2 Susceptibility of *Staphylococcus* bacteria to antibiotics

Fifty-four identification S aures isolates were evaluated against some common antibiotics, as shown in appendix table (3),

A Sensitivity test was conducted for the *Staphylococcus* isolate as the causative agent of the cases and the sensitivity patterns of the isolates. the results showed that the preferred drug for treatment is Amikacin, the sensitive rate of Amikacin (98.14%) isolates was sensitive to it and the reason for its effectiveness is due to its lack of use local, and the reason for the lack of use was that this bacterium could not adapt to it and then resist it this antibiotic belongs to the group of Aminoglycoside antibiotics and is used to treat severe infections caused by Gentamicin and Tobramycin. thus, it is distinguished from the rest of the antibiotics that make protein, which inhibit growth. the group of amino glycoside antibiotics participates in being poorly absorbed by the digestive system. therefore, they are taken by muscle or intravenously, and all of them are excreted through the kidneys include the appearance of blood in diuresis and frequent urination times, and then kidney failure. these serious side effects have occurred from the use of antibiotics belonging to this group, especially the number of elderly patients or patients who suffer from burns that are accompanied by a malfunction therefore, the concentration of the antibiotic in the blood must the monitored in such cases (Mims et al., 2004). The sensitive rate of Clindamycin was (90.74%), in the current

study the sensitive rate of Ciprofloxacin is (68.51%). The availability of a wide range of fluoroquinolone medications namely Ciprofloxacin, allowed for the efficient treatment of infections caused by *S.aureus* strains , these strains quickly developed resistance to these drugs(Pourmand *et al*., 2014). But it still low and this may be explained by the fact that local isolates didn't develop high resistance to this antibiotic due to its limited use in comparison with penicillin and also because Ciprofloxacin is a broad-spectrum antibiotic. In the current study show the rate of sensitive to Trimethoprim was (92.59%). The result show that the sensitive rate of *S.aureus* isolates to vancomycin is (96.29%) .the result of present study agreed with a study done by(Salman and Ali,2017) who mentioned that the complete low resistance to Vancomycin(9.1%) .The establishment and subsequent spread of Vancomycin resistance is seen as a problematic scenario that adds to the difficulty of treating and most Vancomycin be reserved to treat isolates that are resistant to other antibiotics. The percentage of sensitive to Nitrofurantion was high, reaching (96.29%), and to Gentamycin reaching (92.59%). All isolates are tested to Chloramphenicol, the results showed that the sensitive rate for Chloramphenicol (92.59%) the result of the present study agreed with a study done by (Petrillo et al., (2021) found all isolates are susceptible to chloramphenicol (100%) topical chloramphenicol is an excellent first line choice of therapy for suspected or proven.

4-6-3 Susceptibility of *Streptococcus* bacteria to antibiotics

Twenty-five identification *S. pneumonia*, isolates were evaluated against 8 common antibiotics, as shown in appendix table (4), 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 widely used for many decades to treat variety of infections. Tetracycline is considerably more potent than tetracycline and is able to function in bacteria expressing resistance to tetracycline, 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, Vancomycin is a glycopeptide antibiotic that is widely used to treat serious infections, it binds to the dipeptide D-Ala4-D -Ala 5 of lipid II and pre-vents trans glycosylation and transpeptidation catalyzed by PBP2and PBP2a and antagonizes peptidoglycan remodeling (zeng *et al*.,2016)

4-7 *Zingiber officinale Rhizome* Non sonicated extract

The results of the chemical detection of *zingiber officinale Rhizome* showed the presence of active compounds, such as turbines, glycoside, alkaloids, soaps and volatile oils this is consistent with the findings of the researcher (Ahmed,2000) as the *zingiber officinale Rhizome* non sonicated extract is considered one of the substances that inhibit the growth of bacteria because it contains many effective substances including phenols and volatile oils.

4-8 Investigation of the effect of non-sonicated aqueous *Zingiber officinale* on *Staphylococcus, streptococcus, pseudomonas* bacteria

We made two non-sonicated from the *zingiber officinale rhizome*, non-sonicated aqueous extract and non-sonicated ethanolic extract, and we made 3 concentrations of each non sonicated extract (10,20,40) mg/ml and we treated with each concentration 3 types of bacteria, Staphylococcus, *Streptococcus, Pseudomonas*

It was noticed that the non-sonicated aqueous *of zingiber officinale Rhizome* had an effect on isolates, and with (10,20,40) mg/ml concentrations of the non-sonicated, except for concentration 10 mg/ml. 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 mg/ml they showed different inhibition zones, and according to the types of isolates, the zones of inhibiting ranged according to the following

After the inhibitory effectiveness of the *zingiber officinale* Rhizome non sonicated extract was investigated against the *staphylococcus* isolates, and after selecting the most isolates and using bacterial culture by diffusion in the holes, it was observed that the water non sonicated of *zingiber officinale* rhizome had an effect on these isolates and with different concentration of the non-sonicated extract as for the concentrations 20,40 mg/ml, it showed different inhibition zone, and according to the types of isolates.

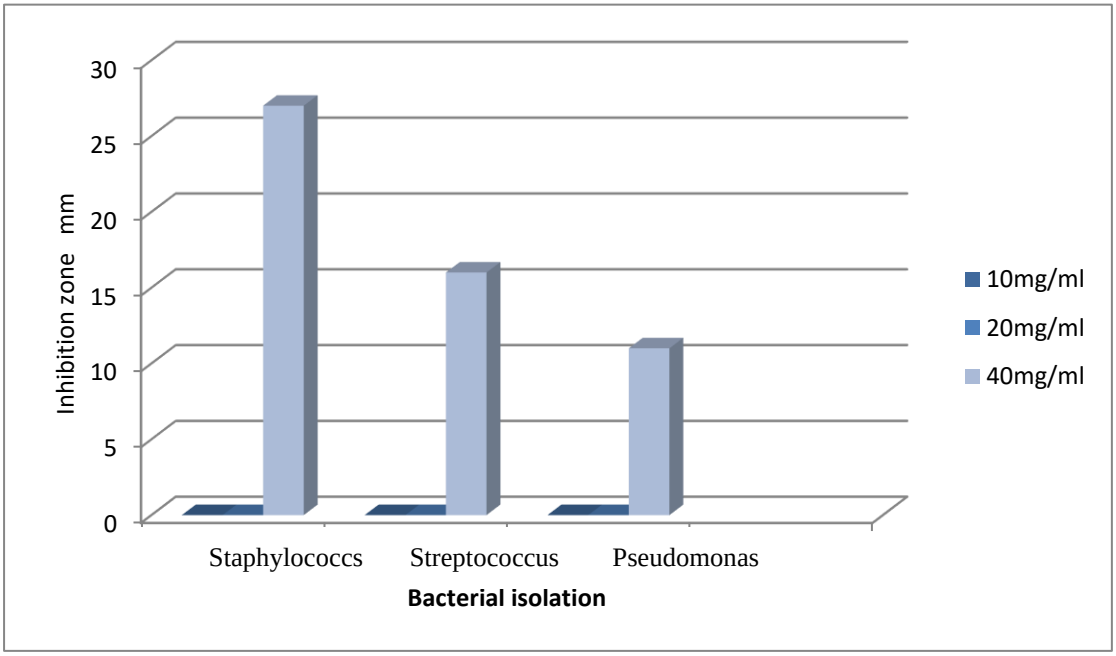

Figure (4-4) show effect *zingiber officinale* aqueous non sonicated extract on bacterial isolates.

