NEAR EAST UNIVERSITY
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DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

Gas chromatography-mass Analysis of Most Active Plant Extracts Compounds against Bacteria Isolated from Surgical Wound Sites in Erbil City

MSc. THESIS

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APPROVAL

We certify that we have read the thesis submitted by Diyari Abubaker titled" **Gas chromatography-mass Analysis of Most Active Plant Extracts Compounds against Bacteria Isolated from Surgical Wound Sites in Erbil City"**

"And that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Diyari Abubaker

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ÖZET

Erbil Şehrindeki Cerrahi Yara Bölgelerinden İzole Edilen Bakterilere Karşı En Aktif Bitki Ekstrakt Bileşiklerinin Gaz Kromatografisi-Kütle Analizi

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Ameliyat sonrası veya cerrahi yara enfeksiyonu, özellikle hastanede yatan hastalar arasında üçüncü en sık bildirilen nozokomiyal enfeksiyon ve herhangi bir ameliyat geçiren hastalar için en sık görülen komplikasyonlardan biri olarak kabul edilir. Öte yandan, bu tür enfeksiyonları tedavi etmek için alternatif bir antimikrobiyal ajan olarak doğal ürünlere olan ilgi, son birkaç yılda, özellikle birden fazla antibiyotik sınıfına karşı bakteriyel direncin ortaya çıkmasıyla birlikte artmıştır. Çalışmanın amacı, Irak Kürdistan bölgesi Erbil ilçesinde bulunan bir sevk hastanesinde mikroorganizmaların prevalansını değerlendirmek ve doğal ve sentetik ürünlerin cerrahi alan enfeksiyonlarından (CAE) izole edilen mikroorganizmalara karşı antibiyotik aktivitesini test etmektir. Örnekler, Haziran 2022'den Ekim 2022'ye kadar cerrahi yara enfeksiyonu belirtileri ile Rzgari hastanesine başvuran 50 hastadan rastgele toplandı. Salgılanan irin veya eksüdadan örnekler, aseptik ortamlarda iki steril sürüntü ile her hastanın cerrahi yarasından alındı, sürüntülerden biri smear hazırlamada, diğeri kültür amaçlı kullanıldı. Tüm test ve analiz işlemleri Cihan Üniversitesi Biyoloji Bölümü'nde gerçekleştirildi. Sonuç: 50 şüpheli enfeksiyon vakasından toplam 29 irin numunesi (%58) pozitif olarak belirlenirken, 21 numunede (%42) herhangi bir bakteri üremesi belirtisi görülmedi. İzole edilen 29 bakterinin tamamı gram pozitif bakterilerdi ve 28 örnek (%96,5) staphylococcus spp. ve sadece 1 örnek (%3,45) lactobacillus olarak tanımlandı. İzole edilen mikroorganizmanın test edilen çeşitli antibiyotiklere karşı dirençli olduğu ve ayrıca test edilen bitki özlerine rastgele duyarlılık gösterdiği bulundu.

Çalışmada daha fazla kadın olmasına rağmen (%52) erkeklere (%48) kıyasla. Ancak enfeksiyonun olumlu sonuçları erkeklerde (%52) kadınlara göre (%48) daha fazla görüldü. Tarçın, zencefil ve turp özütü CG-MS kullanılarak tanımlandı ve 3-Hidroksibenzoik asit, benzoik asit, gallik asit, siringik asit, izovanillik asit, protokateküik asit, kateşin, kafein, epikateşin ve kersetin için pozitif bir sonuç etiketlendi. Çalışmada test edilen bitki ekstraktı yüksek mikrobiyolojik aktiviteler göstermiştir. Ek olarak, bu çalışmadan elde edilen bilgiler, hastanedeki epidemiyolojik ve terapötik etkileri olabilecek cerrahi alan enfeksiyonlarının (CAE) mikrobiyal etiyolojisinin daha iyi anlaşılmasına olanak tanıdı. Bulaşıcı hastalıklar söz konusu olduğunda, çeşitli antimikrobiyal maddelere karşı bakterinin duyarlılığına ve direncine dikkat edilmesi önerilir. Duyarlılık modelinin, bitki ekstraktının antimikrobiyal aktivitesini belirlemek için tek kriter olamayacağını vurgulamak önemlidir, çünkü prosedür in vitro yapıldı ve hastanın immünolojik durumu ve klinik durumu hesaba katılmadı. Bu çalışma, çalışılan türlere karşı aktiviteden sorumlu bileşenlerin daha fazla izolasyonu için bitki ekstraktının seçiminde temel bilgiler sağlayabilir.

