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STUDY OF THE SYNERGISTIC EFFECT OF SAUSSUREA COSTUS AND CINNAMOMUM CASSIA EXTRACTS WITH RESISTANT ANTIBIOTICS ON THE TONSILLITIS CHILDREN'S PATHOGENS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Noor Khalid Ismael ALSAMRAAI

ABSTRACT

STUDY OF THE SYNERGISTIC EFFECT OF SAUSSUREA COSTUS AND CINNAMOMUM CASSIA EXTRACTS WITH RESISTANT ANTIBIOTICS ON THE TONSILLITIS CHILDREN'S PATHOGENS

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Master of Science in Biology

Advisor: Prof. Dr. Ülkü Nihan YAZGAN TAVŞANOĞLU

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The study included the collection of 60 samples of children between 1-10 years with tonsillitis from both genders, for the period between 15/11/2021 to 15/2/2022, from Samarra General Hospital and private clinics in Iraq. The statistical analysis showed significant differences of infection between male and female, at 63.33% and 36.66%, respectively. As for age groups, the highest infection rate was in the 5-6-year-old group with a rate of 36.66%. 53 samples gave a bacterial growth rate of 88.33%, and bacterial isolates were diagnosed by a set of microscopic and biochemical tests. The results of this diagnosis showed that *Staphylococcus aureus* was one of the most common bacterial isolates in tonsillitis, with a rate of 60.37%, *Streptococcus viridanse* 13.20%, *Bacillus* 9.43%, *Streptococcus pyogenes* 7.54%, *Pseudomonas aerginosa* 5.66%, and *Klebsiella pneumoniae* 3.77%. Isolates, were tested for sensitivity to ten antibiotics. The inhibitory effect of two types of hot and alcoholic aqueous plant extracts of cinnamon and coctus on isolated bacteria at concentrations (12.5, 25, 50, 100, 200, 400) mg / mL were studied. The results of the current study showed the superiority of alcoholic extracts over hot aqueous extracts in inhibiting the growth of bacterial isolates. This is a new finding for Iraq regarding the synergistic blend of these plants with bacteria resistant.

2022, 57 pages

Keywords: Tonsillitis*, Saussurea costus, Cinnamomum cassia,* Antibiotics

ÖZET

SAUSSUREA COSTUS VE CINNAMOMUM CASSIA EKSTRAKTLARININ DİRENÇLİ ANTİBİYOTİKLERLE ÇOCUKLARDA TONSİLİT PATOJENLERİ ÜZERİNDEKİ SİNERJİK ETKİSİNİN İNCELENMESİ

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Biyoloji, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Ülkü Nihan YAZGAN TAVŞANOĞLU

Eş Danışman: Dr. Öğr. Üyesi Osama NADHOM NIJRIS

Bu çalışma, 15/11/2021 - 15/2/2022 tarihleri arasında Irak'ta bulunan Samarra Genel Hastanesi ve özel kliniklerden her iki cinsiyetten bademcik iltihabı olan 1-10 yaş arası 60 hastadan toplanan örneklerin sonuçlarını içermektedir. İstatistiksel analizlere göre, sırasıyla %63.33 ve %36.66 oranlarında erkek ve kız çocukları arasında enfeksiyon açısından anlamlı bir fark göstermiştir. Yaş gruplarına göre en yüksek enfeksiyon oranı %36,66 ile 5-6 yaş grubundadır. 53 numune (%88,33) bakteri üreme oranı vermiş ve bakteri izolatları bir dizi mikroskobik ve biyokimyasal testle teşhis edilmiştir. Buna göre, *Staphylococcus aureus*'un %60,37, *Streptococcus viridanse* %13,20, *Bacillus* %9,43, *Streptococcus pyogenes* %7,54, *Pseudomonas aerginosa* %5,66 ve *Klebsiella pneumoniae* %3,77 ile tonsillitte en sık görülen bakteriyel izolatlardan olduğunu göstermiştir. İzolatlar, on farklı antibiyotiğe karşı duyarlılık açısından test edilmiştir. Tarçın ve Hint priminin iki tür sıcak ve alkollü sulu bitki ekstraktının izole edilmiş bakteriler üzerinde (12.5, 25, 50, 100, 200, 400) mg / mL konsantrasyonlarında inhibitör etkisi incelenmiştir. Mevcut çalışmanın sonuçları, bakteriyel izolatların büyümesini engellemede alkollü ekstraktların sıcak sulu ekstraktlara göre üstünlüğünü göstermiştir. Bakterilere direnç antibiyotiklerle bu bitkilerin sinerjistik karışımına ilişkin Irak için yeni bir bulgudur.

2022, 57 sayfa

Anahtar Kelimeler: Tonsilit, *Saussurea costus*, *Cinnamomum cassia*, Antibiyotikler

PREFACE AND ACKNOWLEDGEMENTS

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Noor Khalid Ismael ALSAMRAAI
Çankırı-2022

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LIST OF SYMBOLS

%	Percent
BP	Base pair
CFU	Colony forming unit
ºC	Celsius degrees
GR	Gram
L	Liter
MOL	Mole
µG / ML	Microgram / Milileter
N	Normal
PPM	One in a Million
VER	Version
β	Beta

LIST OF ABBREVIATIONS

ACC	Acetyl-coa carboxylase
ANOVA	Analysis of Variance
ARDS	Acute respiratory distress syndrome
ASO	Antistreptolysin O
CLSI	Clinical and laboratory standards ınstitute
CT	Computed tomography
D.W	Distilled water
DMOS	Dimethylsulphoxide
EBV	Epstein-barr virus
EPS	Extra cellular polymers substance
GP	General practitioner
HCL	Hydrochloric acid
HIV	*Human immunodeficiency virus*
ICU	Intensive care unit
KOH	*Potassium hydroxide*
MDR	Multiple drug resistance
MIC	Determination of minimum inhibitory concentratio
MR-VP	Methyl Red / Voges-Proskauer
WHO	World health organization

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1. INTRODUCTION

Tonsillitis is a kind of pharyngial infection that often develops rapidly at the top of the throat and manifests as sore throats, fevers, swollen tonsillitis, stomach problems, and lymphadenitis (Klug *et al.* 2016). The disease primarily spreads through the air (Windfuhr *et al.* 2016). Tonsillitis is most common in children between the ages of 5 and 15, and 15% to 30% of children suffer from sore throat complications, which are primarily caused by group A beta-hemolytic streptococci (GABHS) (Schroeder 2003).

To ease the symptoms and prevent complications, paracetamol, ibuprofen, and penicillin can be used (Windfuhr *et al.* 2016). In children with recurrent tonsillitis, penicillins, cephalosporins, or macrolides may be commonly used. These drugs reduce the incidence of disease (De and Anari 2018). Whether a person recovers with or without medicine, fifty percent of symptoms vanish within three days and eighty percent within one week. Antibiotics shorten the length of symptoms by approximately 16 hours (Spinks *et al*. 2013). The emergence of resistance to these antibiotics constitutes a serious situation. Therefore, plant extracts like *Saussurea costus* and *Cinnamomum cassia* can be used as a well-known natural herb with a medical history in the treatment of tonsillitis in children (Pandey *et al*. 2007).

The purpose of this study is to examine the preventive effects of *Cinnamomum cassia* and *Saussurea costus* plant extracts against bacteria with high antibiotic resistance that cause tonsillitis in children. The study was planned by following the steps below. *i*)Isolation and identification of bacteria that cause tonsillitis from the throat; ii) a susceptibility test for Amikacin, Gentamicin, Ciprofloxacin, Tetracycline, Imipenem, Chloramphenicol, Meropenem, Cefixime, Levofloxacin, and Amoxicillin/Clavulanic acid for bacterial isolates; iii) alcoholic and aqueous extraction for *Cinnamomum cassia* and *Saussurea costus*; iv) measuring the minimum inhibitory concentration of two plant extracts (alcoholic and aqueous) through a series of dilutions; v) measuring the synergistic minimum inhibitory concentration of some antibiotics used and the plant extract (alcoholic and aqueous).

2. LITERATURE REVIEW

Group A streptococcal bacteria or a viral infection are the most common causes of tonsillitis. Constant or recurrent tonsillitis is more common in children than adults (Table 2.1). According to our knowledge, there is no study examining the association between chronic tonsillitis and tooth decay in children aged 4-5 years in Iraq and the Middle East (Zaid and Ahmed 2016).

Table 2.1 Causative organisms of tonsillitis (Walijee *et al.*2017).

Bacterial	**Viral**
Group A Streptococci	Rhinovirus
Non-Group A Streptococci	Influenza A
Neiserria gonorrhoea	Adenovirus
Mycoplasma pneumoniae	Herpes simplex virus
Chlamydia pneumoniae	Epstein– Barr virus
Corynebacterium diphtheriae	Metapneumovirus, Respiratory syncytia, Virus, Parainfluenza

Tonsilitis can be defined as part of the spectrum of pharyngitis due to infection of the pharynx (Fig. 2.1) in both genders and age groups, and is widely seen in autumn and winter (Macnamara 2008). In 50–80% of cases, the cause of tonsillitis is caused by viruses such as Epstein-Barr virus (EBV), herpes simplex, influenza, and rhinovirus. In 5–36% of the cases, A beta-hemolytic streptococcus (GABHS) causes the infection. *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae* can also infect the tonsils and pharynx. In tonsillitis, not only viruses or bacteria but also fungi (Candida) may cause sore throats in immunocompromised patients. There is no evidence to suggest that bacterial tonsillitis is more severe than a viral infection and can cause strep throat, and it is difficult to differentiate between clinical symptoms (Bartlett *et al*. 2015).

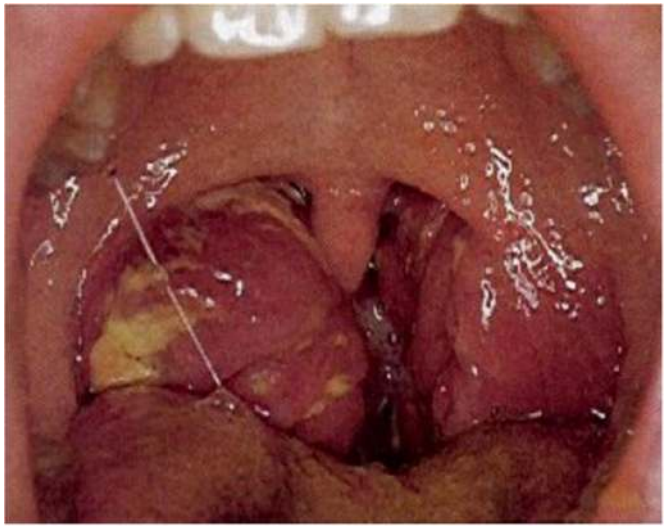

Figure 2.1 Acute bacterial Tonsillitis (Alasmari *et al*. 2017)

Bacterial tonsillitis can be caused by aerobic pathogens in unvaccinated patients; bacteria should be worthy (Berger *et al*. 2016). Recurrent tonsillitis has also been linked to tuberculosis, and clinicians should examine patients' risk (Jadia *et al*. 2010). White blood cells in the tonsils produce inflammatorycytokines such phospholipase to eliminate viruses or bacteria, which, in turn, causes a fever (Manthiram *et al*. 2018).

2.1 Symptoms, Signs and Diagnosis

Anterior cervical tenderness, tonsil secretions, sore throat, lymph nodes, and fever are common signs of acute tonsillitis. Patients may also experience secondary dysphagia as a result of swollen tonsils and should be evaluated for these symptoms. In addition to these symptoms, patients should be evaluated for their vaccination status and whether there is any discharge from the tonsils, such as mucus (Gottlieb *et al.* 2018). When dealing with any sort of ear, noise, or tongue (ENT) issue, it's important to check the ears and nose thoroughly (Gottlieb *et al.* 2018). Tonsillitis is caused by viral infections that manifest as a cough, a drippy nose, hoarseness, and mouth or throat ulcers (Bochner *et al.* 2017). Tonsillitis has a wide range of possible diagnoses, including pharyngitis, retropharyngeal abscess, epiglotitis, Ludwig's angina, and the presence of a dental abscess or peritonitis, primary human immunodeficiency virus (HIV), Coxsackie virus, and Epstein-Barr virus. As a side effect of strep throat, it is possible to tell the

difference based on a patient's medical history and other clinical signs (Gottlieb *et al.* 2018).

Increased levels of secreted phospholipase mean people with tonsillitis are more likely to have an abnormal fatty acid metabolism (Ezzedini *et al.* 2013). The most common viral causes of tonsillitis are the common cold-causing rhinovirus, respiratory syncytial virus, adenovirus, and coronavirus. The virulence of other viral causes such as Epstein-Barr virus (which causes mononucleosis), cytomegalovirus, hepatitis A, rubella, and others is typically low and rarely causes complications. However, it can be more serious and require medical attention (Georgalas *et al.* 2009). The bacterial infection is usually caused by group A beta-hemolytic streptococci (GABHS) but can also be caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenza* (Wang *et al*. 2017).

GABHS is one of the most common bacterial causes of pharyngitis in children, primarily affecting school-aged children (ages 5-15) in late fall, winter, and spring, and many clinicians only consider GABHS in the context of pharyngitis because of its potential and frequency of inducing acute rheumatic fever. However, one study reported that 8% of the children aged 3 months to 5 years with pneumonia were positive for GABHS in troat culture (Shulman *et al*. 2012). These bacteria can cause various skin and soft tissue infections, and some of them can be life-threatening if not untreated (Pitetti *et al*. 2001). GABHS is transmitted through saliva, nasal secretions, food, and water, and when infection occurs, the usual incubation time period can take about 2 to 4 days. The pathogenity of GABHS is regulated by various factors, including streptolysin O, streptolysin S, streptokinase, proteases, deoxyribonucleic acid (DNAases A, B, C, and D), and hyaluronidase (Bisno *et al*. 1995). Streptolysin O and streptolysin S. Streptolysin O is a toxin similar to Streptolysin O that causes cell membrane demage. Hemolysins produced by GABHS strains may be harmful to the membranes of pleomorphic leukocytes, platelets, and subcellular organelles. Many other extracellular materials cultured by GABHS can promote liquefaction of pus and spread of streptococcus through tissue layers (Bisno *et al*. 1995).

In the treatment of tonsillitis, throat culturation is still the best diagnostic test choice. Although the test is simple to do and highly sensitive, it is directly related to the quality of the sample and the method used to process it. Apart from the test, the tonsils and the posterior pharyngeal wall should be checked for signs of infection (Kurtz *et al.* 2000).