As for *staphylococcus* bacteria, the effect of non-sonicated aqueous extract was weak on bacteria. at a concentration 10mg/ml and 20 mg/ml the bacteria not effect, and at a concentration of 40 mg/ml, the inhibition zone was (27) mm

As for *streptococcus*, the effect of non-sonicated aqueous extract was also weak on them. as a concentration of 20 mg/ml, there was no effect of non-sonicated aqueous extract on inhibiting the growth of the isolate. at a concentration of 40 mg/ml the zone of inhibition was slightly higher and amounted to (16) mm

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

From this we conclude that the *staphylococcus* bacteria were the most effective species with non sonicated aqueous extract of *zingiber officinale* Rhizomes , followed by the *streptococcus* bacteria in the terms of susceptibility , while the *pseudomonas* bacteria were lees affected by the non-sonicated extract of *zingiber officinale* Rhizomes a study conducted by (Hussein and Mohammed 2021) that the non-sonicated extract is ineffective when the diameter of the inhibition zone is less than 0.9mm , and it is partially effective when these measurements are between (0.9-12) mm, and it is effective between (13-

18) mm, and it is very effective when the diameter of the inhibition zone is 18 mm or more.

4-9 Investigation of the inhibitory activity of non sonicated ethanolic extract of *zingiber officinale Rhizomes* by well diffusion method

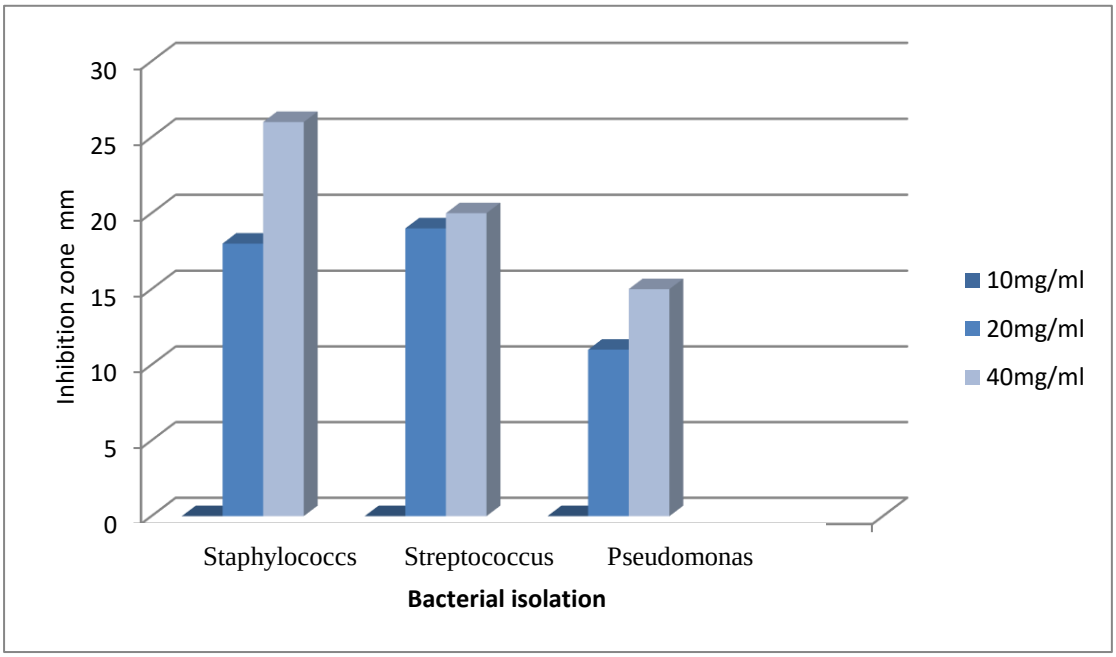

Figure (4-5) effect of non-sonicated ethanolic extract on bacterial isolates.

The results shown in Table (5) in Appendix showed the inhibitory activity of non sonicated ethanolic extract of *zingiber officinale Rhizomes* against the isolates *Staphylococcus*, *Streptococcus*, and *Pseudomonas* bacteria at different concentration (10,20,40) mg/ml.

At a concentration of 10 mg/ml, *staphylococcus* bacteria did not appear to be affected by non-sonicated ethanolic. Non sonicated ethanolic extract showed a very high inhibitory activity against *staphylococcus* bacteria, where the zones of inhibition were 18 mm at a concentration of 20 mg/ml and was 26 mm at a concentration 40 mg/ml.

As for *streptococcus* bacteria, at a concentration of 10 mg/ml, no inhibition of the bacteria appeared. at a concentration of 20 mg/ml, the inhibition zone was (19) mm, while a concentration of 40 mg/ml was more effective in inhibiting the growth of bacteria, as the zone of inhibition was (20) mm, While the *pseudomonas* bacteria had the least effect on non-sonicated ethanolic extract for concentrations 10 mg/ml no inhibition appeared, but at a concentration 20 mg/ml the inhibition zone was (11) mm and in concentration 40 mg/ml the diameter of inhibition zone was (15) mm.

These results are consistent with what was indicated by Zaika (1988) , who obtained an increase in the inhibition area by increasing the concentration of the aqueous and the concentration of non-sonicated aqueous and ethanolic extract used of *zingiber officinale* Rhizomes while the effectiveness was at it is best at a concentration of 40mg/ml, as it is in the aqueous and ethanolic non sonicated, and this is concentration with (Sebiono 2011) indicated if an increase in the diameters of the inhibition zone was found with the increase in the concentration of the ethanolic and aqueous non sonicated of *zingiber officinale* Rhizomes the *zingiber officinale* plant treats many microbial disease such as bacterial and fungi infections , and it contains many compounds that possess high anti – microbial effectiveness . it was found that the Rhizomes of the *zingiber officinale* plant contain alkaloids that inhibit the growth of germs (Atai *et al*., 2009) the non sonicated ethanolic extract also carries greater potency then the non-sonicated aqueous extract, and this approaches what (cowan 1999) indicated *zingiber officinale* Rhizome non sonicated have a high effectiveness in inhibiting the growth of bacteria isolated from clinical cases because they contain tannins that

have the ability to stimulate phagocytic cells and strengthen immunity in the body the presence of alkaloids has a bactericidal effect due to it is ability to Integrated into DNA strand , as well as interfere with the necessary metabolic pathways and physiological activity . (philipson *et al*., 1987) studies indicated that the phenolic substances in *zingiber officinale* non sonicated extract have a role in the effectiveness of these pharmacological non sonicated, and the compound zingiber officinal has antioxidant properties (Bahandari *et al*.,2005) shogaols and zingerone is present in small amount in fresh *zingiber officinale*, but it is present in a greater amount in dried *zingiber officinale* (Jolad *et al*, 2004) it has been shown that zingiber officinale has a pharmacological activity against *staphylococcus*, *streptococcus* and *pseudomonas* (Jagetia *et al*., 2003) the ethanolic non sonicated extract of *zingiber officinale Rhizomes* has an inhibitory effect on the growth o bacteria because it contains zingiberene and farnescene active substance such as turbines that have a role in the direction of Gram- negative and gram positive bacteria . these compounds work to renew the cell wall or weaken the vital activity of the cell. Effective transport of ions and salts across the membrane.

Table (4-11) Inhibitory ability of *zingiber officinale* non sonicated extract on bacterial isolates isolated from otitis media

Ethanolic non sonicated extract *pseudomonas*	Aqueous non sonicated extract *pseudomonas*	Ethanolic non sonicated extract *streptococcus*	Aqueous non sonicated extract *streptococcus*	Ethanolic non sonicated extract *staphylococcus*	Aqueous non sonicated extract *staphylococcus*	Concentration mg/ml
12.00	**2.00**	**17.67**	**2.33**	**22.33**	**24.00**	**40**
8.67	**4.00**	**14.67**	**4.67**	**15.00**	**2.67**	**20**
2.33	**2.00**	**2.67**	**2.33**	**2.33**	**3.00**	**10**
4.141	**n.s**	**4.172**	**n.s**	**5.313**	**5.058**	**LSD**

The results of using aqueous and ethanolic *zingiber officinale* non sonicated extract inhibitors against different types of bacteria, and as shown in the tables above, showed that there were significant differences between the different concentrations. It can be noted that the aqueous non sonicated extract of *zingiber officinale* showed an inhibitory effect against the *Pseudomonas* bacteria at concentration10 mg/ ml. This was the lowest diameter of inhibition against *staphylococcus* and *streptococcus* bacteria at concentrations 10,20,40 mg/ml. The highest effect was against *staphylococcus* bacteria, where the inhibition rate reached 24.00. As for sonicated ethanolic, it had an inhibitory effect on the isolates used in this study, as the diameter of the inhibition zone towards *staphylococcus* bacteria was 22.33 at a concentration of 40mg/ml, in addition to its effect towards the rest of the isolates.