Anahtar Kelimeler: cerrahi alan enfeksiyonları, *Staphylococcus aureus*, *Staphylococcus epidermidis*, antimikrobiyal direnç, GC-MS, bitki ekstraktı.

ABSTRACT

Gas chromatography-mass Analysis of Most Active Plant Extracts Compounds against Bacteria Isolated from Surgical Wound Sites in Erbil City

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Background and objectives: Postoperative or surgical wound infection specifically is considered the third-most frequently reported nosocomial infection among hospitalized patients and one of the most frequent complication for patients undergoing any type of operations. On the other hand, interest in natural product as an alternative antimicrobial agents to treat such infection, has grown over the past several years, especially with the appearance of bacterial resistance against multiple class of antibiotics. The objective of the study was to evaluate the prevalence of microorganisms as well as to test the antibiotics activity of natural and synthetic products against microorganism isolated from surgical site infections (SSIs) in a referral hospital located in Erbil district Kurdistan region-Iraq.

Methods: Samples were collected randomly from 50 patients presenting to Rzgari hospital with signs of surgical wound infection from June 2022 till October 2022. Samples from the secreted pus or exudate were obtained from each patient's surgical wound by the mean of two sterile swabs under aseptic settings, where one of the swabs was used for smear preparation while the other was used for culture purposes. All testing and analysis procedures were carried out at the Department of Biology in Cihan University.

Result: out of the 50 cases of suspected infection, a total of 29 pus samples (58%) were identified as positive while 21 sample (42%) showed no sign of any bacterial growth. All of the 29 isolated bacteria were gram positive bacteria, with 28 sample (96.5%) recognized as staphylococcus spp. and only 1 sample (3.45%) identified as lactobacillus bacterium. The isolated microorganism were found to be resistant to a variety of tested antibiotics, while also showing random sensitivity to the tested plant extracts. Although more females were noted in the study (52%) compared to males (48%). Yet positive results of infection were more seen in males (52%) compared to females (48%). Extract of cinnamon, ginger, and radish were identified using CG-MS and a positive result for 3-Hydroxybenzoic acid, benzoic acid, gallic acid, syringic acid, isovanillic acid, protocatechuic acid, catechin, caffeine, epicatechin, and quercetin was labeled.

Conclusion: The plant extract tested in the study showed high microbiological activities. In addition, information obtained from this study allows for a better understanding of the microbial etiology of surgical site infections (SSIs) in the hospital which may have epidemiological and therapeutic implications. In case of infectious diseases, it is recommended to pay attention to the sensitivity and resistance pattern of the presenting bacteria to various antimicrobials agents. It's important to highlight that the sensitivity pattern cannot be the sole criteria to identify antimicrobial activity of the plant extract, because the procedure is held in vitro and it fails to take into account the immunological status and clinical condition of the patient. This work may provide essential information in the selection of plant extract for further isolation of constituents responsible for the activity against the studied species.

Keywords: surgical site infections, *Staphylococcus aureu*s, *Staphylococcus epidermidis,* antimicrobial resistance, GC-MS, plant extract.