Furthermore, rapid antigen detection kits have been developed for the diagnosis of infection of GABHS. Although the kits are generally easy to use, the low sensitivity of the tests, ranging from 77.6% to 98.8%, limits their usage (Gerber and Shulman 2004). The GABHS antigen-positive rapid diagnosis kit can also be used to detect GABHS pharyngitis; a negative test must contain a confirmed ring culture (Cohen *et al*. 2016). Accordingly, the quick antigen diagnosis kits are more expensive than standard throat cultures.

2.2 Treatment and Antibiotic Resistance

Tonsillitis is a self-limiting condition in the majority of patients due to the return of viral etiology. Acute tonsillitis is treated with supportive therapy, such as analgesics and fluids, but patients may occasionally require hospitalization (Bartlett *et al.* 2015). Non-steroidal anti-inflammatory drugs (NSAIDs) are medicines that can help relieve pain and other symptoms (Stelter 2014). Antibiotics are usually used to treat patients at risk for bacterial pharyngitis. Originally, an antibiotic was defined as a chemical produced by one microbe that inhibits the growth of other germs. However, synthetic technologies have altered this concept. Antibiotics are substances created by microbes or similar substances that impede the growth of other germs at low doses (Szczepanowski *et al.* 2009).

Streptococcus pyogenes is the most prevalent cause of bacterial tonsillitis, and penicillins are typically the most effective antibiotic treatment (Sidell *et al*. 2012). Disadvantages of using antibiotics include developing resistance to antibacterials, gastrointestinal system symptoms, diarrhea, and cost (Pelucchi *et al.* 2012). In addition, antibiotics have little negative effect compared to their effectiveness in reducing suppurative complications and duration of symptoms (Gottlieb *et al.* 2018). The control

of infectious diseases poses a serious situation due to the increase in the number of microorganisms resistant to antimicrobial agents. Infections caused by resistant microorganisms often do not respond to the usual treatment, which can put them at risk of prolonged sickness or death. The main cause of antibiotic resistance is a DNA mutation in the bacteria (Laxminarayan 2003).

2.3 Treatment with the Plant Extracts

In the treatment of asthma and cough, as well as cholera, chronic skin sickness, and rheumatism, the roots of *Saussurea coctus* are employed (Pandey *et al.* 2007). In India, *S. costus* can be used alone or in conjunction with other medicines in the Indian medical system (Ayurveda, Siddha, and Unani). In the book of Hagers Handbuch der Pharmazeutischen Praxis (Blaschek et al. 1998), they describe the botany, chemical components, pharmacological properties, and uses of *S. costus*. Due to the importance of plants covering traditional and folklore medicinal uses, botanical characteristics, phytochemistry, and pharmacology (Pandey *et al.* 2007), there has been a great interest by various researchers.

Recent studies have confirmed that *Cinnamomun cassia* has a wide range of pharmacological effects, including antineoplastic, anti-inflammatory and analgesic, anti-diabetic and anti-obesity, anti-bacterial and antiviral, cardiovascular protection, cellular protection, neuroprotective, immune-regulating, and anti-tyrosinase effects. activity and other effects. However, recent studies on *C. cassia* are still incomplete and more in-depth investigations into nutritional therapy, health products, toxicity, and side effects are required, and more bioactive components and potential pharmacological effects need to be explored in the future (Mishra and Srivastava 2022). It is also believed that cinnamon can be utilized to cure a wide range of oral health issues, including gum disease and foul breath (Mishra and Srivastava 2022).

2.3.1 *Saussurea costus*

The Asteraceae family includes about one thousand genera and thirty thousand species worldwide, of which about 177 genera and 1052 species are found in India alone. The genus Saussurea DC, which is of the same family, contains about 300 species worldwide, of which 61 species are found in India. It is imported from India to Iraq and sold dried by herbalists. Some of the common and ethnologically important species of this genus found in India are listed in Table 2.2.

Table 2.2 Scientific classification of *Saussurea costus* (Kuniyal *et al.* 2021)

Kingdom	**Plantae**
Clade	Tracheophytes
Order	Asterales
Family	Asteraceae
Genus	*Saussurea*
Species	*S. costus*

Several researchers have reported that the different biological activities of *Saussurea costus* (Figure 2.2) and different extracts of this plant exhibited anti-inflammatory, hepatoprotective, anti-ulcer, anti-carcinogenic, and anti-immune properties in different test models in vitro and in vivo (Kuniyal *et al.* 2021).

Figure 2.2 *Saussurea costus* (Kuniyal *et al.* 2021)

2.3.2 *Cinnamomum cassia*

Cinnamon is a tropical evergreen tree that is native to South Asia but is now found all over the world. It has a strong smell and has been used in Chinese medicine and traditional cooking for a long time (Figure 2.3).

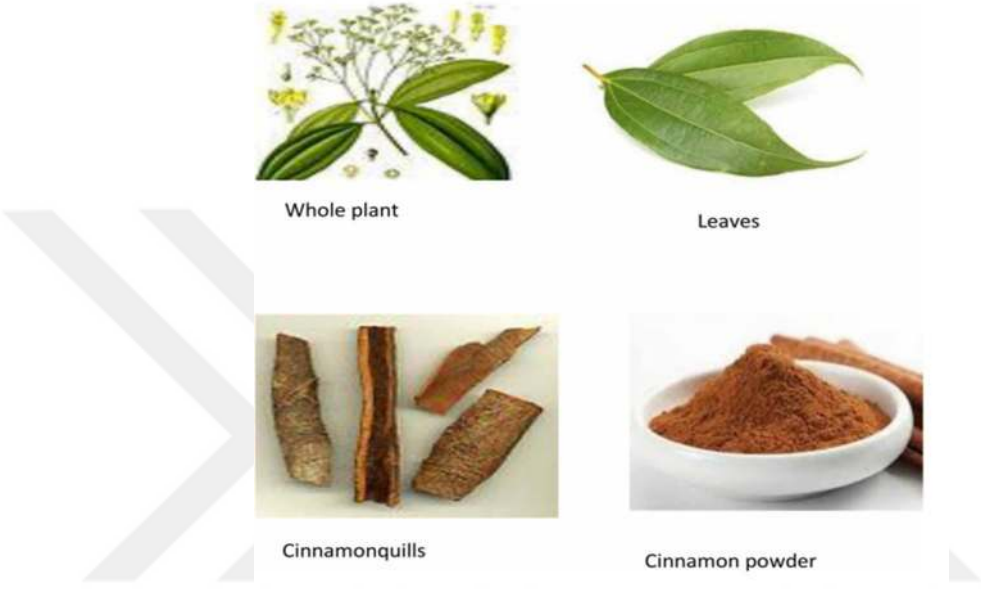

Figure 2.3 *Cinnamomum cassia* (Mishra and Srivastava 2022)

Cinnamomum verum and *Cinnamomum cassia*, the most prevalent species in India and Sri Lanka, are aromatic evergreen trees and bushes, with various components of cinnamon, especially bark and leaves, having been used for medicinal and culinary purposes in India and Sri Lanka for thousands of years. It is used in folk medicine to alleviate the symptoms of rheumatic diseases as well as nausea, indigestion, and other digestive disorders, as well as to treat coughs and diarrhea. It was also used in Iraq as an extract against many pathogenic bacterial species such as *E. coli, S. typhimrium, P. aeruginosa,* and *S. aureus*, as well as against yeasts and fungi such as *C. albicans* (Tekwu *et al*. 2012). It is used around the world as a flavoring factor in a variety of foods, including sweets, desserts, snack foods, beverages, and chewing gum, plus it is commonly used in soaps and perfumes due to its unique aroma (Mishra and Srivastava 2022).

Table 2.3 Scientific classification of *Cinnamomum cassia* (Mishra and Srivastava 2022)

Kingdom	**Plantae**
Clade	Tracheophytes
Order	Laurales
Family	Lauraceae
Genus	*Cinnamomum*
Species	*C. cassia*

Medicinal and flavorful, cinnamon is a plant with a distinct perfume and taste. Cinnamon's primary active ingredients are polyphenols and volatile essential oils. Vanilla, caffeine, gallic, protocatechuic, coumaric, and ferulic acid are some of the physiologically active components contained in cinnamon essential oil (Singh *et al.* 2007). Because of the essential oil's active components, it had a distinct flavor and aroma. The key bioactive components in each portion of the plant, including bark, leaves, and root has been given in Table 2.4 (Sree Satya Nandam and Vangalapati 2012).

Table 2.4 Major bioactive constituents is vary in different part of plan (Sree Satya Nandam and Vangalapati 2012)

Parts of Plant	**Major bioactive constituents**
Leaves	Cinnamaldehyde:1-5%Eugenol: 70-95%
Bark	Cinnamaldehyde:65-80% Eugenol: 5-10%
Root	Camphore: 60%

3. MATERIALS AND METHODS

3.1 Sampling

The swap samples were collected from 60 patients suffering from tonsillitis at Samarra General Hospital and private clinics between the period from 11/15/2021 to 2/15/2022. The profile of the patients included all genders and age groups from 1-10 years. The patient was diagnosed clinically by the specialist doctor. The samples were collected by using sterile cotton swabs containing gel swab medium from patients. All samples were transported using a sterile and cool box to protect the samples from contamination.

3.2 Samples Culture

All samples were cultured on blood agar, nutrient broth agar and MacConkey agar medium for the primary isolation, and then incubated at 37° C for 24 to 48 hours (Forbes *et al.* 2007). The culture media was prepared according to the supplied company's instructions, based on the information on the packages, and sterilized with a sterilizer at 121° C and a pressure of 15 pounds/inch2 for 15 minutes. After pouring the media, it was incubated at 37° C for 24 hours to ensure that it was not contaminated, and then stored in the refrigerator until use (Forbes *et al*. 2007).

For the synthetic culture media, the blood base agar medium was made according to the manufacturer's instructions after sterilizing with an ionizer and chilling to 45° C. Five percent of human blood was then added. (Forbes *et al*. 2007). The urea agar medium was made by dissolving 24 g of Urea Base Agar in 950 mL of distilled water, pouring the solution into tubes, sterilizing them at 121° C for 15 minutes, allowing them to cool to 45-50° C, and then filtering in 50 mL of 40 percent sterile urea solution (Forbes *et al*. 2007).

3.3 Identification of Bacterial Isolates

Morphological and biochemical assays were used to identify bacterial isolates. The diagnosis of bacterial isolates included the following:

3.3.1 Initial bacteriological diagnosis

The colonies growing on the surface of culture media for the initial culture were initially characterized depending on the characteristics of the cultures in terms of shape, size, smell, color, texture, and hemolysis on blood agar medium, fermented by lactose sugar on MacConkey agar medium, and the characteristics of the bacterial cells were studied by examination and microscopy after staining them with Gram stain and then examining them under the oil lens of a light microscope to observe the size and shape of bacterial cells and the way they collect and interact with Gram stain (forbes *et al*. 2007). It was obtained ready-made and used to differentiate between gram-positive and gram-negative bacteria (Forbes *et al.* 2007).

3.3.2 Biochemical tests

a) Catalase test

This test was conducted by transferring a portion of the young colonies on nutrient agar to a clean glass slide using a sterile loop and then adding a drop of hydrogen peroxide (H2O2) to the bacterial culture. A positive test is shown by the emergence of gas bubbles within a few seconds of administration (Cappuccino and Welsh 2018).

Utilizing the catalase reagent, the capacity of bacterial isolates to generate the catalase enzyme was evaluated. The reagent was produced by dissolving 3 mL of hydrogen peroxide (H2O2) in 97 mL of distilled water to achieve a 3 percent reagent concentration. (Brown and Smith 2017).

b) Oxidase test

This test was carried out by transferring part of the young colony from the fed agar with a sterile wooden stick to a filter paper saturated with oxidase reagent tetramethyl -p-phenyl diammine dihydrochloride at a concentration of 1%, the appearance of violet color indicates a positive test (Cappuccino and Welsh 2018).

Using the oxidase reagent, the ability of the bacterial isolate to produce the oxidase enzyme was determined during the oxidase test. The reagent was produced by combining 1 g of Tetramethyl-para-phenylene-diamine dihydro chloride with 100 mL of sterile distilled water (Brown and Smith 2017).

c) Indol test

The test was carried out by inoculating tubes containing peptone water with the bacteria to be tested and incubated for 24-48 hours at a temperature of 37° C, then 0.5 mL of Kovacs reagent was added to the inoculated tubes. The appearance of a pink or dark red ring after adding the reagent indicates a positive test (Brown and Smith 2017).

This Kovacs reagent were prepared by dissolving 5g of the P-dimethyl-amino benzaldehyde in 75 mL of isoamyl alcohol, and then 25 mL of HCL was added and kept in a sterile container in the refrigerator until use, the reagent was used in the indole ring formation test (Brown and Smith 2017).

d) Methyl red test

This assay was conducted to investigate the ability of the bacterial isolate to ferment glucose sugar and form organic acids, inoculated tubes containing MR-VP medium with bacterial colonies, it was incubated for 24-48 hours and at 37° C after that, 5 drops of methyl red reagent were added to each tube, the change in the color of the medium to red indicates a positive test (Mahon *et al*. 2011).

The reagent was used to detect the complete dissolution of sugar. It prepared by dissolving 0.1 mL of methyl red dye in 300 mL of ethyl alcohol at a concentration of 95%, and the volume was completed to 500 mL with distilled water (Brown and Smith 2017).

e) Voges proskaur test

This test was conducted to investigate the ability of the bacterial isolate to produce acetone, as MR-VP medium was inoculated with a young bacterial colony that was incubated for 48 hours at a temperature of 37° C, then 6 drops of 5% alpha-naphthol and 3 drops of potassium hydroxide were added to each tube with a good shake, the appearance of a carmine pink color indicates a positive test (Mahon *et al*. 2011).

The reagent consists of two solutions, A and B The first solution was prepared by dissolving 40g of potassium hydroxide in 100 mL of distilled water, and the second solution was prepared by adding 50 g of alpha-naphthol in 100 mL of Absolute Ethanol, this reagent was used in the vogas-Proskaure to detect the ability of bacteria to produce acetone (Brown and Smith 2017)**.**

f) Citrate utilization test

The habitats of the citrate medium were inoculated with bacterial isolates and then incubated for 18-24 hours at 37° C. The change of the color of the medium from green to blue indicates the positivity of the test and the ability of bacteria to consume citrate as the sole source of carbon (Alfred 2005).

g) Kligler test

The test was conducted by inoculating cleared agar habitats with the bacterial culture by plotting and stabbing and then incubating at 37° C for a period of 24 hours, by changing the color of the medium by adding drops of phenol red reagent to yellow, or the medium remains without a change in red color, or the color of the bottom of the

medium changes without changing the color of the surface oblique part of the medium, the formation of a black precipitate is evidence of the production of gas isolates (Forbes *et al*. 2007).

h) Urease test

The test was carried out by inoculating the urea medium with the bacterial culture by plotting on the surface of the medium and incubating at 37° C for a period of 24 hours, the change in the color of the medium from yellow to pink indicates the ability of bacteria to produce the urea enzyme that breaks down urea into ammonia (Obeid and Abbas 2015).