4-10 Investigation of the Inhibitory Activity of An Aqueous Solution of Different *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 particles, and we treated them with three types of *Staphylococcus, 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 the effect of an aqueous

solution of *zingiber officinale* sonicated at three concentrations on the types of isolates under study.

For *staphylococcus* bacteria, the diameters of the inhibition zones ranged from 18 mm at a concentration of 300µg/ml, and 25 mm at a concentration of 500πg/ml, but at a concentration of 100µg/ml we did not find any inhibition as for the *streptococcus* bacteria, no effect of the solution was shown at a concentration of 100 µg/ml, as in *staphylococcus* bacteria. at a concentration of 300 µg/ml, the diameter of the inhibition zones was 18 mm, and in the concentration of 500µg/ml, the diameter of the inhibition zone was higher, as it was 25mm.

As for the *pseudomonas* bacteria, it had a weak effect in front of the aqueous solution of nano- zingiber officinale, where at a concentration of 100µg/ml and a concentration of 300µg/ml, no inhibition appeared in the growth of bacterial isolates, but at a concentration of 500µg/ml inhibition of bacterial growth appeared, and the diameter of the inhibition n zones was 21 mm.

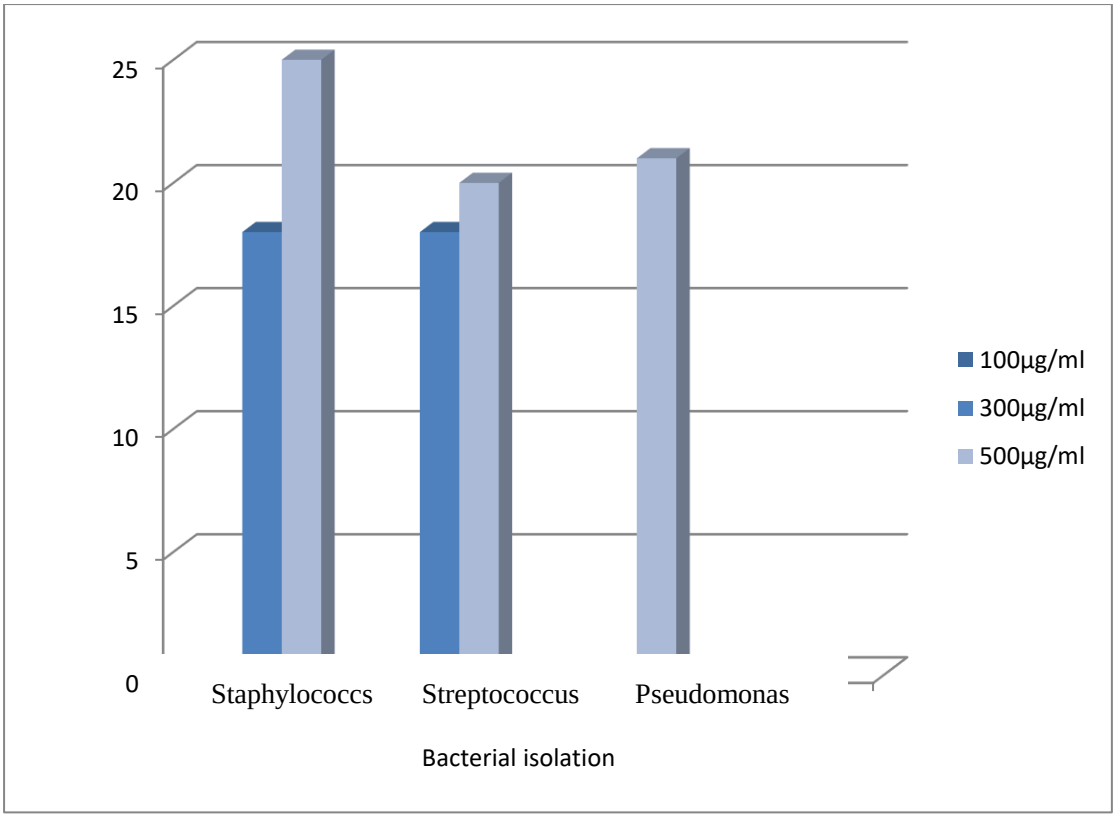

Figure (4-6) show effect of aqueous solution of different *zingiber officinale Rhizome* sonicated on bacterial isolate

4-11 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 nano- ethanolic zingiber officinale solution was investigated at different concentration towards the isolates of *staphylococcus*, *streptococcus*, and *pseudomonas* the effect of the various isolates was observed, as shown in figure 5. As it turned out that the *staphylococcus* bacterium was affected by the ethanolic *zingiber officinale* sonicated, it is highest inhibition value was 27mm at a concentration of 500µg/ml, and at a concentration of 300µg/ml the diameter of the inhibition zone decreased to 25mm, but at a concentration of 100µg/ml the diameter of the inhibition zone was less than that, reaching 15 mm. 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 .

As for the *pseudomonas* bacteria, there was also a clear effect of the *zingiber officinale* sonicated ethanol solution, where different inhibition zones appeared, and according to the concentration, at a concentration of 500µg/ml the stabilization zones was 15 mm and at a concentration of 300µg/ml only10 mm, it was less than that, reaching 30mm, and at a concentration of 100 µg/ml the bacteria 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 (hassan and siham 2014) 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

(2013) Ali) including the compounds of tenanted, saponins , flavonoids , and volatile oils as these compounds have an effective effect in inhibiting the growth of microorganisms (Al-jeuuri , 1994) , and the phenolic compounds present in them have an effective role in inhibiting microorganism growth even at low concentration (Degtayrova and pochinko 1990).

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, (Huang *et al*., 2017; Bahadar *et al*., 2016). 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 special receptors on the bacterial cell wall and vectors in connection with the transport of their molecules into the cell to stop the action of enzymes, coenzymes and other molecules, effective biology (Mitschrs *et al*.,1992).

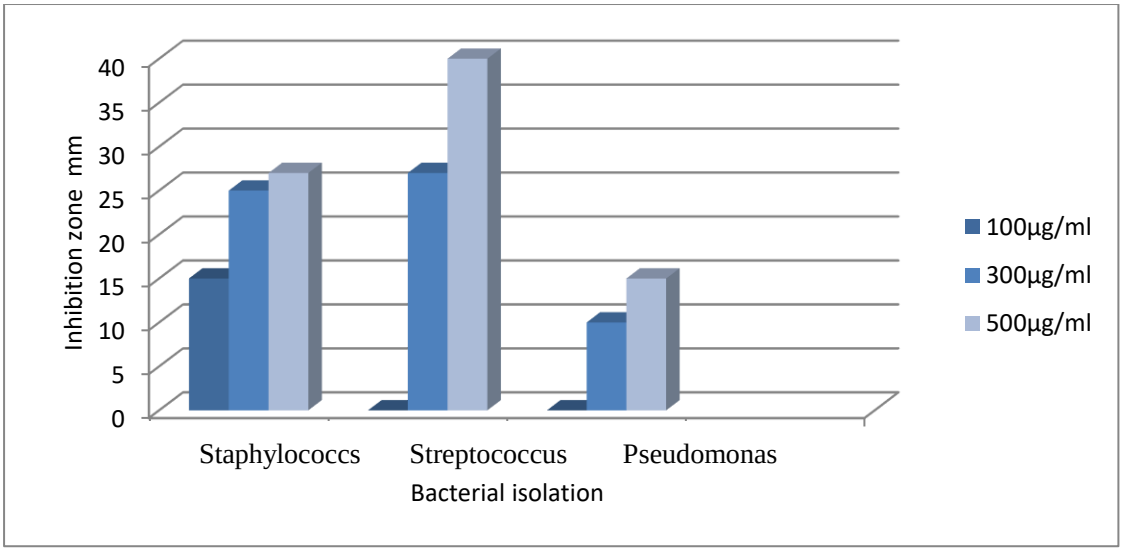

Figure (4-7) show effect of ethanolic solution of different zingiber officinale Rhizome sonicated on bacterial isolates