LIST OF CONTENTS

List of tables

List of figures

List of diagrams

List of symbols

%	Percentage
C°	Degree Celsius
α	Alfa
β	Beta
δ	Delta
♂	Male
♀	Female
σ	Sigma factors
σ	Sigma
+	Psitive
-	Negative
=	Equal

List of abbreviation

µg/mL	Microgram per milliliter
µl	Microliter
µm	Micrometer
MTBG	4-Methylthio-3- butenyl-glucosinolate-4
HETE	5-hydroxyeicosatetraenoic acid
AGEs	Aminoglycoside modifying enzymes
agr	Accessory gene regulator
CAT	Chloramphinecol acetyl transferases
CAUTI	Catheter-associated urinary tract infections
CLSI	Clinical and Laboratory Standards Institute
cm	Centimeter
CNS	Central nervous system
CO2	Carbon dioxide
CoNS	Coagulase-negative *Staphylococci*
CPS	Central pain syndrome
DHPS	Dihydropterooate synthase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
e.g.	For example
ECM	Engine control module
EMC	Electromagnetic compatibility
EPS	Extracellular Polymeric Substances
ESBLs	Extended-spectrum beta-lactamases
et al	And others

Fig	Figure
g	Gram
GC	Gas Chromatography
GSK	Glycogen synthase kinase
h	Hour
H2O2	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
ICU	Intensive care unit
IMDs	Implanted medical devices
kDa	kiloDalton
LC	Liquid Chromatography
LO	Lipoxygenase
m	Meter
MAE	Microwave assisted
Mb	Megabyte
mcg	Microgram
MDR	Multidrug resistance
mg	Milligram
mg/ml	Milligram per millimeter
MHA	Mueller Hinton agar
MICs	Minimum inhibitory concentrations
ml	Milliliter
mm	Millimeter
MRSA	Methicillin-resistant *Staphylococcus* aureus
MSCRAMMs	Microbial surface components recognizing adhesive matrix

NaCl	Sodium chloride
NCCLS	National Committee for Clinical Laboratory Standards
nm	Nanometer
NO	Number
O2	Oxygen
PBP	Penicillin Binding Protein
PDR	Pandrug Resistant or Pan-Resistant
PGF2	prostaglandins-F2
PKC	Protein Kinase C
PTP1B	Protein tyrosine phosphatase 1B
QS	Quorum-Sensing
R	Resistance
RAP	purified antifungal proteins
RNA	Ribonucleic acid
Rs	Raphanus sativus
Rs-AFP1	Raphanus sativus-alfa fetoprotein 1
Rs-AFP2	Raphanus sativus-alfa fetoprotein 2
s	Second
sar	Staphylococcus accessory regulator
sarA	*Staphylococcal* accessory regulator A
sarH1	*Staphylococcal* accessory regulator H1
sarT	*Staphylococcal* accessory regulator T
SFE	Supercritical fluid extraction
SMX/TMP	Trimethoprim/Sulfamethoxazole
SSI	*Surgical Site Infection*
SSP.	*Species*
SSSS	Staphylococcal scalded skin syndrome

T	Test strain
TLC	Technique high quality
TSS	Toxic shock syndrome
TSST-1	Toxic shock syndrome-1
TXB2	Thromboxane –B2
UAE	Ultrasound-assisted extraction
UV	Ultraviolet
VISA	Vancomycin insensitive S. aureus
WRE	White radish root extract
XDR	Extensive Drug Resistant
etc.	And other similar things
PTP1B	Protein tyrosine phosphatase 1B
Sig	Sigma
VRSA	vancomycin resistant *S. aureus*
Era.	Evoked response audiometry
C.Z	Condition zero
GC mass	Gas chromatography-mass