This urea medium was prepared by dissolving 20 g of Urea powder in 50 mL of distilled water to obtain 40% concentration, and then sterilized by filtering using Millipore filters (Forbes *et al*. 2007).

i) Carbohydrate fermentation test

The test was conducted to investigate the ability of the isolates to ferment types of sugars by inoculating tubes containing the sugars fermentation medium with the young bacterial colony, incubated at 37° C for 24-48 hours, the change of the color of the medium from red to yellow indicates the occurrence of fermentation (Abboud 2017).

j) Mannitol fermentation test

This test was selective for *Staphylococcus aureus*, as this test was conducted to reveal their ability to ferment mannitol sugar, mannitol salt Agar was inoculated with the young bacterial cultures and incubated at 37° C for 24 hours, the change in the color of the medium from pink to golden yellow indicates a positive test (Macfaddin 2000).

3.4 Maintanance of Bacteria Isolates

Bacterial isolates were preserved in two ways: Short-term preservation: Bacterial isolates were kept on Slant nutrient medium in the refrigerator at 4° C, taking into account the isolates were renewed periodically every month by reactivating them in the heart and brain infusion medium and re-growing them on a tilted culture medium to ensure their survival throughout the study period (Angshumanjan *et al*. 2016).

Long-term preservation: the diagnosed bacterial isolates were preserved in a sterilized heart-brain infusion medium, sterilized by sterilizer, and distributed in tubes, at an amount of 4 mL per tube to preserve their genetic characteristics and ensure that their characteristics would not change, then add 1 mL of glycerol 20% to the tubes and shake the tube well to top and bottom and save them at -20° C (Green 2015).

3.5 Antibiotics Susceptibility Test

To examine the resistance of antibiotics to bacterial isolates, susceptibility of all isolates was tested using the Disc Diffusion Methods. Way according to modified Kirby-Baure method described by the World Health Organization (Morello *et al*. 2006). Ten kinds of antibiotic discs was used by using agar Muller-Hinton's medium, as a suspension of bacterial isolates was prepared, a single colony of isolates under study was kept in tubes containing Normal saline solution and these tubes were incubated at a temperature 37° C for a period of time, 18-24 hours, and then the growth level in the tubes has been compared with the tube containing 0.5 MacFarland's solution, which is approximately equal to 1.5 X 10^{8} cells / mL, and using a sterile cotton swab, bacteria were spread on Muller-Hinton's broth medium and left for 15 minutes to dry the dishes, then, the antibiotic discs were distributed by means of sterile forceps on the surface of the medium, and the dishes were incubated for 24 hours at a temperature of 37° C, then the results were read by measuring the diameter of the inhibition zone using a standard ruler and comparing it with the standard tables (CLSI 2020), in order to know the resistant, intermediate and sensitive bacteria to the antibiotics.

As in table 3.1, the Macfarland solution consists of two solutions: **Solution A**: consists of dissolving 1.175 g of barium chloride (BaCl) and filling the volume with 100 mL of distilled water. **Solution B**: It consists of adding 1 mL of concentrated sulfuric acid to 100 mL of distilled water. The standard solution is prepared by adding 0.5 of solution A to 99.5 of solution B, mixing well, and placing it in an airtight, sterile vial until used. It gives an approximate number of bacterial cells when performing a sensitivity test according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2020). Normal saline solution was prepared by dissolving 0.85 g of sodium chloride in 90 mL of distilled water, completing the volume to 100 mL, then sterilizing with osmosis and keeping at 4° C until use (Forbes *et al.* 2007).

Table 3.1 Antibiotics disk used in the study (CLSI 2020)

Antibiotics	**Content (µg)**	**Inhibition diameters**		
		S	**I**	**R**
Amikacin (AK)	30	≥17	15-16	≤14
Ciprofloxacin (CIP)	5	≥25	19-24	≤18
Amoxicillin / Clavulanic acid (AMC)	30	≥18	14-17	≤13
Levofloxacin (LEV)	5	≥22	15-21	≤14
Cefaxime (CFM)	5	≥19	15-17	≤14
Tetracycline (TEV)	30	≥19	16-18	≤15
Meropenem (MEM)	10	≥20	16-19	≤15
Imipenem (IMP)	10	≥22	19-21	≤18
Gentamycin (CN)	10	≥15	13-14	≤12
Chloramphenicol (KF)	30	≥17	15-16	≤14

3.6 Plants Collection and Extractions

The plants were purchased from local markets in Samarra. The plants were kept in a shaded place after rinsed and dried in the laboratory to prevent the from rotting. After grinding with an electric grinder, it was stored in moisture free and airtight containers. The extracts of plants were prepared based on the method of Al-Fartusie *et al*. (2019), with a weight of 10 gm of dry powder for each plant and 100 mL of hot distilled water (aqueous) or ethanol (alcoholic) and left after 24 hours in the refrigerator. Afterward, the extrants obtained after passing through the Whatmann No.1 filter storde in sterile, opaque bottles in the refrigerator until used.

For testing the inhibitory activity of plant extracts against bactearial species, the agar diffusion method was used by drilling on the center of Muller Hinton agar with a cork borer to make holes of equal dimensions to prevent overlapping damping diameters, with a diameter of 6 mm to contain plant solutions at a rate of 60 µl per hole after spreading 0.1 mL of the bacterial barrier, on the medium to test the sensitivity of bacteria to plant extracts at concentrations (12.5, 25, 50, 100, 200, and 400) mg/mL of the food medium, then the dishes were left in the refrigerator for one hour for the dispersal of the plant solutions, and then the dishes were incubated at 37° C for 24 hours, the results were read by measuring the diameter of the inhibition zone (Al-Fartusie *et al*. 2019).

To test the inhibitory activity of antibiotic powders against bacterial isolates, a series of graded dilutions of antibiotic powders were prepared as in Table 3.2, and the specified dilutions were taken at 3.12, 6.25, 12.5, 25, 50, and 100 mg/mL. For the purpose of testing the sensitivity of bacterial isolates to antibiotic powders, the medium of the Muller Hinton agar was prepared in a volumetric flask and sterilized by the sterilizer at a temperature of 121° C. For a quarter of an hour and at a pressure of 15 pounds/ang 2, after cooling to a temperature of 45° C, the center of the Muller was poured into sterile petri dishes and left until it solidified, the plates were then incubated in the incubator at a temperature of 37° C for 24 hours. The data were recorded for the inhibition zones on the next day using a standard ruler, and then they were compared with the results of the inhibitory activity of the extracts under study against the isolated bacterial species (WHO 2006).

Table 3.2 Types of antibiotic powders

Antibiotics	Manufacturer and country of origin
Amoxicillin / Clavulanic acid	Samarra Drugs Industry (SDI)- Iraq
Chloramphenicol	Samarra Drugs Industry (SDI)- Iraq
Tetracycline	Samarra Drugs Industry (SDI)- Iraq
Levofloxacin	Samarra Drugs Industry (SDI)- Iraq

3.7 The Synergistic Effects of Plant Extracts and Antibiotics

The synergistic effects of aqueous and alcoholic extracts with antibiotics were studied by the method of diffusion in agar by drilling and following the method (Passat 2012). The mixture of 60 microliters of the plant extract with the lowest inhibitory concentration of the antibiotic was placed in each well on a medium (Muller-Hinton Agar) inoculated with bacteria and the dishes were incubated at 37° C, then for 24 hours, and the diameter of the damping area around the well was measured in millimeters, and the synergistic reaction was observed or not.

3.8 Statistical Analysis

The results were statistically analyzed by using the minitab statistical program and by applying the ANOVA test. The arithmetic means were compared with Duncan's multiple ranges test 0.05 probability and 0.01, and the Chi-Square test was also applied to determine the differences between the studied groups (SAS 2012).

4. RESULTS AND DISCUSSION

4.1 Isolation

From the 60 samples collected, 53 (88.33%) of them gave a positive bacterial growth, while 7 (11.57%) did not give growth (Chi-Squire (X^2) = 42.533, *P*-value = 0.0008). The reason for the non-growth of these samples, may be due to the fact that the infection was viral, fungal, or parasitic, or that the bacteria in the infection may be of an anaerobic type (Mahdi and Mahdi 2017). According to the results, the infection rate of males was higher than that of females, at a rate of 38 (63.33%) and 22 (36.66%), respectively, from tonsillitis infections. However, the statistical analysis showed significant differences of infection between male and female (Chi-Squire (X^2) = 8.53, *P*-value = 0.003). However, the results obtained by Al-Tameemi *et al*. (2020) showed that the females are more susceptible to infection than males.

The age of patients ranged from 1-10 years. The highest infection rate among the age groups was 36.66% at age group 5-6, 25% at the age group 7-8 years. 16.66% at age groups 3-4, 11.66% at age groups 9-10 years, while the lowest percentage, 10%, at the age groups 1-2. The findings were concur with the previous studies suggesting some reasons for his acute tonsillitis study on children in schools; such low immunity in the children, overcrowded class rooms and poor ventilation inside the school as well as low immunity in the children influence the acute tonsilitis (Vijayashree *et al.* 2014). The majority of people who get bacterial pharyngotonsillitis are children and adolescents between the ages of 5 and 15 (Khadilkar and Ankle 2008). There is an increase in oral microbes during the childhood age because of the different contacts at home or school or day care facilities. This causes an increase in the frequency of infections in the mouth. The gender of those undergoing tonsillectomy was not clearly differentiated, however (Lamaro-Cardoso *et al*. 2009). Pharyngotonsillitis recurrence has been documented in 75.5% of patients, according to some research. Tonsillectomy is usually indicated by recurrent pharyngotonsillitis and/or enlargement of the tonsils, as well as sleep apnea, snoring, and breathing pauses (Stuck *et al.* 2008).

Table 4.1 Distribution of sources of infection and percentage of isolated bacteria according to age groups

Age groups	**Tonsilitis**	
	n	**%**
1-2	6	10
3-4	10	16.7
5-6	22	36.7
7-8	15	25
9-10	7	11.7
Total	**60**	**100**
Chi-Square = 18.125, *P*-value = 0.001		

4.2 Identification of Bacterial Isolates

Bacterial isolates were identified by colony morphology and microscopic exam and biochemical tests. These tests were conducted to diagnosis the isolated bacterial species and explained in materials and methods section. The obtained results were as follows, results show in table 4.4.

Table 4.2 Morphology and microscopy characteristics of bacteria isolated from from tonsillitis (Growth: +, no growth: -)

Bacteria species	**Medium**			**Appearance characteristics**	**Gram Stain**
	MacConkey	**Blood**	**Mannitol**		
S. aureus	-	Beta hemolysis	Gold to yellow and fermentation mannitol	small round shaped clusters or pairs	+
S. pyogenes	-	Beta hemolysis	-	small round shaped chains or pairs	+
S.viridanse	-	Alfa hemolysis	-	small round shaped chains or pairs	+
Bacillus sp.	-	Beta hemolysis	-	a short rod appears singly or pairs	+
K. pneumoniae	pink mucosa	Grayish mucosa colonies	-	a short rod appears singly or pairs	-
P. aeruginosa	pale colonies	Greyish white, β-Hemolysis (in some strains)	-	a short rod appears singly or pairs	-

Table 4.3 Biochemical tests used in the diagnosis of gram-negative bacteria (Y: Yellow acidic reaction, R: Red alkaline reaction, negative: - , positive: +)

Bacteria	**IMVIC indole, methyl red, Voges-Proskauer test, Citrate test**				**Catalase**	**Oxidase**	**Kligler iron agar**				**Motility**
	IND	**MR**	**VP**	**C**			**slant**	**butt**	**H2S**	**gas**	
K. pneumoniae	-	-	+	+	+		-	Y	Y	-	+
P. aeruginosa	-	-	-	+	+		+	R	R	-	-

Table 4.4 Biochemical tests used in the diagnosis of gram-positive bacteria (S: sensitive)

Bacteria	**Catalase**	**Oxidase**	**Coagulase**	**novobiocin**	**hemolysin**
S. aureus	+	-	+	S	β
S. pyogenes	-	-	-	-	β
S.viridanse	-	-	-	-	α
Bacillus sp.	-	-	-	-	β

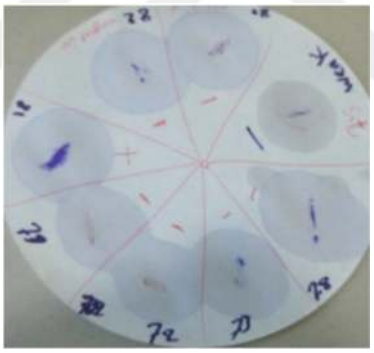

Figure 4.1 Oxidase Test (Purpule Color Positive)

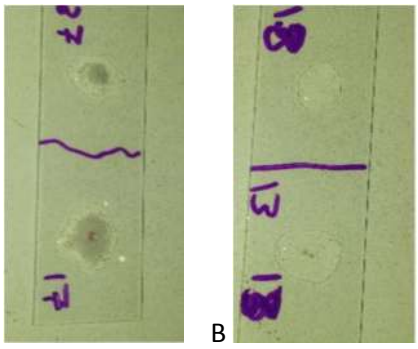

Figure 4.2 Catalase Test (A: Positive Result, B: Negative Result)

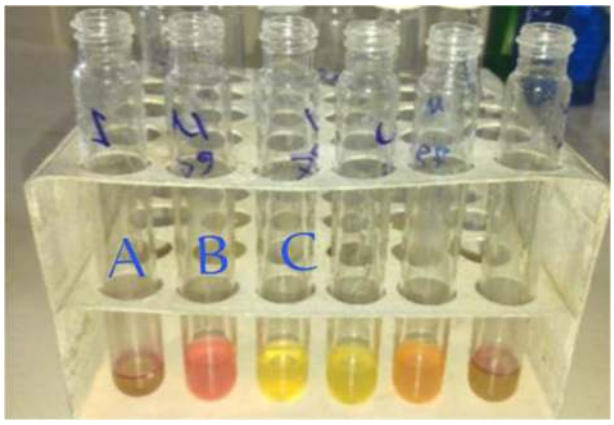

Figure 4.3 A: Indole Test, B: Methyl Red Test, C: Voges Proskauer

Figure 4.4 Citrate Test (Positive Result: Blue Color, Negative Result: Green Color)

Figure 4.5 Different Types of Bacteria on Kligler Iron gar

4.3 The Result of The Preparation and Proportions of The İsolated Bacterial Species

Of the 53 isolated samples of the patients, 6 strains of bacteria were found 4 types of gram-positive bacteria *S. aureus, S. pyogenes, S. viridanse* and *Bacillus* and 2 species Gram-negative bacteria *K. pneumonia* and *P. aeurginosa* causing the tonsillitis. The most common isolated species causing bacterial infections was *S. aureus* 32 (60.37%), *S. viridanse* 7 (13.20%), *Bacillus* 5 (9.43%), *S. pyogenes* 4 (7.54%), *P. aergionosa* 3(5.66%), *K. pneumonia* 2 (3.77%), respectively. Results of the study were in an agreement with Al-Tameemi *et al*. (2020) and Cavalcanti *et al*. (2019) those who isolated *S. aureus* bacteria from tonsils with the highest percentage.