4-12 Characterization of *Zingiber officinale* sonicated

4-12-1 Analysis by field Emission scanning electron microscopy (FESM)

SEM was used to confirm the morphology and size of the *zingiber officinale* sonicated, shape *zingiber officinale* sonicated with size range between (10.20µm – 27.28µm) were reported. Figure (4-4) representative SEM micrograph of *zingiber officinale* sonicated. SEM is one type of electron microscope that takes the image to the specimens by scanning with a high- energy packages of electrons in a raster scan pattern. The electron interacts with the atoms that can make up the specimens producing signals that contains information about the

specimen's surface topography, synthesis, and other properties such as electrical conductivity (verma and maheshwari,2018). The sizes and shapes of biogenic sonicated can be controlled by exchanging the bio-reeducation conditions, including the type of culture and organism, nature of the medium and incubation time (vetchinkina *et al*., 2019).

Figure (4-8) showed the FESEM characteristic of sonicated *zingiber officinale* and showed the diameters of sonicated of *zingiber officinale* (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 (Raut et al ., 2013).

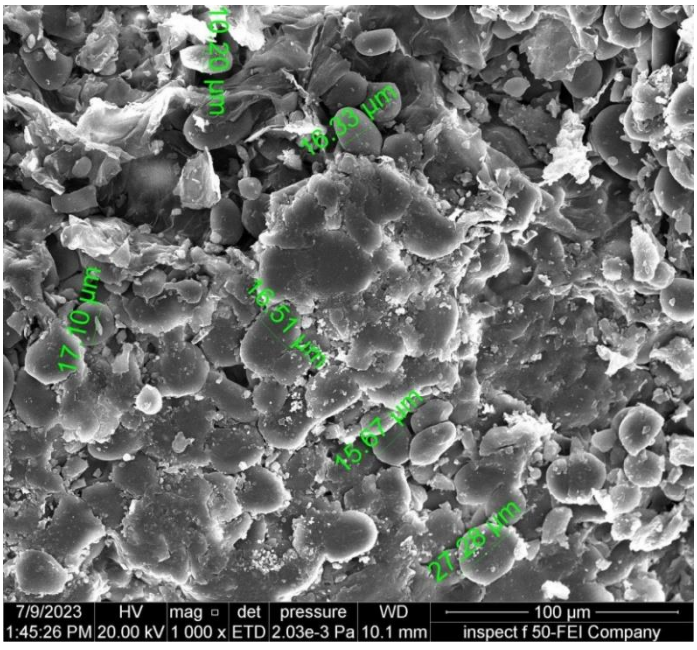

Figure (4-8) FESEM image of sonicated of zingiber officinale Rhizome plant

4-12-2 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

$x=(k*\lambda)/(\beta*\cos\acute{O})$

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

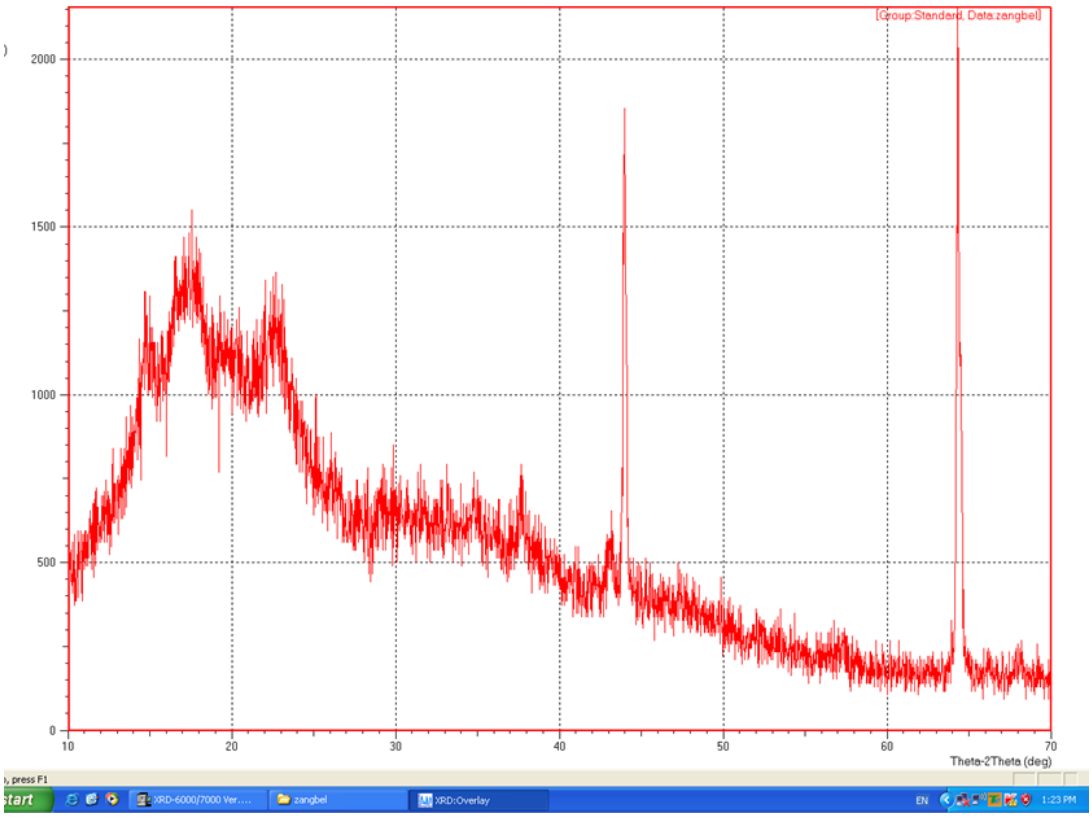

Figure (4-9) XRD pattern of Rhizome zingiber officinale nanoparticles

4-12-3 Fourier Transform Infrared Spectroscopy (FITR)

FITR analysis it was used to obtain information about chemical compounds involved stabilization and reduction of Zingiber officinale nanoparticles. FITR spectra of Zingiber officinale sonicated (powder and

with ethanol) showed the present of peak at 3393.57 cm-1 can be assigned to hydroxyl (OH) group. 2922.58 cm-1 was assigned to Alkane (C-H stretching). 1633.51 cm-1 present of (C=C stretch binding carboxyl stretch protein). 1364.64 cm-1, 1144.07cm-1 assigned to methyl (C-H). 1073.34 cm-1 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.