Chapter One

1. Introduction

1.1 Background

All medications used to treat illnesses are made from two primary sources. The first category includes synthetic medications, while the second one includes active compounds, known as natural products obtained from microorganisms or plant extracts (Mishra & Tiwari, 2011). On the other hand, since the beginning of time, people have used plants for many purposes, including food production, perfume and seasoning, equipment, medicine, and shield assembly. Many ailments have been attempted to be treated, especially with the extracts produced from medicinal plants. As a result, the field of medicine has emerged (Diken, 2009). Because they do not have a significant adverse effect and have multiple beneficial effects including antimicrobial effect, natural medications made from plants are frequently more appealing than synthetic treatments. Due to this, research on herbal medicines, which have had a long-standing impact on medicine, has grown to be quite interesting. Moreover, plants and their extracts have been used for generations to treat wounds and skin conditions (Mensah et al., 2006). Postoperative or surgical wound infection specifically is thought to be the third most typical nosocomial infection among hospitalized patients and one of the most frequent complications for patients undergoing any type of operations (Mangram et al., 1999). In addition, because of the financial burden and negative impact on the quality of life of the patient, the existence of a wound places a significant strain on health and social care providers (Welsh, 2018), where postoperative surgical wound site infections are linked to higher rates of morbidity and mortality, longer hospital stays, and higher healthcare spending (Weigelt et al., 2010). Although skin is frequently occupied by normal flora, such as coagulase-negative staphylococci class that includes Staphylococcus Aureus and Epidermidis, and usually presents limited virulence capacity, and particularly the skin of patients inside the hospital and who received antimicrobial therapy is not infrequently colonized by significant healthcare- connected infections, like Gram-negative non- glucose catalysing bacteria such as Pseudomonas aeruginosa, yeasts, these microorganisms alone are routinely not able to cause an infection (Wu et al., 2011; Grice & Segre, 2011). Skin constitutes a protective barrier and during minor or major episodes that compromise the integrity of the skin's surface like

incisions, colonizing germs are regularly established to the skin and the beneath tissue, a condition known as opportunistic infection. Additionally, external germs from sources including water, dirt, or contaminated surfaces can contaminate skin abrasions and wounds causing subsequent infection (Kanitakis, 2002), (Ki & Rotstein, 2008). Meanwhile, antibiotics have made a substantial positive difference worldwide, notably lowering the rate of sickness and fatality brought on by bacterial infections in humans (Rossolini et al., 2014). However, antibiotics have been abused and inappropriately used by the general public, leading to an increase in antibiotic resistance rates in a number of bacteria. Thus, Antibiotic resistance has been identified as a significant worldwide health problem in recent years. As a result, attempts have been generated to combat the threat of antibiotic endurance during also the investigation of different springs of antimicrobial compounds, like medicinal plants (Anand et al., 2019).

Cinnamaldehyde and trans-cinnamic acid, for instance, are active metabolites of cinnamon bark that has showed some antibacterial events against harmful Gram-positive and Gram-negative microorganisms by causing cell membrane damage. Therefore, constituting an alternative strategy to treat bacterial illnesses that are resistant to many different types of treatment (Vasconcelos et al., 2018). Cinnamon extract compounds also exert tyrosine phosphatase 1B (PTP1B) inhibitory activity. They lower the blood glucose level by promoting the ß cells function to produces insulin, normalizing its concentration (Allen et al., 2013).

Radish (Raphanus sativus L.) on the other hand, has a great low-cost and commercial value in many regions of the world where different parts of the radish like the roots, seeds and leaves are implemented in medicinal devotions following active compounds extraction (Nadkarni, 1976). The radish is also a natural source highly rich in iron, calcium and sodium among commonly consumed vegetables (Kaymak et al., 2010). Raphanin, a watery extracts of the radish seeds that is active in blocking the germination of seeds from various plants has been demonstrated to be an effective antibacterial principle (Ivánovics & Horváth, 1947).