Table 4.5 Number and Percentage to Bacterial Species Isolated from tonsillitis

Source isolation **Type of isolation**	**Tonsillitis**	
	No	**%**
S. aureus	32	60.37
S. pyogenes	4	7.54
S. viridanse	7	13.20
K. pneumoniae	2	3.77
P. aergionosa	3	5.66
Bacillus	5	9.43
Total	53	100
Chi-Square = 59.502, *P*-value=0.00003		

4.4 The Results of Antibiotics Susceptibility Test

A sensitivity test for bacteria isolated from tonsilitis was found *S. aureus* showed 80% sensitivity with chloramphenicol, 90% with Amikacin, 78% with Gentamycin, 85% with Meropenem, 95% with Tetracycline, and the highest sensitivity to Ciprofloxacin and Amoxicillin / Clavulanic acidwas 97% and 96, respectively. The lowest sensitivity effect was 0% with Cefixime (table 4.6). In the current study, *S. pyogenes* showed sensitivity for Amikacin, Ciprofloxacin, Gentamycin, Meropenem, Tetracycline, and Levofloxacin 100%, 88%, 75%, 95%, 85%, and 90%, respectively, while the resistance was 100% with Amoxicillin/Clavulanic acid and Chloramphenicol.

The sensitivity of *P. aeruginosa* to antibiotics, the highest effect was found for Tetracycline, Ciprofloxacin, Levofloxacin, and Meropenem 94%, 98%, 85%, and 89%, respectively. While *P. aeruginosa* was resistant to Amikacin, Imipenem, Gentamycin, Cefixime, Amoxicillin/Clavulanic acid and Chloramphenicol 100%, 85%, 90%, 79%, 96%, and 95%, respectively.

K. pneumonia were resistant to Tetracyclin, Imipenem, Gentamycin, Cefixime, Amoxicillin / Clavulanic acidand Chloramphenicol 80%, 85%, 90%, 87%, 75% and 100% respectivey, and showed sensitivity to, Amikacin, Ciprofloxacin, Levofloxacin and Meropenem, 100%, 97%, 85% and 95%, respectively.

Bacillus showed sensitivity to Amikacin, Imipenem, Ciprofloxacin, Gentamycin, Cefixime, Tetracyclin, Levofloxacin, Amoxicillin / Clavulanic acidand Meropenem, 100%, 85%, 78%, 87%, 90%, 82%, 98%, 95% and 70%, respectively and the species showed 4% resistant to Chloramphenicol.

Figure 4.6 Effect antibiotics discs on *Bacillus*

S. viridanse showed sensitivity to Amikacin, Ciprofloxacin, Tetracyclin, Levofloxacin and Meropenem 80%, 70%, 89%, 98% and 100%, respectively, and the bacteria showed resistance to Imipenem, Gentamycin, Cefixime, Chloramphenicol, Amoxicillin / Clavulanic acid90%, 80%, 86%, 90%, 75%, respectively.

The antibiotic gentamicin has been tested on *P. aeruginosa* isolates and the results showed that the isolates were resistant to antibiotic, while half was sensitive which was agree with Khan *et al.* (2017) and Presad *et al.* (2017). However, the majority of isolates were sensitive to the antibiotic at a rate of by 84%, which consistent with the result of Kumar *et al.* (2014). Chloramphenicol had an average effect on isolates; these results were close to those of Argawl *et al.* (2017).

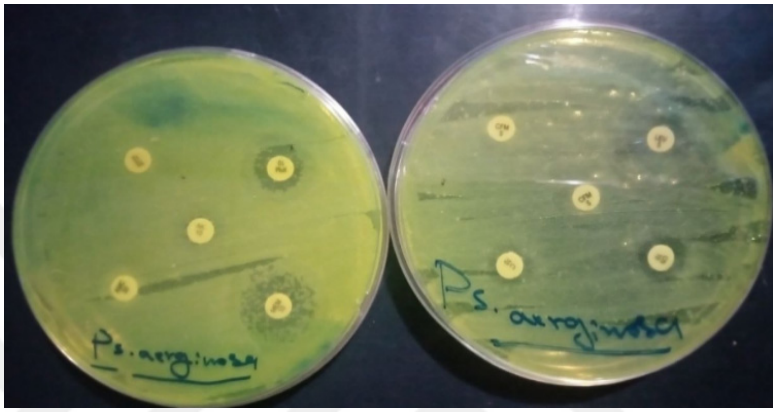

Figure 4.7 Effect antibiotics discs on *P. aerginosa*

The results showed that, the majority of isolates were resistant to cefixime which in line with the finding of Abdullah *et al.* (2011) and Agrawal *et al.* (2017). While, *K. pneumonia* showed a high sensitivity to cefixime, which is in agree with Khan and Ahmed *et al*. (2016).

Figure 4.8 Effect antibiotics discs on *K. pneumoniae*

Bacterial resistance to antibiotics is caused by its production of β-lactamases, which cause antibiotic resistance by altering the target site. Tetracycline resistance is also caused by the presence of genes in chromosomes that serve to protect the target site from antagonistic action or through the efflux pump that exits from the tetracycline. The cause of resistance to cephalosporins is the bacterial production of an enzyme class C β-lactamases that degrade cephalosporins, especially the third generation (Munita and Arias 2016).

The results of this study showed that most isolates were sensitive to Amikacin, Ciprofloxacin, Tetracyclin, Levofloxacin and Meropenem the results were in agreement with previous studies, in this study tetracycline show sensitivity to the bacteria isolation, the more effect on gram positive bacteria the results of the current study of tetracycline have converged with results Rao *et al.* (2014).

Table 4.6 Sensitivity ratios (R: resistant, I: intermediate, S: sensitive) of bacterial isolates under study to antibiotics

Antibiotics	mg/disk	*S. pyogenes*		*S. aureus*		*K. pneunomia*		*S. viridanse*		*P. aeruginosa*		*Bacillus*	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amikacin (AK)	30	0	100	100	0	20	80	0	100	10	90	0	100
Imipenem (IPM)	30	10	90	20	80	15	85	10	90	15	85	85	15
Ciproflaxin (CIP)	10	88	12	98	2	97	3	70	30	98	2	78	22
Gentamycin (CN)	10	75	25	78	22	10	90	20	80	10	90	87	13
Cefixime (CFM)	5	14	86	0	100	13	87	14	86	21	79	90	10
Tetracyclin (TE)	10	85	15	95	5	20	80	89	11	94	6	82	18
Levofloxacin (LEV)	5	90	10	5	95	85	15	98	2	85	15	98	2
Meropenem (MEM)	10	95	5	85	15	95	5	100	0	98	2	70	30
Chloramphenicol (C)	30	0	100	80	20	0	100	10	90	5	95	4	96
Amoxicillin-Clavulanic (AMC)	30	0	100	96	4	25	75	25	75	4	96	95	5

4.5 Effect of Cinnamomum cassia and Saussurea costus Extracts on Bacteria Isolates

The results indicated that there was an inhibitory activity against bacterial isolates with different inhibition diameters. There were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic Cinnamon extract on *S. aureus* by comparing the arithmetic averages of the type of extracts, in the table 4.7, it represents numbers with similar small letters in the same column or similar capital letters in the same row have no significant differences between them.

The concentrations of the aqueous extract of cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg/mL, gave the rates of inhibition diameters 10 mm, 8 mm, 0 mm, 0 mm, 0 mm, and 0 mm, respectively, while they gave the rates of inhibition diameters of 10 mm, 9 mm, 8 mm, 7 mm, 6 mm, and 6 mm, respectively for the alcoholic cinnamon extract. It was observed that the highest inhibitory activity of the cinnamon water extract was at a concentration of 400 mg/mL with an average inhibition diameter of 10 mm, while the lowest inhibitory activity was at a concentration of 200 mg/mL with an inhibition diameter of 8 mm against the above bacteria.

While cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 10 mm, and the lowest inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 6 mm. While the Costus extract showed that there were significant differences between the inhibitory effect of aqueous and alcoholic extract on *S. aureus*, the concentrations of the aqueous extract of Costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 14, 12, 10, 10, 8, and 8 mm, respectively for the alcoholic Costus extract. Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg / mL with an average inhibition diameter of 14 mm, and the lowest average inhibitory activity was at a concentration of 12.5 mg / mL with an average inhibition diameter of 8 mm against the above bacteria. There were significant differences between Cinnamon and Costus extracts, and showed

that there were significant differences between extract and antibiotics by comparing the arithmetic averages of the extracts and antibiotics.

The alcoholic and aqueous extracts of cinnamon and costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin, and Amoxicillin/Clavulanic acid at concentrations of 100, 50, 25, 12.5, 6.25, and 3.12 mg/mL. The rates of inhibition diameters were found to be 35, 33, 30, 28, 20, 15 mm, respectively with Levofloxacin, 30, 25, 20, 18, 15, 10 mm, respectively with Chloramphenicol, 30, 25, 24, 23, 20, 18 mm, respectively with Tetracyclin, and 45, 40, 35, 30, 20, 10 mm, respectively with Amoxicillin/Clavulanic acid.

Table 4.7 The inhibitory action against *S. aureus* by aqueous and alcohol extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	10	8	0	0	0	0	3.0 ^{c}	5.35 ^{A}
	Ethanol	10	9	8	7	6	6	7.7 ^{b}	
Costus	Water	0	0	0	0	0	0	0.0 ^{d}	5.15 ^{A}
	Ethanol	14	12	10	10	8	8	10.3 ^{a}	
Mean of Con.		8.5 ^{a}	7.25^{a}	4.5^{b}	4.25^{b}	3.5 ^{b}	3.5^{b}		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.8 The inhibitory action against *S. aureus* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	45	40	35	30	20	10	30.0 ^{A}
Chloramphenicol	30	25	20	18	15	10	19.67 ^{C}
Tetracycline	30	25	24	23	20	18	23.33 ^{B}
Levofloxacin	35	33	30	28	20	15	26.83 ^{B}
Mean of Con.	35.0 ^{a}	30.7 ^{b}	27.2 ^{bc}	24.7 ^{c}	18.75^{d}	13.3^{e}	

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

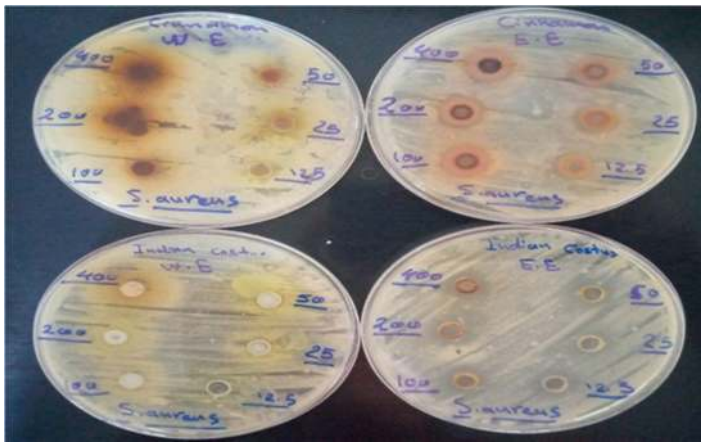

Figure 4.9 Effect extracts on *S. aureus*

There were no significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability levels between the inhibitory effect of aqueous and alcoholic cinnamon extracts on *S. pyogenes* by comparing the arithmetic averages of the type of extracts. The concentrations of the aqueous extract of cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg/mL, gave the rates of inhibition diameters of 20, 15, 13, 12, 0, 0 mm respectively, while they gave the rates of inhibition diameters of 15, 14, 13, 12, 10, 0 mm respectively for the alcoholic cinnamon extract.

It was observed that the highest inhibitory activity of the cinnamon water extract was at a concentration of 400 mg/mL with an average inhibition diameter of 20 mm, while the lowest inhibitory activity was at a concentration of 50 mg/mL with an inhibition diameter of 12 mm against the above bacteria. While cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 15 mm, and the lowest inhibitory activity was at a concentration of 25 mg/mL with an average inhibition diameter of 10 mm. While the costus extract showed that there were significant differences between the inhibitory effects of aqueous and alcoholic extracts on *S. pyogenes* by comparing the arithmetic averages of the types of extracts. The concentrations of the aqueous extract of costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 16, 15, 14, 14, 13, and 12 mm respectively for the alcoholic costus extract. Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 16 mm, and the lowest average inhibitory

activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 12 mm against the above bacteria.

We showed that there were significant differences between Cinnamon and Costus extracts comparing the arithmetic averages of the plant and showed that there were significant differences between extract and antibiotics by comparing the arithmetic averages of the extracts and antibiotics, we find that the alcoholic and aqueous extracts of the cinnamon and the Costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin and Amoxicillin / Clavulanic acidat concentrations 100, 50, 25, 12.5, 6.25 and 3.12 mg / mL, gave the rates of inhibition diameters 38, 35, 33, 30, 28, 25 mm respectively with Levofloxacin, 35, 30, 28, 26, 22, 18 mm respectively with Chloramphenicol, 25, 20, 16, 15, 13, 12 mm respectively with Tetracyclin and 30, 27, 24, 20, 18, 0 mm respectively with Amoxicillin/Clavulanic acid.