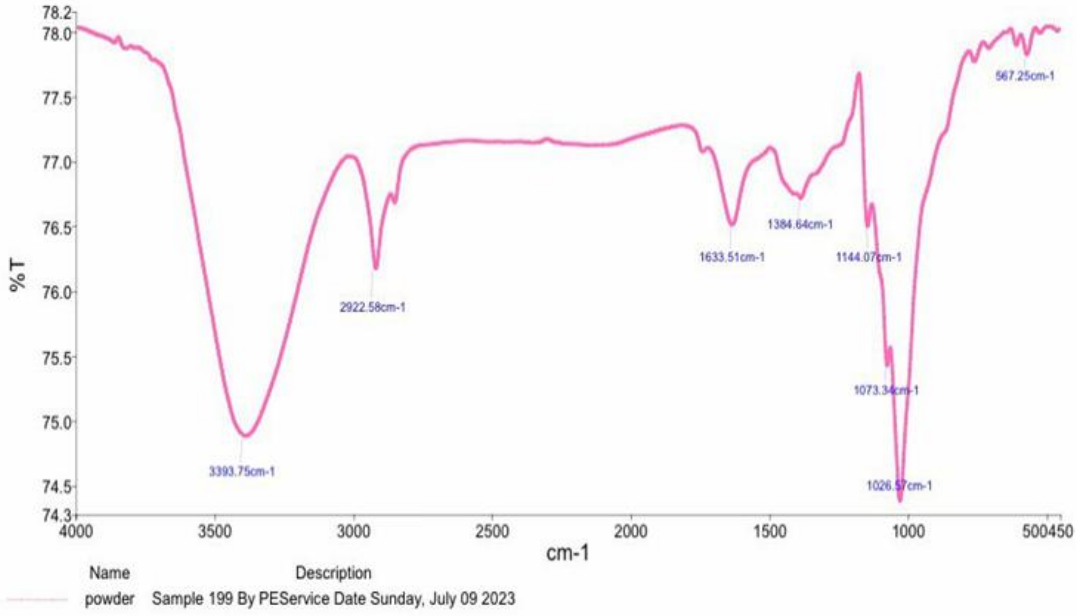

Figure (4-10) FTIR spectra of sonicated zingiber officinale

The absorbance peaks present in the sonicated zingiber officinale specimens with water 3436.41 cm-1 related to Amine's doublet for NH stretch. 1634.97cm-1 present of C= C stretch binding carbonyl stretch protein. 1384.56 cm-1 1152.33 cm-1, and1102.53 cm-1 associated to inorganic ions. 1077.17 related to Primary inorganic ion stretch C-O. the formation of zingiber officinale crystallite is cubic as the absorbance peak is present between range of 500 -1000 cm (pei *et al*., 2010), in addition

to protein and biomolecules such as fatty acid may play a key role in controlled sonicated zingiber officinale morphology and size (Dobias et al., 2011).

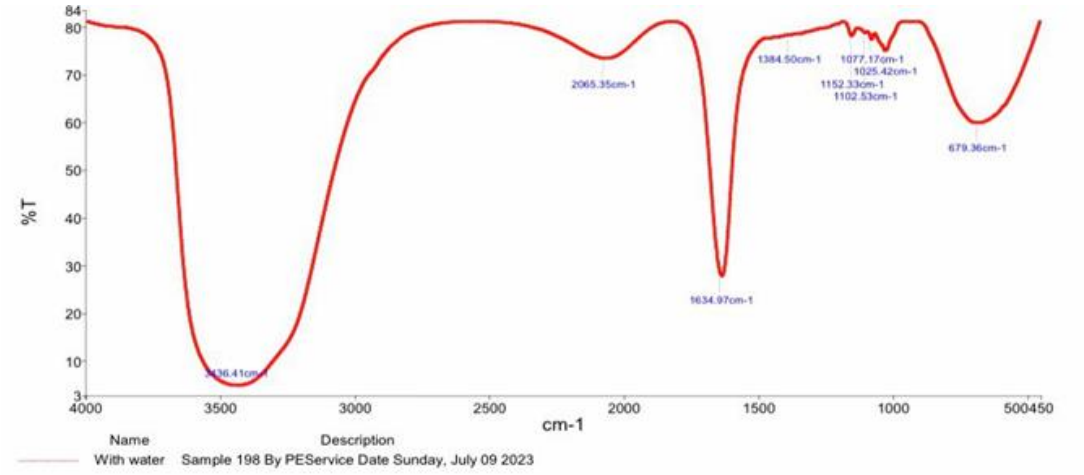

Figure (4-11) FTIR spectra of non sonicated zingiber officinale with water

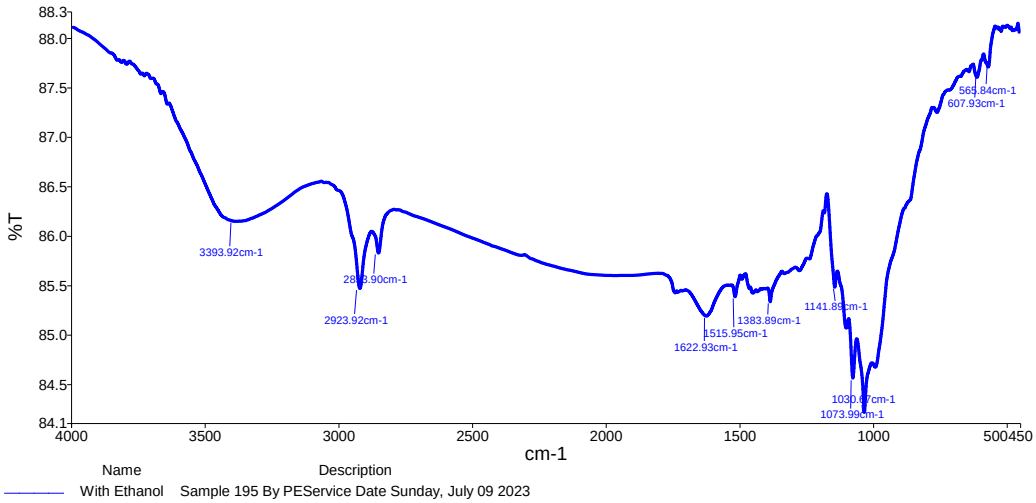

Figure (4-12) FTIR spectra of sonicated zingiber officinale with ethanol

4-13 Detecting of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MIC) of sonicated

The MIC was detected by microtiter plate method. Different concentration of Rhizome of zingiber officinale sonicated raining 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 *Staphylococcus* and *Streptococcus* bacteria were (64µg/ml) and (128µg/ml) respectively, table (4-12)

Table (4-12) MIC and MBC of zingiber officinale rhizome sonicated by microtiter plate against pathogenic

Test organisms	Minimum Inhibition Concentration (MIC) of rhizome of zingiber officinale sonicated mg/ml	Minimum bactericidal Concentration (MBC) of rhizome of zingiber officinale nanoparticles mg/ml
Staphylococcus	64	128
Streptococcus	64	128
Pseudomonas	32	64

Table (4-13) Inhibitory ability of zingiber officinale sonicatedon bacterial isolates isolated from otitis media

Ethanolic sonicated Pseudomonas	Ethanolic sonicated Pseudomonas	Ethanolic sonicated streptococcus	aqueous nano streptococcus	Ethanolic sonicated staphylococcus	aqueous sonicated staphylococcus	concentration
12.67	**19.00**	**34.00**	**17.33**	**23.33**	**19.67**	**500**
12.67	**3.67**	**20.33**	**15.00**	**21.33**	**15.33**	**300**
3.00	**2.33**	**4.00**	**3.00**	**11.33**	**4.00**	**100**
4.141	**4.507**	**8.502**	**4.388**	**6.597**	**6.142**	**LSD**

The results of Table (4-13) showed a clear effect of sonicated aqueous and ethanol of *zingiber officinale* Rhizome on the direction of some bacterial isolates, and showed that there was significant difference between the different concentration of these nano non sonicated. The minimum direction was (2.33) on the direction of the Pseudomonas bacteria ,and the proportion was directly proportional between the concentration and the diameter of the area .The inhibition was consistent with what was indicated by (Zaika1988),who obtained an increase in the inhibition zone by increasing the concentration of non-sonicated aqueous and ethanolic used for *zingiber officinale* rhizomes .The inhibitory effectiveness was at its best at a concentration of 500,as it rose to 34.00 with non-sonicated ethanolic extract towards *streptococcus* bacteria.