Lastly, ginger (zingiber officinale), a lasting plant in the family Zingiberaceae, has thick tuberous rhizomes that are aromatic, yellowish in nature and exert many medicinal properties. In the world of spices and flavorings, ginger is one of the most often employed and well-liked ingredients. Additionally, in Ayurvedic, Tibb-Unani, Chinese, and Islamic herbal treatments, it has been utilized as a therapy for ages where ginger was preferred as a way to irradiate a variety of

conditions, like colds, arthritis, nausea, hypertension, constipation, migraines, indigestion and many others (Bode & Dong, 2011). Despite the fact that antimicrobial activity of ginger was detected in the essential oils, the abiotic stresses, which include environmental stress factors like light, moisture, temperature, soil nutrients, and ozone, as well as living organisms stress factors like plant eaters, insects, microorganisms, and human factors like pollution, could change the excellence as well as the amount of the bioactive phytochemical materials in the ginger rhizome, which could manifest as variations in antibacterial results (Abdalla & Abdallah, 2018).

1.2 objectives

1- To identify microorganisms isolated from surgical wounds

2- To test the antibiotics activity of different natural products compounds

Chapter Two

2. Literature Review

2.1 Introduction

A significant public health problem is the rise of antibiotic-resistant microbes, especially in hospitals and other healthcare environments (Spellberg et al., 2008). However, more than half of the Earth's population still relies on herbal remedies as their main form of therapy, according to the World Health Organization. Therefore, the use of native goods as an alternative to conventional therapy in the healing and treatment of many ailments including surgical site infection has increased over the past several decades owing also to their low toxicity and their potential cheaper charge compared to synthetic drugs (Saeed & Tariq, 2007).

2.2 surgical wound infection

Infection that adversely impact the incision or deep tissue at the operation site are known as surgical-site infections. In patients who receive implanted components, it often occurs up to thirty later surgery or up to one year after surgery. Despite improvements in prophylaxis, SSIs remain a substantial clinical problem since they are associated with high mortality such as life threatening bacteremia and morbidity like chronic pain and discomfort, as well as an increase in overall health cost due to an extended length of hospital stay (Owens & Stoessel, 2008). In addition, according to studies, surgical wound infections are the third most prevalent infection acquired from the hospital representing 14% to 17% of all infections obtained from the hospital and 38% of nosocomial infections in patients undergoing surgery (NNIS, 2004). According to the Centers that control diseases like the CDC, wound infection following surgery complicate 5% of the almost 30 million procedures carried out annually (Bratzler, & Hunt, 2006). The varied nature of these infections makes studies on the extent of SSI challenging. Post-operative wound infection prevalence and incidence rates differ significantly across procedures, hospitals, doctors, patients, and geographic locations. According to a study, unhygienic surgery had a

higher prevalence of surgical site infections (SSI) than contaminated surgery (27.3%), clean contaminated surgery (19.3%), and clean surgery (14.3%), with the link being statistically significant (Ameh et al., 2009). The infections in charge of many surgical wound infection come from the patient's own flora. However, although aerobic Gram positive cocci are the most prevalent pathogens, complex illnesses usually involve Gram-negative bacilli and anaerobic bacteria. Yet, depending on the type of surgery, specific bacteria may be involved where *Staphylococcus aureus* and *Staphylococcus Epidermidis* were seen as the most often isolated bacterium (Owens & Stoessel, 2008).

2.2 Antimicrobial Activity

Since before discovered about the existence of bacteria, the idea that even some plants had therapeutic characteristics or even would include what we would now regard to as antimicrobial principles was widely held. Many research teams in the domain of medicinal and aromatic florae have been focusing on the search for new drugs targeting bacteria, specifically after the evolution of antibiotic resistant drawback, throughout the last few decades. Recio et al. compiled a list of 75 species in which the authors had established the activity of the extract together with both the spectrum of and the principles underlying this activity after reviewing the most pertinent studies on this topic written between 1978 and 1988. Gram positive bacteria are the most perceptive germs, and the review generally revealed that phenolic compounds are the main active chemical in these plants (Redo et al., 1989). In addition, 115 publications were found in PubMed between 1966 and 1994 that discussed the antibacterial properties of medicinal plants. However, this number reached an increase of more than two fold in the next decade between 1995 and 2004 to reach 307. These numbers show how there is a growing demand for this kind of study among the scientific community's group that focuses on studying plants' therapeutic characteristics (Rios & Recio, 2005). On the other hand, although the overuse of antibiotics in clinical practice is generally acknowledged to be the root cause of antibiotic resistance, some scientists have hypothesized that the widespread use of biocides, particularly in consumer items like farms and households, may also play a role (Russell et al., 1998).