Table 4.9 The inhibitory action against *S. pyogenes* by aqueous and alcohol extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	20	15	13	12	0	0	10.0 ^{b}	10.33 ^{A}
	Ethanol	15	14	13	12	10	0	10.7 ^{b}	
Costus	Water	0	0	0	0	0	0	0.0 ^{c}	7.00 ^{B}
	Ethanol	16	15	14	14	13	12	14.0 ^{a}	
Mean of Con.		12.75 ^{a}	11.0^{a}	10.0^{a}	9.5^{a}	5.8^{b}	3.0^{b}		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.10 The inhibitory action against *S. pyogenes* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	30	27	24	20	18	0	19.8 ^{C}
Chloramphenicol	35	30	28	26	22	18	26.5 ^{B}
Tetracycline	25	20	16	15	13	12	16.8 ^{C}
Levofloxacin	38	35	33	30	28	25	31.5 ^{A}
Mean of Con.	32.0 ^{a}	28.0 ^{b}	25.3 ^{bc}	22.8 ^{cd}	20.3^{d}	13.7^{e}	

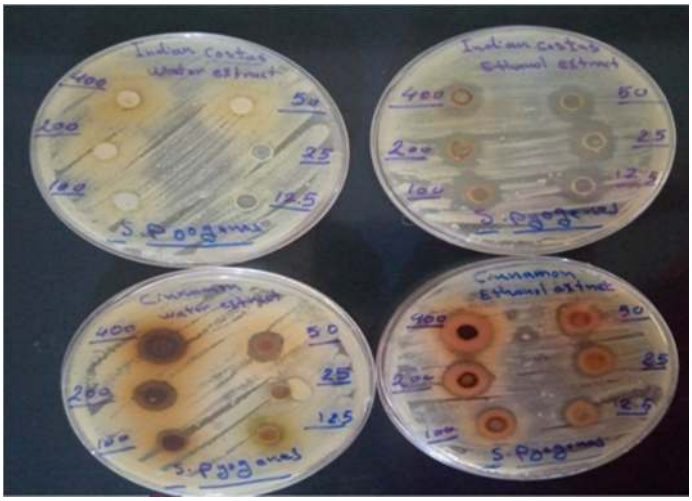

Figure 4.10 Effect extracts on *S. pyogenes*

There were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic cinnamon extract on *S. viridanse* by comparing the arithmetic averages of the type of extracts (table 4.11). The concentrations of the aqueous extract of cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg/mL, gave the rates of inhibition diameters 16, 13, 10, 0, 0, 0 mm respectively, while they gave the rates of inhibition diameters of 22, 20, 18, 17, 16, 14, mm respectively for the alcoholic cinnamon extract. It was observed that the highest inhibitory activity of the cinnamon water extract was at a concentration of 400 mg/mL with an average inhibition diameter of 16 mm, while the lowest inhibitory activity was at a concentration of 100 mg/mL with an inhibition diameter of 10 mm against the above bacteria.

While cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 22 mm, and the lowest inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 14 mm. While the Costus extract showed that there were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic extract on *S. viridanse* by comparing the arithmetic averages of the type of extracts.

The concentrations of the aqueous extract of costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 20, 18, 16, 15, 14, and 13 mm respectively for the alcoholic costus extract. Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 20 mm, and the lowest average inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 13 mm against the above bacteria.

There were significant differences between Cinnamon and Costus extracts comparing the arithmetic averages of the plant and showed that there were significant differences between extract and antibiotics against the above bacteria by comparing the arithmetic averages of the extracts and antibiotics. The alcoholic and aqueous extracts of cinnamon and the Costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin, and Amoxicillin/Clavulanic acid, at concentrations 100, 50, 25, 12.5, 6.25 and 3.12 mg / mL, gave the rates of inhibition diameters 40 38, 34, 30, 25, 20 mm respectively with Levofloxacin, 30, 28, 26, 24, 20, 18 mm respectively with Chloramphenicol, 27, 25, 24, 20, 18, 10 mm respectively with Tetracyclin and 35, 30, 28, 26, 23, 18 mm respectively with Amoxicillin / Clavulanic acid.

Table 4.9 The inhibitory action against *S. viridanse* by aqueous and alcohol extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	16	13	10	0	0	0	6.5 ^{b}	12.2 ^{A}
	Ethanol	22	20	18	17	16	14	17.8 ^{a}	
Costus	Water	0	0	0	0	0	0	0.0 ^{c}	8.00 ^{B}
	Ethanol	20	18	16	15	14	13	16.0 ^{a}	
Mean of Con.		14.5 ^{a}	12.8^{ab}	11.0^{bc}	8.0^{cd}	7.5^{d}	6.8^{d}		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.10 The inhibitory action against *S. viridans* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	35	30	28	26	23	18	26.7 ^{B}
Chloramphenicol	30	28	26	24	20	18	26.7 ^{B}
Tetracycline	27	25	24	20	18	10	20.7 ^{C}
Levofloxacin	40	38	34	30	25	20	31.2 ^{A}
Mean of Con.	33.0 ^{a}	30.3 ^{ab}	28.0 ^{bc}	25.0 ^{c}	21.5^{d}	16.5^{e}	

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

There were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic Cinnamon extract on *Bacillus* by comparing the arithmetic averages of the type of extracts. The concentrations of the aqueous extract of Cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg / mL, gave the rates of inhibition diameters 15, 12, 10, 0, 0, 0 mm respectively, while they gave the rates of inhibition diameters 20, 18, 16, 14, 13, 12 mm respectively for the alcoholic Cinnamon extract. It was observed that the highest inhibitory activity of the Cinnamon water extract was at a concentration of 400 mg / mL with an average inhibition diameter of 15 mm, while the lowest inhibitory activity was at a concentration of 100 mg / mL with an inhibition diameter of 10 mm against the above bacteria.

While cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 20 mm, and the lowest inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 12 mm. While the costus extract showed that there were significant differences between the inhibitory effect of all concentrations of aqueous and alcoholic extract on *Bacillus* by comparing the arithmetic averages of the type of extracts. The concentrations of the aqueous extract of costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 15, 14, 13, 12, 10, and 8 mm respectively for the alcoholic costus extract. Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter

of 15 mm, and the lowest average inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 8 mm against the above bacteria.

There were significant differences between Cinnamon and Costus extracts comparing the mean of the plant and there were significant differences between extract and antibiotics against the above bacteria by comparing the mean of the extracts and antibiotics. The alcoholic and aqueous extracts of cinnamon and costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin and Amoxicillin/Clavulanic acidat concentrations 100, 50, 25, 12.5, 6.25 and 3.12 mg/mL, gave the rates of inhibition diameters of 35, 30, 28, 26, 24, 20 mm respectively with Levofloxacin, 28,26, 24, 20, 18, 16 mm respectively with Chloramphenicol, 30, 27, 25, 23, 22, 20 mm respectively with Tetracyclin and 30, 25, 22, 20, 16, 15 mm respectively with Amoxicillin/Clavulanic acid.

Table 4.11 The inhibitory action against *Bacillus* by aqueous and alcohol extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	15	12	10	0	0	0	6.2 ^{c}	10.8 ^{A}
	Ethanol	20	18	16	14	13	12	15.5 ^{a}	
Costus	Water	0	0	0	0	0	0	0.0 ^{d}	6.0 ^{B}
	Ethanol	15	14	13	12	10	8	12.0 ^{b}	
Mean of Con.		12.5 ^{a}	11.0^{a}	9.8^{ab}	6.5^{bc}	5.8^{c}	5.0^{c}		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.12 The inhibitory action against *Bacillus* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	30	25	22	20	16	15	21.3 ^{B}
Chloramphenicol	28	26	24	20	18	16	22.0 ^{B}
Tetracycline	30	27	25	23	22	20	24.5 ^{AB}
Levofloxacin	35	30	28	26	24	20	37.2 ^{A}
Mean of Con.	33.8 ^{a}	27.0 ^{b}	24.8 ^{bc}	23.3 ^{cd}	20.0^{de}	17.8^{e}	

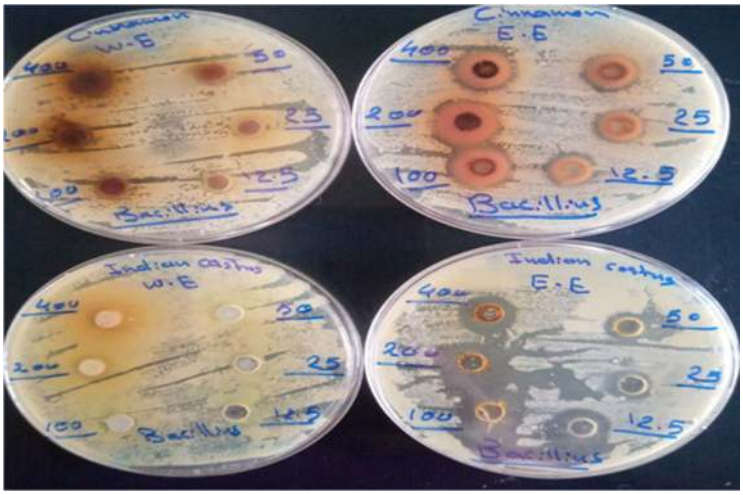

Figure 4.11 Effect extracts on *Bacillus*

The results of the effect of cinnamon and costus extracts on gram positive bacteria were consistent with the results of Abdulrasheed *et al*. (2019), who showed that the alcohol and aqueous cinnamon extract had an inhibitory activity against *S. aureus*. Furthermore, the ethanolic extract comparatively higher antimicrobial activity than the aqueous extract where the highest concentration of 100 mg/mL gave inhibitory activity with a diameter of 20 mm, while the lowest concentration of 20 mg / mL gave the lowest inhibition with a diameter of 9 mm, with ethanolic extract while the highest concentration of 100 mg/mL gave inhibitory activity with a diameter of 11 mm, while the lowest concentration of 20 mg/mL gave the lowest inhibition with a diameter of 8 mm. Nematollahi *et al*. (2020) was also showed that cinnamon extracts has a high inhibitory activity against *S. aureus.* A study showed that cinnamon has high inhibition *S. pyogenes* (Ács *et al*. 2019), and the extract of cinnamon has a high efficacy against *S. pyogenes* (Singh *et al.* 2020).

Accordingly, these findings are in line with Nematollahi *et al*. (2020) who found that cinnamon oil has an inhibitory activity against *Bacillus* ssp. The results of costus extract consistent with the findings of Majeed *et al*. (2019) who showed that the alcohol and aqueous costus extract had an inhibitory activity against *S. aureus* , Majeed *et al*. (2019), *S. pyogenes* (Vaidya and Shingadia 2020), and also *Bacillus*. The results of current study are consistent with these previous studies.

There were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic cinnamon extracts on *P. aeroginosa* by comparing the arithmetic averages of the type of extracts. The concentrations of the aqueous extract of cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg / mL, gave the rates of inhibition diameters 15, 10, 0, 0, 0, 0 mm respectively, while they gave the rates of inhibition diameters 14, 13, 12, 10, 10, 8 mm respectively for the alcoholic cinnamon extract.

It was observed that the highest inhibitory activity of the cinnamon water extract was at a concentration of 400 mg/mL with an average inhibition diameter of 15 mm, while the lowest inhibitory activity was at a concentration of 200 mg/mL with an inhibition diameter of 10 mm against *P. aeroginosa*. While cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 14 mm, and the lowest inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 8 mm. The Costus extract showed that there were significant differences between the inhibitory effect of aqueous and alcoholic extract on *P. aeroginosa* by comparing the arithmetic averages of the extracts. The concentrations of the aqueous extract of Costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 15, 12, 10, 8, 6, and 6 mm respectively for the alcoholic Costus extract. Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 15 mm, and the lowest average inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 6 mm against the above bacteria. There were significant differences between Cinnamon and Costus extracts comparing the arithmetic averages of the plant and showed that there were significant differences between extract and antibiotics against the above bacteria.

The alcoholic and aqueous extracts of cinnamos and Costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin and Amoxicillin/Clavulanic acidat concentrations 100, 50, 25, 12.5, 6.25 and 3.12 mg/mL, gave the rates of inhibition diameters 40, 35, 32, 30, 28, 25 mm respectively with Levofloxacin, 30, 28, 26, 24, 22, 20 mm respectively with

Chloramphenicol, 20, 15, 14, 13, 12, 10 mm respectively with Tetracyclin and 35, 32, 30, 28, 15, 0 mm respectively with Amoxicillin/Clavulanic acid.

Table 4.13 The inhibitory action against *P. aeruginosa* by aqueous and alcoholic extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	15	10	0	0	0	0	4.2 [b]	7.7 [A]
	Ethanol	14	13	12	10	10	8	15.2 [a]	
Costus	Water	0	0	0	0	0	0	0.0 [c]	4.8 [B]
	Ethanol	15	12	10	8	6	6	9.5 A	
Mean of Con.		11.0 [a]	8.8[b]	5.5[c]	4.5[c]	4.0[c]	3.5 [c]		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.14 The inhibitory action against *P. aeruginosa* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	35	32	30	28	15	0	23.3 [B]
Chloramphenicol	30	28	26	24	22	20	25.0 [B]
Tetracycline	20	15	14	13	12	10	14.0 [C]
Levofloxacin	40	35	32	30	28	25	31.7 [A]
Mean of Con.	31.3 [a]	27.5 [ab]	25.5 [bc]	23.8 [cd]	19.3[de]	13.8[e]	

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

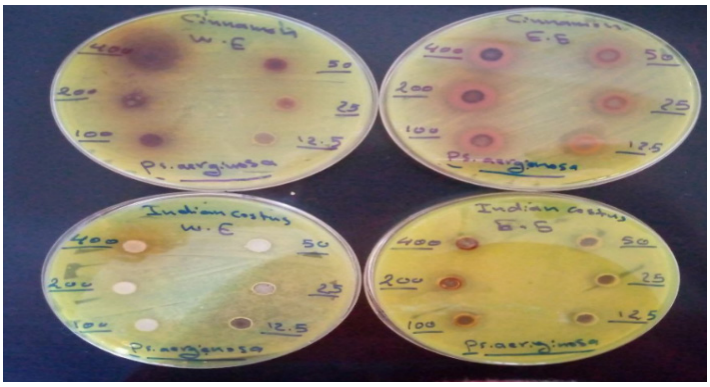

Figure 4.12 Effect extracts on *P. aereuginosa*

There were significant differences according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level between the inhibitory effect of aqueous and alcoholic Cinnamon extract on *K. pneumonia* by comparing the arithmetic averages of the type of extracts. The concentrations of the aqueous extract of cinnamon, which are 400, 200, 100, 50, 25, 12.5, mg/mL, gave the rates of inhibition diameters of 14, 12, 0, 0, 0, 0 mm respectively, while they gave the rates of inhibition diameters 15, 14, 13, 12, 10, 10 mm respectively for the alcoholic Cinnamon extract. It was observed that the highest inhibitory activity of the cinnamon water extract was at a concentration of 400 mg/mL with an average inhibition diameter of 14 mm, while the lowest inhibitory activity was at a concentration of 200 mg/mL with an inhibition diameter of 12 mm against the above bacteria. The cinnamon alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 15 mm, and the lowest inhibitory activity was at a concentration of 12.5 mg/mL with an average inhibition diameter of 10 mm. While Costus extract showed that there were significant differences between the inhibitory effect of aqueous and alcoholic extract on *K. pneumonia* by comparing the arithmetic averages of the type of extracts.