Conclusions and

Recommendations

Conclusion

1- The result of our study showed that different bacterial species exhibited different sensitivities towards the extract of *Zingiber officinale* rhizome
2- *Staphylococcus aureus* that cause otitis infection was significantly inhibited by non-sonicated *Zingiber officinale* rhizome in ethanolic extract, as compared to *Streptococcus pneumonia* and *Pseudomonas aeruginosa*
3- *Streptococcus pneumonia* that also isolated from otitis infection patients was the most influenced to sonicated ethanolic extract of *Zingiber officinale* rhizome at low concentration
4- *Zingiber officinale* rhizome have antibacterial activity against pathogenic bacteria with different chemical structure when analyzed under Infrared spectroscopy

Recommendations

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

2- Study the cytotoxic effect of crude extract on normal cell and cancer cell

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Appendices

Table (1): Description resistance and sensitive of antibiotics on bacteria

Antibiotics	Inhibition zone (ml)	Resistance	Inter mediate	Sensitive
Pseudomonas				
Gentamycin	10	≤ 12	13-14	15
Imipenem	15	≤15	16-18	≥19
	Staphylococcus			
Vancomycin	13	≥10	18-20	≥15
Gentamycin	13	≤12	13-14	≥15
Amikacin	18	≤14	15-16	≥15
Flucloxacillin	17	≥14	15-16	≥17
Chloramphenicol	20	≤12	13-17	≥18
	Streptococcus			
Tetracycline	10	≤18	19-22	≥23
Gentamycin	20	≤15	16-18	≥19
Chloramphenicol	21	≤12	18-20	≥21

Table (2):Phenotypic of antibiotic susceptibility of *Pseudomonas* Isolation

Isolates	IEL	CN	CAZ	PRL	CIP	NX	MEM
1	R	R	R	R	R	S	R
2	R	R	R	R	R	S	S
3	R	S	R	R	R	R	R
4	S	R	R	R	S	R	R
5	R	R	R	R	S	R	S
6	R	R	R	R	S	S	S
7	R	R	R	R	S	R	S
8	R	R	R	R	R	S	S
9	R	S	S	R	S	S	R
10	S	R	R	R	S	R	R
11	R	R	R	R	R	S	S
12	R	S	R	R	R	R	R
13	R	R	R	R	S	S	S
14	R	R	R	R	S	R	S
15	S	S	R	R	R	S	R
16	R	R	IN	R	S	R	S
17	R	S	R	R	S	S	R
18	R	R	R	R	R	S	R
19	R	S	R	R	R	S	S
20	R	R	R	R	S	R	S
21	R	R	R	R	R	S	S

Abbreviations: [Imipenem (IEL), Gentamicin (CN), Ceftazidime (CAZ), Piperacillin (PRL), Levofloxacin (NX), Meropenem (MEM)]

Table (3):henotypic of antibiotic susceptibility of *Staphylococcus* Isolation.

Isolates	VA	AK	CD	TM	CIP	F	GEN	C
1	S	S	S	R	S	S	S	S
2	S	S	S	R	S	S	S	S
3	S	R	R	R	S	S	S	S
4	S	S	S	R	S	S	S	S
5	S	S	S	R	S	S	S	S
6	S	S	S	R	R	S	S	S
7	S	S	S	R	R	S	S	S
8	S	S	S	R	S	S	R	S
9	S	S	S	IN	S	S	S	S
10	S	S	S	R	S	S	S	R
11	R	S	S	R	R	S	S	S
12	S	S	S	R	S	S	R	S
13	S	S	S	S	S	S	S	S
14	S	S	S	R	R	S	S	S
15	S	S	S	R	S	S	S	S
16	S	S	S	R	R	S	S	S
17	S	S	R	R	S	S	S	S
18	S	S	S	R	S	S	S	S
19	S	S	S	R	R	S	S	S
20	S	S	S	R	S	S	S	S
21	S	S	S	R	S	S	S	S
22	S	S	S	R	S	R	S	S
23	S	S	S	R	S	S	S	S
24	S	S	S	R	R	S	S	S
25	S	S	S	R	R	S	S	S
26	S	S	S	R	S	S	S	S
27	S	S	S	R	S	S	S	S
28	S	S	S	R	R	S	S	S
29	S	S	R	R	S	S	S	S
30	S	S	S	R	S	R	S	S
31	S	S	S	R	S	S	S	S
32	S	S	S	R	S	S	S	S
33	S	S	S	R	S	S	S	R
34	S	S	S	R	S	S	S	S
35	S	S	S	R	S	S	S	S
Isolates	VA	AK	CD	TM	CIP	F	GEN	C
36	S	S	S	R	S	S	S	S

37	S	S	S	R	S	S	S	S
38	S	S	S	R	S	S	S	S
39	S	S	S	R	R	S	S	S
40	S	S	S	R	S	S	S	S
41	S	S	R	R	S	S	R	R
42	S	S	S	R	R	S	R	S
43	S	S	S	R	S	S	S	S
44	S	S	S	R	R	S	S	S
45	R	S	S	R	R	S	S	S
46	S	S	R	S	R	S	S	S
47	S	S	S	R	S	S	S	R
48	S	S	S	R	S	S	S	S
49	S	S	S	R	R	S	S	S
50	S	S	S	S	S	S	S	S
51	S	S	S	R	R	S	S	S
52	S	S	S	R	S	S	S	S
53	S	S	S	R	R	S	S	S
54	S	S	S	R	S	S	S	S

Abbreviations: [Vancomycin (VA), Amikacin (AK), Clindamycin)CD) , Trimethoprim (TM), Ciprofloxacin(CIP), Nitrofurantoin(F), Gentamicin (GEN),Chloramphenicol]

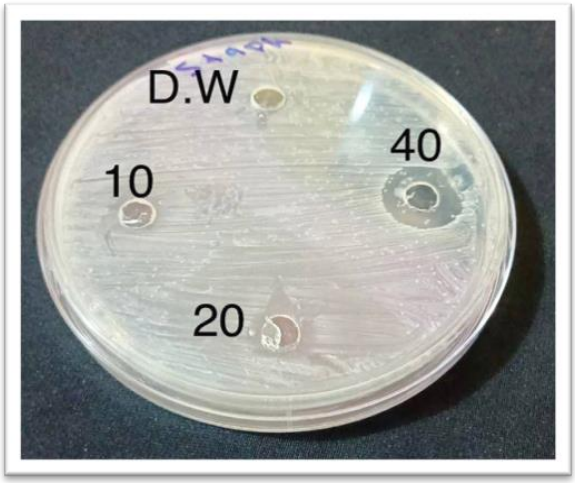
Table (4): Phenotypic of antibiotic susceptibility of *Streptococcus* Isolation

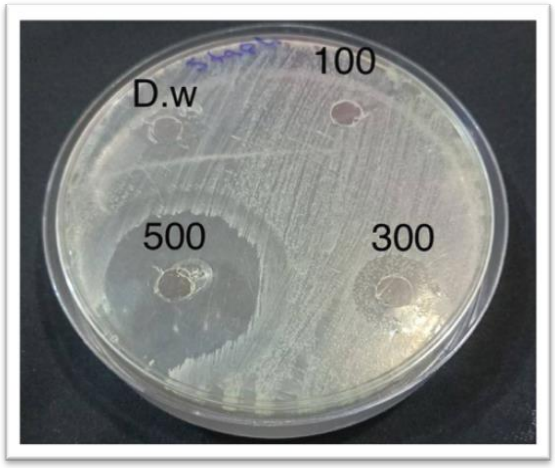
Isolates	VA	TE	CD	GEN	E	AZM	C
1	S	R	R	S	R	R	S
2	S	R	R	S	R	R	S
3	S	S	R	S	R	R	S
4	R	R	R	S	R	R	S
5	S	R	R	S	R	R	R
6	S	S	R	S	S	R	S
7	S	R	R	S	R	R	S
8	S	R	S	S	R	R	S
9	S	S	R	S	R	R	S
10	R	R	R	S	R	R	S
11	S	S	R	S	R	R	R
12	S	R	R	S	R	R	S
13	S	R	R	S	R	R	S
14	R	R	R	S	R	R	R
15	S	S	R	S	S	R	S
16	S	R	R	S	R	R	S
17	R	R	S	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)]