Antimicrobials are substances that either eliminate or stop the growth of bacteria. As a result, antimicrobial activity is crucial in the fight against many diseases brought on by microbes. Some plants' bioactive components, that are the physiologically active secondary metabolites of plants is represented by plant polyphenols which include saponins, tannins, steroids, flavonoids, anthraquinones, glycosides, triterpenes, and phytosterols, alkaloids as well as reducing sugars, have been linked to their antibacterial activity ((Saleh et al., 2015). Other than having a direct antibacterial impact, biologically active plant compounds can activate the host's natural defenses by altering the immunological response; they may also shield cells and tissue from oxidative stress; and they may promote tissue regeneration and repair (Amparo et al., 2020). However, the direct destruction of the objected bacterium is not the only approach of plant products' antibacterial action when emphasizing on their antimicrobial effects. The active syntheses of bushes and herbs can reduce the infectivity of the bacterial intruder or force it to be further susceptible to antibiotic action, for example, by interfering with antimicrobial resistance methods, by altering the metabolism of the bacterial compartment, by regulating gene display, or by interfering with countless molecular goals in the bacterial unit (Khameneh et al., 2019).

Numerous strategies of phenolics' antibacterial achievement were previously identified, including interactions with bacterial proteins and cell walls, injury to cytoplasmic coatings, declined flexibility of membranes, and inhibition of nucleic acid production, cell wall synthesis, and energy metabolism (Cushnie & Lamb, 2011; Daglia, 2012; Gyawali & Ibrahim, 2014). For instance, due to their potential impact on the nonmevalonate pathway, which in the majority of bacteria, acts as a supplementary carbon source and is crucial for the formation of cell membrane structures (including Gram-negative bacteria), terpenoids are acknowledged to perform a status in some plants' antibacterial activities (Agyare et al., 2014).

On the other hand, bacteria reproduce sheltered by a self- constructed biofilm medium made of bacterial sugar from inside the cell, proteins, and extracellular liberated nucleic acids in a unicellular form known as a biofilm on solid surfaces or air-liquid interfaces (Donlan & Costerton, 2002) and research on plant phenolics' antibiofilm activity has shown that, in addition to their harmful effects on bacteria, they also have "softer" effects that suppress biofilms by affecting bacterial regulatory systems like quorum sensing or other global regulator systems, without having any impact on bacterial growth (Silva et al., 2016).

2.3 Antibiotics and the Mechanisms of Resistance to Antibiotics

Before the development of antibiotics, it was impossible to cure and ultimately bacterial illnesses including meningitis and bacteremia were definitely lethal. Sadly, the development of antibiotic-resistant bacteria has been accelerated in recent decades due to the abuse and misuse of antibiotics as well as societal and economic issues, rendering pharmacological therapy ineffective. Currently, antibiotic resistance accounts for at least 700,000 annual deaths worldwide (AMR). The World Health Organization (WHO) estimates that without new and improved treatments, this figure might increase to 10 million by 2050, emphasizing a serious health issue (WHO, 2019).

The ability of bacteria to resist the effects of antibiotics or biocides that are used to kill or control them is the simplest definition of bacterial resistance. In other words, antimicrobial resistance, conforming to the World Health Organization, is an instinctive occurrence that come about when microorganisms stop reacting to antibiotics that they were previously vulnerable to and that were effective in treating diseases brought on by these germs (WHO, 2019). Drug resistance makes illnesses more difficult or impossible to treat, raising the danger of the spread of fatal infectious diseases. However, despite all the efforts to discover new drugs, with time, antibiotic-resistant strains to the newly discovered agent are frequently isolated as well. It is clear from the information above that every effort made to discover new antibiotics that can eradicate MDR is failing. For this reason, complimentary supplies of antimicrobial means, such as active compounds in plants are being investigated (Anand et al., 2019) combined with thorough examination concerning the mechanism of bacterial resistance (Mancuso et al., 2021).