The concentrations of the aqueous extract of Costus, which are 400, 200, 100, 50, 25, 12.5 mg/mL, do not have any inhibition, while they gave the rates of inhibition diameters 14, 13, 12, 10, 10, 10 mm respectively for the alcoholic costus extract. The Costus alcohol extract gave the highest inhibitory activity at a concentration of 400 mg/mL with an average inhibition diameter of 14 mm, and the lowest average inhibitory activity was at a concentration of 12.5 mg / mL with an average inhibition diameter of 10 mm against the above bacteria. There were significant differences between Cinnamon and Costus extracts comparing the arithmetic averages of the plant and showed that there were significant differences between the effect of extract and antibiotics against the above bacteria. The alcoholic and aqueous extracts of cinnamos and costus have shown a weak inhibitory effect compared to the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin, and Amoxicillin/Clavulanic acid at concentrations 100, 50, 25, 12.5, 6.25 and 3.12 mg/mL, gave the rates of inhibition diameters 50, 48, 46, 44, 42, 40 mm respectively with Levofloxacin, 32, 30, 28, 25, 23, 20 mm respectively with Chloramphenicol, 27, 25, 24, 23, 20, 18 mm respectively with

Tetracyclin and 45, 43, 38, 35, 30, 25 mm respectively with Amoxicillin/Clavulanic acid.

Table 4.15 The inhibitory action against *K. pneumonia* by aqueous and alcoholic extracts

Type of Extract		**Concentrations (mg / mL)**							
		400	200	100	50	25	12.5	Mean of extract type	Mean of plant
Cinnamon	Water	14	12	0	0	0	0	4.3 [b]	8.3 [A]
	Ethanol	15	14	13	12	10	10	12.3 [a]	
Costus	Water	0	0	0	0	0	0	0.0 [c]	5.8 [B]
	Ethanol	14	13	12	10	10	10	11.5 [a]	
Mean of Con.		10.8 [a]	9.8[a]	6.3[b]	5.5[b]	5.0[b]	5.0 [b]		

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

Table 4.16 The inhibitory action against *K. pneumonia* by antibiotics

Type of Antibiotics	**Concentrations (mg / mL)**						
	100	50	25	12.5	6.25	3.12	Mean of antibiotic type
Amoxicillin / Clavulanic acid	45	43	38	35	30	25	36.0 [B]
Chloramphenicol	32	30	28	25	23	20	26.3 [C]
Tetracycline	27	25	24	23	20	18	26.7 [C]
Levofloxacin	50	48	46	44	42	40	45.0 [A]
Mean of Con.	38.5 [a]	36.5 [ab]	34.0 [ab]	33.0 [b]	28.8[c]	25.8	

*Numbers with small letters in the same column or similar capital letters in the same row have no significant differences between them according to Duncan's multiple ranges test at the 0.05% and 0.01% probability level

The effectiveness of cinnamon extract against *P. aeruginosa* and the higher ethanolic extract in antimicrobial activity than the aqueous extract is consistent with the previous findings (Abdulrasheed *et al*. 2019). The highest concentration of 100 mg/mL gave inhibitory activity with a diameter of 16 mm, while the lowest concentration of 20 mg/mL gave the lowest inhibition with a diameter of 9 mm with ethanolic extract. The highest concentration of 100 mg/mL gave inhibitory activity with a diameter of 13 mm, while the lowest concentration of 20 mg/mL gave the lowest inhibition with a diameter

of 7 mm. The cinnamon extracts has a high inhibitory activity against *P. aeruginosa* (Nematollahi *et al*. 2020), *K. pneumoniae* (Oulkheir *et al*. 2017, Meshaal *et al*. 2021).

The effectiveness of Costus extract against *P. aeruginosa* are consistent with the previous results (Majeed *et al*. 2019), while the results showed inconsistent with the findings of Majeed *et al.* (2019) on the the effectiveness of the aqueous extract of the costus and the ineffectiveness of the ethanolic extract against *K. pneumoniae*.

It is seen that the effect of antibiotics exceeds the inhibitory effect of the mentioned plant extract. There is a clear discrepancy in the level of inhibition between plant extracts compared to the antibiotics. This study's findings about the advantage of antibiotics over plant extracts concur with those of Singh *et al*. (2020), who demonstrated the superiority of Gentamycin over the alcoholic extract of cinnamon. It has been claimed that cinnamon extract, essential oils, and their constituents hinder the growth of bacteria via anti-quorum sensing effects by disrupting the cell membrane, altering the lipid profile, inhibiting ATPases, cell division, motility, and biofilm formation (Vasconcelos *et al*. 2018). The antibacterial activity is related to the presence of plant components such as phenols and flavonoids in the crust extract (Yamamato and Gaynor 2002). *Cinnamon cassia* mainly contains bio-oils and other derivatives and the crust of cinnamon contains 65–80% cinnamaldehyde, and 5%–10% eugenol (Rao and Gan 2014). The antibacterial efficacy of cinnamon is directliy linked to its main ingredient, cinnamaldehyde and this compound inhibits acetyl-CoA carboxylase (Meades *et al*. 2010). Acetyl-CoA carboxylase (ACC) catalyzes the fatty acid biosynthesis, which is essential for many important biological process, including the synthesis and maintenance of cell membranes (Gago *et al*. 2011).

4.6 Synergistic Action Between Extracts and Antibiotics

The synergistic action of water and alcoholic extracts of *Cinnamon* and *Costus* with the antibiotics under study against isolates. The results showed that there is a discrepancy in the effect of the synergistic action on the growth of the bacterial isolate, as it was noted that these extracts of both types and with all their concentrations gave a high synergistic

action with all of the antibiotics with a concentration of 3.12 mg/mL, especially with the antibiotics Levofloxacin, Chloramphenicol, Tetracyclin, and Amoxicillin/Clavulanic acid.

The lowest concentration of this antibiotic, which is 3.12 mg/mL, gave a high efficacy when synergized with all concentrations of aqueous and ethanolic plant extracts of cinnamon and costus. These findings are consistent with the result of El Atki *et al*. (2019) that there were a strong synergistic effect between *Cinnamomum cassia* extract and ampicillin or chloramphenicol towards *P. aeruginosa* and *S. aureus*. Plant-derived compounds can exhibit direct antibacterial activity and/or indirect activity as antibiotic resistance-modifying compounds. Antibiotics increase their effectiveness, reflecting the ability of plant active substances to modify or block the resistance mechanism so that bacteria become sensitive to antibiotics or to antibiotics at low concentrations (Stefanović 2018).

The synergy of antimicrobial drugs is predicated on the idea that the preparation can boost efficacy, decrease toxicity, decrease undesirable side effects, increase bioavailability, decrease the required dose, and limit the emergence of antimicrobial resistance. (Cottarel and Wierzbowski 2007). In recent years, there has been a surge in interest in developing new antibacterial combinations that incorporate natural product classes as key ingredients (Van Vuuren and Viljoen 2011). Cinnamon essential oil works synergistically with amikacin, gentamicin, imipenem, and meropenem against *Acinetobacter baumannii* (Guerra *et al*. 2012). It showed promising results in combination with colistin, a drug used to treat bacterial infections (Utchariyakiat *et al*. 2016). *C. burmannii* essential oil acts synergistically with gentamicin against *S. epidermidis* (Nuryastuti *et al*. 2009) and *C. verum* essential oil showed the possibility of reversing the carrier of the TEM-1 beta-lactamase gene of *E. coli* resistance to piperacillin. The scanning electron micrographs confirmed that the treated bacterial cell surface structure was significantly different from untreated cells.

The lysis of the bacterial cell wall followed by a loss of dense intracellular substance, causing a corrugated surface. Treatment with piperacillin resulted in considerable

damage to the bacterial cell wall, resulting in alterations in the bacterial cell wall size and shape (Yap *et al*. 2015). The synergy of cinnamic acid with vancomycin, ampicillin, amikacin, erythromycin, oxyprofloxacin, it gave high activity against *E. coli* and *S. aureus,* and also the combination of cinnamic acid with ciprofloxacin was more effective against *P. aeruginosa* than if the antibiotics alone (Hemaiswarya and Doble 2010).

Cinnamic acid, which is found in cinnamon, can enhance the action of several antibiotics against *M. avium*. It also has synergistic effects with numerous anti-tuberculosis medications against *Mycobacterium tuberculosis*, including isoniazid, rifampicine, amikacine and clofazimine. The outer cell wall glycopeptidolipid antigen of *M. avium*, which contains trans-cinnamic with phenylalanine, is the mechanism of this inhibitory effect. Because *M. tuberculosis* does not produce these antigens, it suggests that transcinnamic acid appears to target other locations as well (Vasconcelos *et al*. 2018). The study of patterns in antimicrobial efficacy can help find new approaches to address the ever-increasing burden of multidrug-resistant bacterial infections (Bolla *et al.* 2011). Cinnamon and its components have been studied extensively for their antibacterial properties, but very little research has been done on how they affect multi-resistant bacteria (Yap *et al*. 2015).

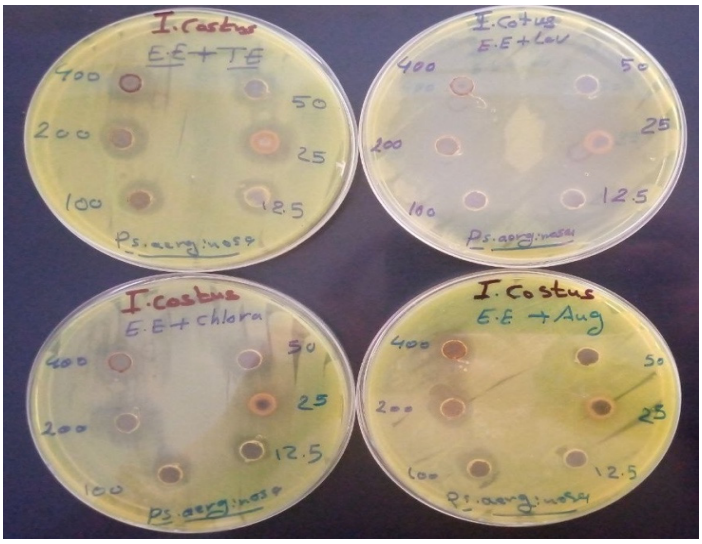

Figure 4.13 Synergistic effect between ethanolic costus extracts and antibiotics on *P. aergenosa*

Table 4.17 Synergistic effect between plant extracts and antibiotics on *S. aureus*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	30	29	27	26	25	24	22.1^{A}	25.1^{A}
		E	20	19	18	17	16	14		
	Coctus	W	30	27	28	27	26	25	28.2^{A}	
		E	35	30	29	28	27	26		
C	*Cinnamon*	W	15	14	13	12	12	10	14.3^{B}	14.1^{B}
		E	20	18	16	15	14	13		
	Coctus	W	16	15	15	14	13	12	13.9^{B}	
		E	16	15	14	13	13	11		
TE	*Cinnamon*	W	20	17	16	15	14	10	15.6^{B}	16.3^{B}
		E	21	18	15	15	14	12		
	Coctus	W	20	18	16	15	14	13	16.9^{B}	
		E	20	19	18	17	17	16		
AMC	*Cinnamon*	W	22	18	16	15	14	13	16.9^{B}	15.5^{B}
		E	21	20	18	17	15	14		
	Coctus	W	17	16	14	13	12	10	14.1^{B}	
		E	17	16	15	14	13	12		
Mean of conc.			21.3^{a}	19.3^{a}	18.0^{ab}	17.1^{ab}	16.2^{b}	14.7^{b}		

(W: water exract; E: Ethanolic extract)

Table 4.18 Synergistic effect between plant extracts and antibiotics on *S. pyogenes*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	30	28	25	20	15	12	20.6^{A}	25.1^{A}
		E	27	25	23	18	14	10		
	Coctus	W	26	25	22	17	16	13	21.4^{A}	
		E	29	27	25	22	20	15		
C	*Cinnamon*	W	22	20	18	15	12	8	14.9^{B}	$14.^{B}$
		E	19	17	14	12	12	10		
	Coctus	W	18	15	14	13	13	11	14.5^{B}	
		E	20	18	16	14	12	10		
TE	*Cinnamon*	W	25	23	22	20	18	15	17.6^{AB}	16.3^{B}
		E	20	17	15	14	12	12		
	Coctus	W	19	16	14	13	11	8	14.9^{B}	
		E	23	20	18	15	12	10		
AMC	*Cinnamon*	W	22	19	17	14	13	11	14.2^{B}	15.5^{B}
		E	18	16	14	14	12	0		
	Coctus	W	10	8	0	0	0	0	8.3^{C}	
		E	18	16	14	12	12	9		
Mean of conc.			21.6^{a}	19.4^{ab}	16.4^{b}	14.6^{bc}	12.8^{cd}	9.6^{d}		

(W: water exract; E: Ethanolic extract)

Table 4.19 Synergistic effect between plant extracts and antibiotics on *P. aereuginosa*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	28	26	25	22	20	15	22.8^{A}	22.7^{A}
		E	30	28	24	23	19	13		
	Coctus	W	26	24	22	18	16	12	22.7^{A}	
		E	32	30	28	24	22	18		
C	*Cinnamon*	W	25	22	20	18	15	12	17.6^{A}	17.8^{B}
		E	23	20	18	16	12	10		
	Coctus	W	22	19	14	12	10	0	17.9^{A}	
		E	28	26	24	22	20	18		
TE	*Cinnamon*	W	20	18	16	14	12	10	12.6^{B}	12.0^{C}
		E	16	14	12	12	11	8		
	Coctus	W	12	10	0	0	0	0	11.5^{B}	
		E	25	23	20	18	16	14		
AMC	*Cinnamon*	W	30	27	25	22	19	15	19.0^{A}	13.7^{C}
		E	20	18	16	14	12	10		
	Coctus	W	18	14	10	8	0	0	8.3^{B}	
		E	16	13	11	10	0	0		
Mean of conc.			23.2^{a}	20.8^{ab}	17.8^{bc}	15.8^{cd}	12.8^{de}	9.7^{e}		

(W: water exract; E: Ethanolic extract)