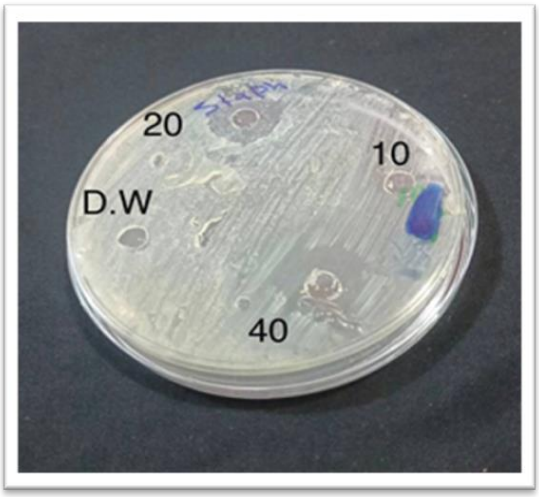
Table(5): Description of inhibitory activity of (aqueous and ethanolic) non sonicated extract and sonicated of zingiber officinale rhizome on bacteria

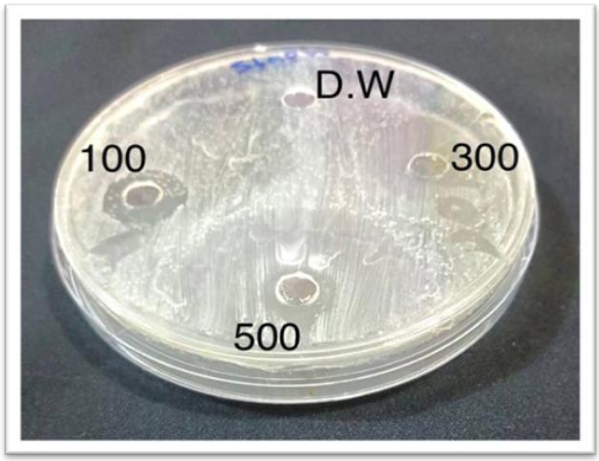
Concentration g/ml	Aqueous non sonicated	Ethanolicnon sonicated	Concentration µg/ml	Aqueous Nano	EthanolicNano
Staphylococcus					
10			100		
20		18	300	18	25
40	27	26	500	25	27
Streptococcus					
10			100		
20		19	300	18	27
40	16	20	500	20	40
Pseudomonas					
10			100		
20		11	300		10
40	11	15	500	21	15



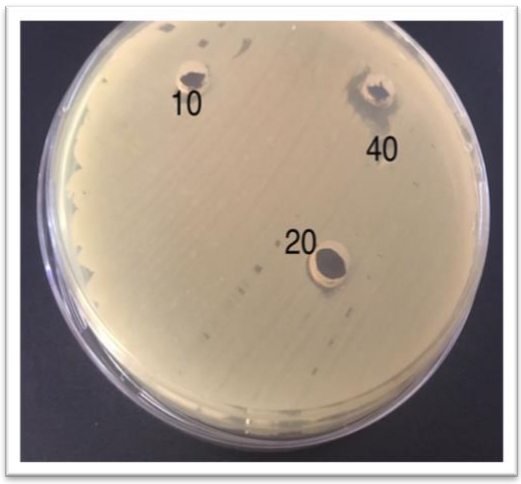


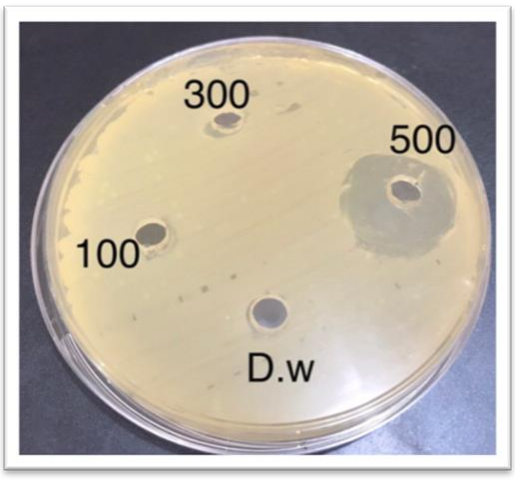
effect of aqueous sonicated and non sonicated extract of zingiber officinale Rhizome on staphylococcus bacteria(mm)



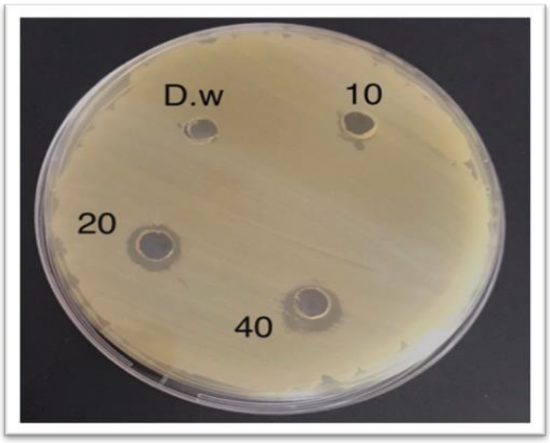


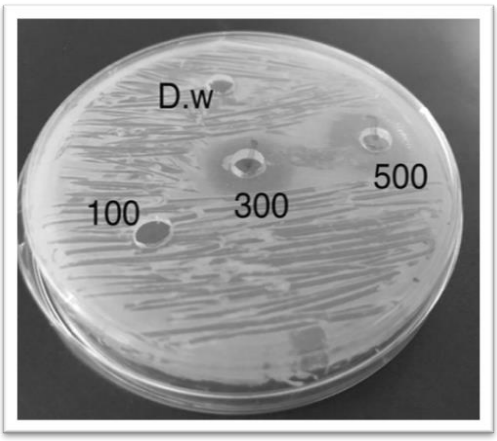
effect of ethanolic sonicated and non sonicated extract of zingiber officinale Rhizome on Staphylococcus bacteria(mm)



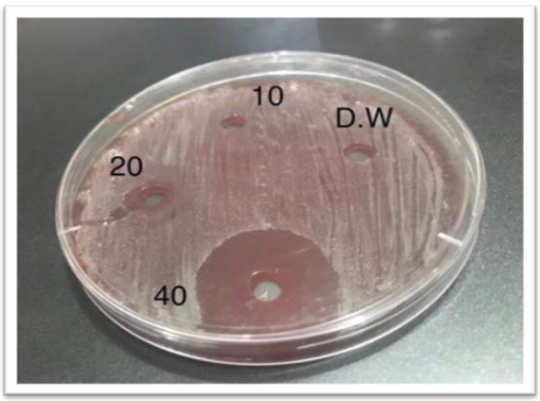


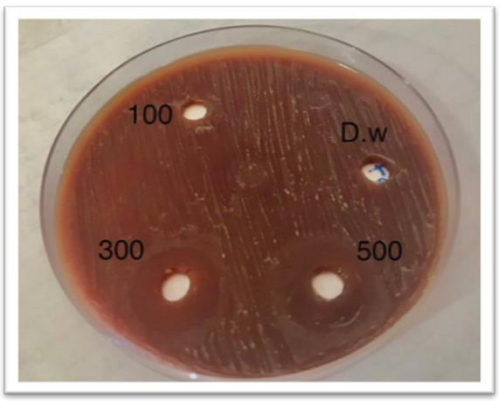
inhibition activity of aqueous solution of zingiber officinale Rhizome sonicated on Pseudomonas bacteria(mm)



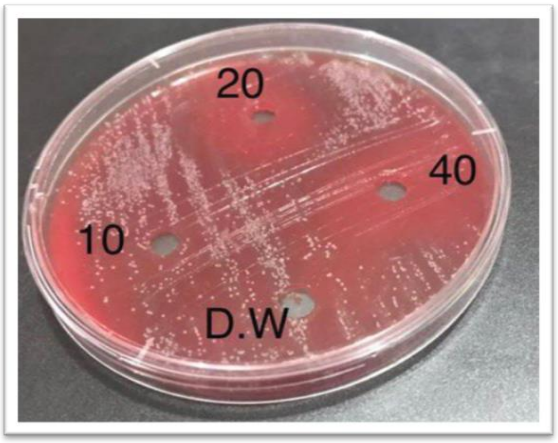


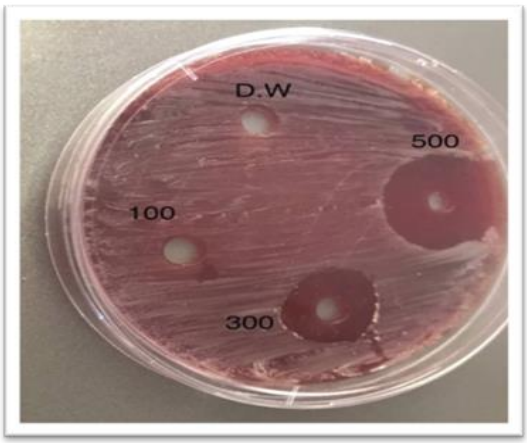
inhibition activity of an ethanolic solution of zingiber officinale Rhizome sonicated and non sonicated extract on Pseudomonas bacteria(mm)





inhibition activity of aqueous solution of zingiber officinale Rhizome sonicated and non sonicated on streptococcus bacteria(mm)





inhibition activity of ethanolic solution of zingiber officinale Rhizome sonicated and non sonicated on streptococcus bacteria(mm)

الخلاصه

أجريت هذه الدراسة لاختبار المستخلص المائي والإيثانولي والمستخلص المائي والإيثانولي لنبات Zingiber officinale Rhizome ضد أنواع البكتيريا المرضية المسببة للأمراض (Staphylococcus، Streptococcus وPseudomonas) المعزولة من مرضى التهاب الأذن.

تم جمع 112 عينة سريرية من مرضى مستشفى الاسكندريه العام ومستشفى مرجان التعليمي من كلا الجنسين والأعمار المختلفة. من 1/أكتوبر/2022 إلى 1/أبريل/2023. أعطت نتائج عزل100 عينة (89.3%) نمواً إيجابياً و12 عينه (10.7%) نموآ سلبيآ من 42 أنثى و 58 من الذكور

تم تشخيص 100 عزلة بكتيرية حسب المظهر والخصائص المزروعة والمجهرية والاختبارات الكيموحيوية وتأكدت باستخدام نظام Vitek2 المضغوط إلى 54 عزلة تنتمي إلى Staphylococcus aureus، و 25 عزلة تنتمي إلى Streptococcus pneumonia، و 21 عزلة تنتمي إلى Pseudomonas aeruginosa

تم إجراء اختبار الحساسية للمضادات الحيوية ضد18 نوعاً من المضادات الحيوية باستخدام طريقة الانتشار القرصي

أظهرت عزلات بكتريا Staphylococcus حساسية للمضاد الحيوي Nitrofurantoin (96.29%)، وللأميكاسين (98.14%)، للفانكومايسين (96.29%)، الجنتاميسين (92.59%) للكلورامفينيكول(92.59%)، الكليندامايسين (92.7)، السيبروفلوكساسين (68.5%) ، ترايميثوبريم (92.59%) .

أظهرت عزلات الزائفة مقاومة للمضاد آميبينيم(85.71%) والبيبرسيلين (100%) للميروبينيم (42%), للجنتاميسين (71.4%) للسيبروفلوكساسين. (47.61%),لليفوفلوكساسين (42.8%).

أظهرت عزلات المكورات العقدية حساسية للكلورامفينيكول (88%)،الجنتاميسين (100%)، للفانكومايسين (80%) ,لكن كانت مقاومة للكلندامايسين (92%)، والتتراسيكلينات (76%)، للأزيثرومايسين (100%)، للإريثرومايسين (88%).

. تم اختبار النشاط المضاد للميكروبات للمحلول المائي والإيثانولي غير الصوتي لنباتات نبات الزنجبيل officinale على بكتريا (Staphylococcus، Streptococcus، Pseudomonas) المعزولة بثلاثة تراكيز 10 ملغم/مل، 20 ملغم/مل، 40 ملغم/مل، وأظهر أن قطر منطقة التثبيط بالنسبة لبكتريا Staphylococcus و Streptococcus و Pseudomonas على التوالي (26، 15،20) ملم للمستخلص المائي غير الصوتي و (27،16،11) ملم للمستخلص الايثانولي غير الصوتي.

تم اختبار النشاط المضاد للميكروبات للمستخلص المائي والإيثانولي الصوتي لنباتات نبات الزنجبيل officinale على بكتريا (Staphylococcus، Streptococcus، Pseudomonas) المعزولة بثلاثة تراكيز 100 ميكروغرام/مل، 300 ميكروغرام/مل، 500 ميكروغرام/مل، وتبين أن قطر منطقة التثبيط بالنسبة لبكتريا Staphylococcus و Streptococcus و Pseudomonas على التوالي (25، 21،20) ملم للمستخلص المائي الصوتي و (15،40،27) ملم للمستخلص الايثانولي الصوتي.

تم دراسة العوامل المضادة للميكروبات من المستخلص المائي والإيثانولي ذو الصوتنة بثلاث طرق مختلفة التحليل الأول بواسطة المجهر الإلكتروني الماسح للانبعاث هذا النطاق من الجسيمات الصوتية بين (10.20-27.28) ميكرومترو الثانية تحليل حيود الأشعة السينية الذي أظهر (23.601-44.015) نانومتر طول الجزيئات والثالث تم استخدام الفحص المجهري الطيفي بالأشعة تحت الحمراء (FTIR) في المدى 400-4000 سم-1، وأظهرت النتائج آنوعاً مختلفاً من المجامبع الفعاله.

تم تحديد التركيز المثبط الأدنى (MIC) والحد الأدنى القاتل المبيد للجراثيم (MBC) للمكورات العنقودية والمكورات العقدية والزائفه وكان 128 مغ/مل MBC و64 مغ/مل للمكورات العنقودية والعقدية ، و64 مغ/ ملMBC و32مغ/مل MIC للالزائفة

وفي هذه الدراسة وجد أن أعلى نسبة إصابة في الفئة (1-15) سنة وعدد الوجبات المصابة بالتهابات الأذن الوسطى أكثر من الإناث. وتبين أن مرضى التهاب الأذن الوسطى في المناطق الحضرية لديهم أعلى نسبة وأقل نسبة لوحظت في المناطق الريفية وأن معدل التهابات الأذن المزمنة أكثر من معدل التهابات الأذن الوسطى الحادة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل /كلية العلوم

قسم علوم الحياة

آلفعاليه آلمضاده للبكتريا لمستخلص جذور آلزنجبيل على بعض البكتريا المعزوله من مرضى التهاب الاذن الوسطى

رسالة

مقدمه الى مجلس كلية العلوم / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم /علوم الحياة

من قبل

ضحى عبد ألامير محمد شنور

بكالوريوس علوم حياة

جامعة بابل (2019)

أشراف

ألاستاذ الدكتور

وجدان رضا محمود

جامعة بابل /كلية العلوم

1445هـ 2024م