Table 4.20 Synergistic effect between plant extracts and antibiotics on *S. viridanse*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	25	22	19	16	14	10	20.3^{A}	19.5^{A}
		E	30	27	26	23	20	15		
	Coctus	W	22	20	17	16	14	11	18.7^{AB}	
		E	26	24	22	20	18	14		
C	*Cinnamon*	W	20	16	12	10	0	0	12.9^{C}	13.3^{B}
		E	23	20	18	15	11	10		
	Coctus	W	20	16	15	12	10	0	13.8^{C}	
		E	22	18	16	14	12	10		
TE	*Cinnamon*	W	20	17	14	12	10	8	17.2^{AB}	15.2^{B}
		E	25	23	22	22	18	15		
	Coctus	W	12	10	0	0	0	0	13.3^{C}	
		E	28	26	25	22	21	15		
AMC	*Cinnamon*	W	20	18	16	13	10	8	18.8^{AB}	16.9^{AB}
		E	30	27	25	22	20	16		
	Coctus	W	19	17	15	12	11	10	15.1^{BC}	
		E	22	20	18	15	12	10		
Mean of conc.			22.8^{a}	20.1^{ab}	17.5^{bc}	15.3^{cd}	12.6^{de}	9.5^{e}		

(W: water exract; E: Ethanolic extract)

Table 4.21 Synergistic effect between plant extracts and antibiotics on *Bacillus*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	21	18	15	13	12	10	16.6^{AB}	16.7^{A}
		E	25	22	20	17	14	12		
	Coctus	W	22	19	18	16	14	10	16.8^{AB}	
		E	26	22	18	14	12	10		
C	*Cinnamon*	W	18	15	12	10	0	0	13.0^{BC}	11.8^{B}
		E	22	20	18	17	14	10		
	Coctus	W	18	15	13	11	10	0	9.8^{C}	
		E	17	13	11	10	0	0		
TE	*Cinnamon*	W	23	22	19	17	15	12	19.3^{A}	17.2^{A}
		E	25	24	22	20	18	15		
	Coctus	W	21	17	15	12	10	0	15.1^{AB}	
		E	23	20	19	17	15	12		
AMC	*Cinnamon*	W	20	17	15	13	10	8	17.4^{AB}	15.6^{AB}
		E	27	25	23	20	17	14		
	Coctus	W	18	16	14	12	10	0	13.8^{BC}	
		E	22	19	17	15	13	10		
Mean of conc.			21.8^{a}	19.0^{ab}	16.8^{b}	14.6^{bc}	11.5^{cd}	7.7^{d}		

(W: water exract; E: Ethanolic extract)

Table 4.22 Synergistic effect between plant extracts and antibiotics on *K. pneumonia*

Antibiotics (3.12 mg)	Type of extract		Conentration (mg / ml)						Mean of extract type	Mean of antibiotics
			400	200	100	50	25	12.5		
LEV	*Cinnamon*	W	43	40	35	32	28	25	36.2^{A}	35.6^{A}
		E	45	43	40	38	35	30		
	Coctus	W	41	37	34	32	28	26	35.0^{A}	
		E	45	42	38	36	33	28		
C	*Cinnamon*	W	22	20	18	15	12	10	17.0^{CD}	16.5^{C}
		E	23	21	19	17	15	12		
	Coctus	W	20	18	16	14	10	0	16.0^{CD}	
		E	25	23	20	18	15	13		
TE	*Cinnamon*	W	20	18	16	14	12	10	15.7^{CD}	14.7^{C}
		E	22	20	17	15	13	11		
	Coctus	W	18	17	15	12	10	0	13.8^{D}	
		E	21	19	17	15	12	9		
AMC	*Cinnamon*	W	30	28	25	23	22	20	25.8^{B}	22.8^{B}
		E	32	30	28	26	24	22		
	Coctus	W	25	23	21	17	15	11	19.8^{C}	
		E	28	26	24	22	15	10		
Mean of conc.			28.8^{a}	26.6^{ab}	23.9^{bc}	21.6^{c}	18.7^{cd}	14.8^{d}		

(W: water exract; E: Ethanolic extract)

5. CONCLUSIONS AND RECOMMENDATION

Our results showed that there is significant differences of infection between genders and the highest percentage of infection detected at age of 5-6 years. Among the observed pathogens of tonsillitis infections, *S. aureus* was found the most common. Most of bacteria isolates were highly sensitive to Amikacin, Ciprofloxacin, Gentamycin, Meropenem, Tetracycline and Levofloxacin. The cinnamon plant had the strongest effect on inhibiting bacterial growth compared to the costus plant. The synergy between plant extracts and antibiotics showed a high inhibitory activity at the lowest concentration of the antibiotic compared to if it was alone. Finally, the current study recommends the use of medicinal plant extracts or their effective compounds in the treatment of various diseases, including tonsillitis and pharyngitis, after clinically proven.

The current study was conducted in Iraq and need to further investigation on the effect of plant extracts. Therefore, it is important ot investigate the side effects of the inhibitory activity of plant extracts of cinnamon and costus. Related to these issue, studies on the effect of extracts and active ingredients of cinnamon and costus should be expanded. Furthermore, in order to determine the mechanism of action of these compounds and their effects on differnt part of bacteria cells, studies on the chaperon effects of plant extracts and active ingredients examined at the genetic level are required.

REFERENCES

Abboud, L. S. 2017. Identification of some virulence factors of Proteus mirabilis isolated from persons with otitis media, Tikrit Journal of Pure Sciences, 8 (22).

Abdullah, F.E., Khatri, P.K., Alzadjali, N.A., Ali, A.D. and Bhagia, G., 2011. Ear infections in Karachi: The frequency and antibiotic resistance of bacterial isolates.

Abdulrasheed, M., Ibrahim, I. H., Luka, A., Maryam, A. A., Hafsat, L., Ibrahim, S., ... and Gidado, M. B. 2019. Antibacterial effect of Cinnamon (Cinnamomum zeylanicum) barks extract on different bacterial isolates. Journal of Environmental Microbiology and Toxicology, 7(1), 16-20.

Ács, K., Balázs, V. L., Kocsis, B., Bencsik, T., Böszörményi, A., and Horváth, G. 2018. Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. BMC complementary and alternative medicine, 18(1), 1-9.

Agrawal, R., Khatri, P., Parihar, R. and Shah, H., 2017. Microbial assessment of chronic suppurative otitis media in a tertiary care center of Rajasthan. Int J Health Sci Res, 7(2), pp.120-126.

Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S., 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of advanced research, 7(1), pp.17- 28.

Alasmari, N. S. H., Bamashmous, R. O. M., Alshuwaykan, R. M. A., Alahmari, M. A. M., Alshahrani, A. A. M., Alqarni, S. A., ... and Alamri, S. O. R. 2017. Causes and treatment of tonsillitis. The Egyptian Journal of Hospital Medicine, 69 (8), 2975-2980.

Al-Fartusie F. S. 2019.Caracterizations of LavenderFlowers Extracts and Study of their effects on peroxidase activity and some microorganisms.Journal of Global pharma technology, vol.11pp.181-191.

Alfred, E. B. 2005. Microbiological application in the laboratory manual in general microbiology. 9th ed. MC Grow Hill companies.

Al-Tameemi, K. A. K. H., Hraishawi, R. M., and Aaiz, F. L. 2020. Isolation, Identification and Evaluation of Resistance of Bacterial Isolates from Patients with Tonsillitis. Annals of Tropical Medicine and Public Health, 23, 23-1017.

Angshumanjana, Anirbanjana, Dipjitdey, Jayantabikashdey, A., and Brookheart RT, Lewis WG, Peipert JF, et al. 2019. Association between obesity and bacterial vaginosis as assessed by Nugent score. Am J Obstet Gynecol; 220:476.e1-11.

Bartlett, A., Bola, S., and Williams, R. 2015. Acute tonsillitis and its complications: an overview. Journal of the Royal Naval Medical Service, 101(1).

Berger A, Meinel DM, Schaffer A, Ziegler R, Pitteroff J, Konrad R, Sing A. 2016. A case of pharyngeal diphtheria in Germany, June 2015. Infection. Oct;44(5):673-5.

Bisno AL. 1995.Streptococcus pyogenes. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. 4th ed. New York: Churchill Livingstone;1786-1810.

Bisno, A. L., Gerber, M. A., Gwaltney Jr, J. M., Kaplan, E. L., and Schwartz, R. H. 2002. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. Clinical infectious diseases, 113-125.

Bochner RE, Gangar M, Belamarich PF 2017. "A Clinical Approach to Tonsillitis, Tonsillar Hypertrophy, and Peritonsillar and Retropharyngeal Abscesses". Pediatr Rev (Review). 38 (2): 82.

Bolla, J. M., Alibert-Franco, S., Handzlik, J., Chevalier, J., Mahamoud, A., Boyer, G., ... and Pagès, J. M. 2011. Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. FEBS letters, 585(11), 1682-1690.

Brown, A. E. and Smith, H. R. 2017. Benson's Microbiological Applications Laboratory Manual in General Microbiology 14th Ed. McGraw-Hill .U S A.

Cappuccino, J. G. and Welsh, C. 2018.Microbiology A la boratory manual. 11th ed. Pearson Education Limited Edinburgh Gate Harlow Essex CM20 2JE. England.

Cavalcanti, V. P., Camargo, L. A. D., Moura, F. S., Melo Fernandes, E. J. D., Lamaro-Cardoso, J., Braga, C. A. D. S. B., and André, M. C. P. 2019. Staphylococcus aureus in tonsils of patients with recurrent tonsillitis: prevalence, susceptibility profile, and genotypic characterization. Brazilian Journal of Infectious Diseases, 23, 8-14.

CLSI , 2020. Epidemiology cutoff values for antifungal susceptibility test . 3nd ed . CLSI supplement M59 . Wayne, PA: Clinical and Laboratory Standards Institute.

Cohen, J. F., Bertille, N., Cohen, R., and Chalumeau, M. 2016. Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database of Systematic Reviews, (7).

Cottarel, G., and Wierzbowski, J. 2007. Combination drugs, an emerging option for antibacterial therapy. Trends in biotechnology, 25(12), 547-555.

De Mand Anari S .2018. "Infections and foreign bodies in ENT". Surgery (Oxf) (Review). 36 (10): 555–556.

El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., ... and Abdellaoui, A. 2019. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. Journal of Advanced Pharmaceutical Technology and Research, 10(2), 63.

Ezzedini R, Darabi M, Ghasemi B, Darabi M, Fayezi S, Moghaddam YJ, et al. 2013. "Tissue fatty acid composition in obstructive sleep apnea and recurrent tonsillitis". Int J Pediatr Otorhinolaryngol. 77 (6): 1008–12.

Forbes , A. B., Sahm, D.F. and Weissfeld, A.S. 2007. Bailey and Scott's Diagnostic microbiology. 12th ed. Mobsy. U.S.A, 1: 266.

Gago G, Diacovich L, Arabolaza A, Tsai SC, Gramajo H. 2011. Fatty acid biosynthesis in actinomycetes. FEMS Microbiol Rev; 35(3):475-97.

Georgalas CC. 2009. Tolley NS, Narula A. Tonsillitis. BMJ Clin Evid. Oct 26;2009.

Gerber, M. A., and Shulman, S. T. 2004. Rapid diagnosis of pharyngitis caused by group A streptococci. Clinical microbiology reviews, 17(3), 571-580.

Gottlieb M, Long B, Koyfman A. 2018. Clinical Mimics: An Emergency Medicine-Focused Review of Streptococcal Pharyngitis Mimics. J Emerg Med. May;54(5):619-.

Green, L. H. 2015. Culturing and Preserving Microorganisms,p. 3944.In: E. Goldman and L. H. Green(ed.), Practical Handbook of Microbiology,3rd ed. CRC Press, New York.

Guerra, F. Q. S., Mendes, J. M., Sousa, J. P. D., Morais-Braga, M. F., Santos, B. H. C., Melo Coutinho, H. D., and Lima, E. D. O. 2012. Increasing antibiotic activity against a multidrug-resistant Acinetobacter spp by essential oils of Citrus limon and Cinnamomum zeylanicum. Natural product research, 26(23), 2235-2238.

Hemaiswarya, S., and Doble, M. 2010. Synergistic interaction of phenylpropanoids with antibiotics against bacteria. Journal of Medical Microbiology, 59(12), 1469-1476.

Jadia S, Chauhan AN, Hazari RS, Maurya AK, Biswas .2010. An unusual cause of recurrent tonsillitis. BMJ Case Rep. Apr 20;2010:2561.

Khadilkar MN, Ankle NR. 2016. Anaerobic bacteriological microbiota in surface and core of tonsils in chronic tonsillitis. J Clin Diagn Res. 10:MC01–3. 15.

Khan M.F and Ramu A . 2018. Spectral characterization of bioactive compounds from Costus pictus and Costus speciosus International Journal of ChemTech Research J. ChemTech. 11(9) 353-363.

Klug TE, Rusan M, Fuursted K, Ovesen T. 2016. Peritonsillar Abscess: Complication of Acute Tonsillitis or Weber's Glands Infection? Otolaryngol Head Neck Surg. Aug;155(2):199-207.

Kuniyal, C. P., Bisht, D. S., Negi, B. S., Singh, S. K., Kaim, J. C., and Sundriyal, R. C. 2021. Production of Picrorhiza kurrooa and Aucklandia costus in Chamoli, Uttarakhand, India. National Academy Science Letters, 44(5), 471-474.

Kurtz B, Kurtz M, Roe M, et al. 2000. Importance of inoculum size and sampling effect in rapid antigen detection for diagnosis of Streptococcus pyogenes pharyngitis. J Clin Microbiol,38:279.

Lamaro-Cardoso J, de Lencastre H, Kipnis A, et al.2009. Molecular epidemiology and risk factors for nasal carriage of Staphylococcus aureus and methicillin-resistant S. aureus in infants attending day care centers in Brazil. J Clin Microbiol. 47:3991–7.

Laxminarayan, R. (2003). ACT Now or Later: The Economics of Malaria Resistance (No. 1318-2016-103262).

MacFaddin, J. F. 2000. Biochemical tests for identification of medical bacteria, williams and wilkins. Philadelphia, PA, 113.

Macnamara M. 2008. Acute and chronic pharyngeal infection In: Gleezon M ed. Scott-Brown’s Otorhinolaryngology, Head and Neck Surgery. London: Edward Arnold:1981-90.

Mahdi, A. G., and Mahdi, R. K. 2017. Isolation and identification of aerobic bacteria that cause tonsillitis from patients of Al-Diwaniyah governorate. Al-Qadisiyah Journal of Pure Science, 22(4), 320-331.

Mahon , C.R., Lehman, D.C. and Manuselis, G. 2011. Textbook of diagnostic microbiology 4th ed., W.B. Saunders Company, China.

Majeed, M. A. A., Ahmad, H. S., Saleh, A., and Hasan, F. K. 2019. Effect of Costus Specious Extract on Some Types of Pathogenic Bacteria. Journal of Global pharma Technology, 11, 481-485.

Manthiram, K., Correa, H., Boyd, K., Roland, J., and Edwards, K. 2018. Unique histologic features of tonsils from patients with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. Clinical rheumatology, 37(5), 1309-1317.

Meades, G., Henken, R. L., Waldrop, G. L., Rahman, M. M., Gilman, S. D., Kamatou, G. P., ... and Gibbons, S. 2010. Constituents of cinnamon inhibit bacterial acetyl CoA carboxylase. Planta medica, 76 (14), 1570-1575.

Meshaal, A. K., Hetta, H. F., Yahia, R., Abualnaja, K. M., Mansour, A. T., Al-Kadmy, I., ... and El-Kazzaz, W. 2021. In Vitro Antimicrobial Activity of Medicinal Plant Extracts against Some Bacterial Pathogens Isolated from Raw and Processed Meat. Life, 11(11), 1178.

Mishra, N., and Srivastava, R. 2022. Therapeutic and Pharmaceutical Potential of Cinnamon. In Research Anthology on Recent Advancements in Ethnopharmacology and Nutraceuticals (pp. 698-710). IGI Global.

Morello, J.K., Mizer, H.E. and Granato, P.A. 2006. Laboratory manual and work book in Microbiology Application to Patient care. 8th ed. McGraw Hill.

Munita, J.M. and Arias, C.A., 2016. Mechanisms of antibiotic resistance. Microbiology spectrum, 4(2).

Nematollahi, Z., Ebrahimi, M., Raeisi, M., Dadban Shahamat, Y., Ghodsi Moghadam, M., Hashemi, M., ... and Shirzad, H. 2020. The Antibacterial Activity of Cinnamon Essential Oil against Foodborne Bacteria: A Mini-Review. Journal of Human Environment and Health Promotion, 6(3), 101-105.

Nuryastuti, T., van der Mei, H. C., Busscher, H. J., Iravati, S., Aman, A. T., and Krom, B. P. 2009. Effect of cinnamon oil on icaA expression and biofilm formation by

Staphylococcus epidermidis. Applied and environmental microbiology, 75(21), 6850-6855.

Obeid, A. H., and Abbas, D. A. H. 2015. Comparative study of antidiarrheal effect of proposed new formulae from D. innoxia hydroalcoholic leave extract against E. coli O: 157 induced diarrhea in goats. Kufa Journal for Veterinary Medical Sciences, 6 (1).

Oulkheir, S., Aghrouch, M., El Mourabit, F., Dalha, F., Graich, H., Amouch, F., ... andChadli, S. 2017. Antibacterial activity of essential oils extracts from cinnamon, thyme, clove and geranium against a gram negative and gram positive pathogenic bacteria. Journal of diseases and medicinal plants, 3(2-1), 1-5.

Pandey, M. M., Rastogi, S., and Rawat, A. K. S. 2007. Saussurea costus: botanical, chemical and pharmacological review of an ayurvedic medicinal plant. Journal of ethnopharmacology, 110 (3), 379-390.

Passat, D. N. 2012. Interactions of black and green tea water extracts with antibiotics activity in local urinary isolated Escherichia coli. Al-Nahrain Journal of Science, 15(3), 134-142.

Pelucchi C, Grigoryan L, Galeone C, Esposito S, Huovinen P, Little P, Verheij T. 2012. Guideline for the management of acute sore throat. ESCMID Sore Throat Guideline Group. Clin Microbiol Infect. Apr;18 Suppl 1:1-28.

Pitetti, R. D., MD., MPH. 2001. Spectrum of Group A Beta-Hemolytic Infections in Children. Pediatric emergency medicine reports.

Rao, P. V., and Gan, S. H. 2014. Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine, 2014.

SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N. C. USA.

Schöfer, H. 2012. Sexually transmitted infections of the oral cavity. Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und Verwandte Gebiete, 63(9), 710-715.

Schroeder, B. M. 2003. Diagnosis and management of group A streptococcal pharyngitis. American family physician, 67(4), 880.

Shulman, S. T., Bisno, A. L., Clegg, H. W., Gerber, M. A., Kaplan, E. L., Lee, G., ... and Van Beneden, C. 2012. Clinical practice guideline for the diagnosis and

management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clinical infectious diseases, 55 (10), e86-e102.

Sidell D, Shapiro NL. 2012. Acute tonsillitis. Infect Disord Drug Targets. Aug;12(4):271-6.

Singh, G., Maurya, S., deLampasona, M. P., and Catalan, C. A. N. 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology, 45(9), 1650–1661.

Singh, J., Singh, R., Parasuraman, S., and Kathiresan, S. 2020. Antimicrobial Activity of Extracts of Bark of Cinnamomum cassia and Cinnamomum zeylanicum. International Journal of Pharmaceutical Investigation, 10(2), 141-145.

Spinks A, Glasziou PP, Del Mar CB .2013. "Antibiotics for sore throat". Cochrane Database Syst Rev (Review) (11): CD000023.

Sree Satya Nandam, S. P. D., & Vangalapati, M. 2012). Optimization of physico-chemical parameters for the extraction of phenolic components from cinnamon species. Journal of Academia and Industrial Research, 1(4), 183-185.

Stefanović, O. D. 2018. Synergistic activity of antibiotics and bioactive plant extracts: A study against Gram-positive and Gram-negative bacteria. Bacterial Pathogenesis and Antibacterial Control, 23, 23-48.

Stelter K,.2014. "Tonsillitis and sore throat in children". GMS Curr Top Otorhinolaryngol Head Neck Surg (Review). 13: 3.

Stuck BA, Windfuhr JP, Genzwurker H, Schroten H, Tenenbaum T, Gotte K. 2008.Tonsillectomy in children. Dtsch Arztebl Int. 105:852–61.

Szczepanowski R, Linke, B, Krahn I, Gartemann KH, Gutzkow T, Eichler W, Puhler A, Schluter A. 2009. Detection of 140 clinically relevant antibioticresistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology-Sgm 155, 2306e2319.

Tekwu, E. M., Pieme, A. C., & Beng, V. P. 2012. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. Journal of ethnopharmacology, 142(1), 265-273.

Utchariyakiat, I., Surassmo, S., Jaturanpinyo, M., Khuntayaporn, P., and Chomnawang, M. T. 2016. Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug

resistant Pseudomonas aeruginosa and the synergistic effects in combination with other antimicrobial agents. BMC complementary and alternative medicine, 16 (1), 1-7.

Vaidya, M., and Shingadia, H. 2020. Antimicrobial activity of Costus speciosus (J. Koieng) Sm. World J Pharm Res, 9(2), 959-963.

Van Vuuren, S., and Viljoen, A. 2011. Plant-based antimicrobial studies–methods and approaches to study the interaction between natural products. Planta medica, 77(11), 1168-1182.

Vasconcelos, N. G., Croda, J., and Simionatto, S. 2018. Antibacterial mechanisms of cinnamon and its constituents: A review. Microbial pathogenesis, 120, 198-203.

Vijayashree MS, Viswanatha B, Sambamurthy BN. 2014. Clinical and bacteriological study of acute tonsillitis.IOSR J Dental Med Sc. 1(2):37-43.

Walijee, H., Patel, C., Brahmabhatt, P., and Krishnan, M. 2017. Tonsillitis. InnovAiT, 10(10), 577-584.

Wang Q, Du J, Jie C, Ouyang H, Luo R, Li W. 2017. Bacteriology and antibiotic sensitivity of tonsillar diseases in Chinese children. Eur Arch Otorhinolaryngol. Aug;274(8):3153-3159.

Windfuhr JP, Toepfner N, Steffen G, Waldfahrer F, Berner R. 2016. "Clinical practice guideline: tonsillitis I. Diagnostics and nonsurgical management". Eur Arch Otorhinolaryngol (Practice guideline). 273 (4): 973–87.

World Health Organization. 2006. The world health report 2006: working together for health. World Health Organization.

Yamamato, S., and Gaynor, C. 2002. Therapeutic potential of inhibitory of the NF. KB pathway in the treatment of inflammation and cancer. Journal of Clinical Investigation, 1, 493-503.

Yap, P. S. X., Krishnan, T., Chan, K. G., and Lim, S. H. E. 2015. Antibacterial mode of action of Cinnamomum verum bark essential oil, alone and in combination with piperacillin, against a multi-drug-resistant Escherichia coli strain. Journal of microbiology and biotechnology, 25(8), 1299-1306.

Zaid, S., and Ahmed, H. 2016. The relationship between severity of dental caries and chronic tonsillitis among Iraqi children. Fac Med Baghdad, 58, 149-52.

APPENDICES

APPENDIX 1. Ethics Committee

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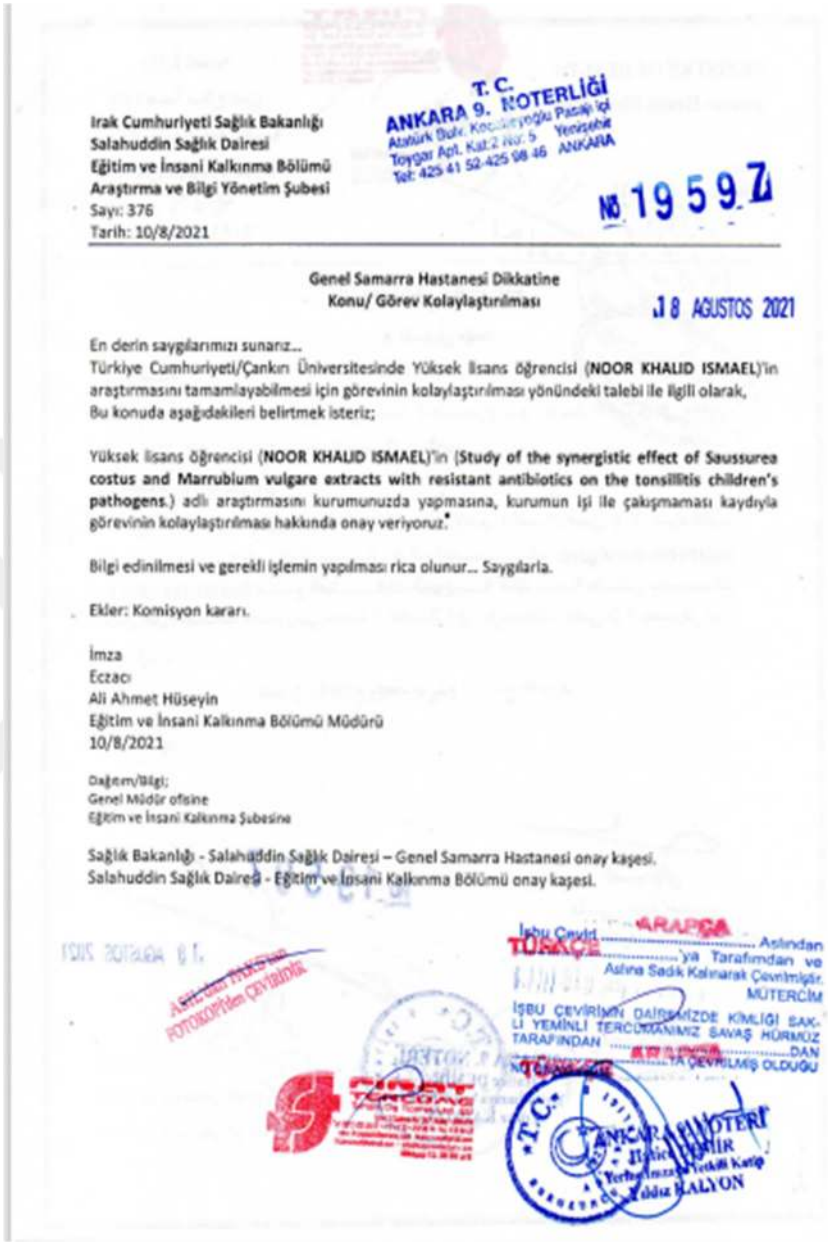

Irak Cumhuriyeti Sağlık Bakanlığı
Salahuddin Sağlık Dairesi
Eğitim ve İnsani Kalkınma Bölümü
Araştırma ve Bilgi Yönetim Şubesi
Sayı: 376
Tarih: 10/8/2021

T. C.
ANKARA 9. NOTERLİĞİ
Atatürk Bulv. Kocatepeoğlu Pasajı İçi
Toygar Apt. Kat:2 No: 5 Yenişehir
Tel: 425 41 52-425 98 46 ANKARA

NO 19597

Genel Samarra Hastanesi Dikkatine
Konu/ Görev Kolaylaştırılması

18 AGUSTOS 2021

En derin saygılarımızı sunarız...
Türkiye Cumhuriyeti/Çankırı Üniversitesinde Yüksek lisans öğrencisi (**NOOR KHALID ISMAEL**)'in araştırmasını tamamlayabilmesi için görevinin kolaylaştırılması yönündeki talebi ile ilgili olarak,
Bu konuda aşağıdakileri belirtmek isteriz;

Yüksek lisans öğrencisi (**NOOR KHALID ISMAEL**)'in (**Study of the synergistic effect of Saussurea costus and Marrubium vulgare extracts with resistant antibiotics on the tonsillitis children's pathogens.**) adlı araştırmasını kurumunuzda yapmasına, kurumun işi ile çakışmaması kaydıyla görevinin kolaylaştırılması hakkında onay veriyoruz.

Bilgi edinilmesi ve gerekli işlemin yapılması rica olunur... Saygılarla.

Ekler: Komisyon kararı.

İmza
Eczacı
Ali Ahmet Hüseyin
Eğitim ve İnsani Kalkınma Bölümü Müdürü
10/8/2021

Dağıtım/Bilgi:
Genel Müdür ofisine
Eğitim ve İnsani Kalkınma Şubesine

Sağlık Bakanlığı - Salahuddin Sağlık Dairesi – Genel Samarra Hastanesi onay kaşesi.
Salahuddin Sağlık Dairesi - Eğitim ve İnsani Kalkınma Bölümü onay kaşesi.

İşbu Çeviri ARAPÇA
TÜRKÇE ..... Aslından
..... 'ya Tarafımdan ve
Aslına Sadık Kalınarak Çevrilmiştir.
MÜTERCİM
İŞBU ÇEVİRİNİN DAİREMİZDE KİMLİĞİ SAK-
Lİ YEMİNLİ TERCÜMANIMIZ SAVAŞ HÜRMÜZ
TARAFINDAN .....DAN
.....A ÇEVRİLMİŞ OLDUĞU

T.C. ANKARA 9. NOTERİ
Yetkili Kaşe
Yıldız KALYON

CURRICULUM VITAE

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Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Biology	2022-Present
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Work Experience

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