2.3.1 Origin of Resistance to Antibiotics

It is feasible to differentiate between two types of resistance in this competition: acquired resistance and natural resistance, which can be further divided into intrinsic and induced resistance.

a) Natural: Natural endurance can either be always existing in the species, this is known as intrinsic, or triggered where the genes are inherently arising in the bacteria, but are only conveyed to resistance stages following antibiotic contact. According to one definition, intrinsic resistance is a trait that all members of a bacterial species possess uniformly, which is unconnected to horizontal gene transfer and unaltered by previous antibiotic exposure (Martinez, 2014; Cox & Wright, 2013).

b) Acquired resistance: By transformation, transposition, and conjugation (all known and also through horizontal gene transfer), changes to their own chromosomal DNA, bacteria can acquire genetic material that confers resistance. Both short - term and long products are possible. Plasmid-mediated transfer of resistance genes is the most prevalent method of acquiring foreign genetic material; bacteriophage-borne transmission is rather infrequent (Coculescu, 2009).

Some germs that are resistant to one medication may also be unaffected by other medications that have the same or a similar mechanism of action. This syndrome is typically perceived in antibiotics with similar chemical structures, such as erythromycin, neomycin-kanamycin, or cephalosporins and penicillins. Though, it occasionally appears in completely unrelated pharmacological categories like in the case of cross-resistance between erythromycin and lincomycin. This could have extrachromosomal or chromosomal origins (Jawetz et al., 1995; Mayer et al., 1995).

Additionally, the majority of the time, multidrug-resistant organisms are bacteria that are no longer treatable with antibiotics. This indicates that the germs can no longer be killed or controlled by a certain antibiotic. The development of harmful bacteria that are resistant to numerous medications was caused by the improper use of antibiotics in therapy. Yet, bacteria

may develop multidrug resistance by one of two ways. The first mechanism include the acquirement of the bacteria to a number of genes, each of which regulates drug antagonism. The second type of resistance, known as multidrug resistance, can also be brought on by altered target shape, enzymatic inactivation, or amplified illustration of the genes that code for several drugs efflux drives. A bacterial strain is deemed multi-drug resistant if it is resilient to three or more kinds of antibiotics, whereas strains are categorized as extensively drug-resistant if they are resistant to all existing antibiotics while reserving one or two antibiotic action, and pan-drug-resistant if they are unaffected by all antibiotics (Nikaido, 2009; Eliopoulos et al., 2008).

2.3.2 Mechanisms of Resistance to Antibiotics

The modifications that take place in the region of the antibiotics link to target areas and the receptor that is attached to the drug are different where they could be ribosomes or other enzymes. The most frequent instances of macrolide antibiotic resistance are those connected to modifications in the ribosomal target. S. aureus, Streptococcus pneumoniae, N. meningitidis, and Enterococcus faecium strains can develop penicillin resistance as well as alterations in penicillin-binding proteins where a significant mechanism in the development of resistance to drugs like beta-lactam, quinolones, glycopeptides, macrolides, tetracycline, and rifampicin includes changes in the target's structural constitution (Ayliffe, 1997; Oppenheim, 1997).

Most Gram-positive and negative microorganisms produce enzymes necessary to break down antibiotics. One of the most crucial resistance mechanisms is this enzymatic inactivation mechanism. In this group, beta-lactamases, aminoglycosides, and modifying enzymes (acetylase, phosphorylase and adenilase enzymes) break down beta-lactam antibiotics, the constantly increased number of these enzymes is able to degrade drugs such as erythromycin and chloramphenicol (Yüce, 2001; Mayer et al., 1995; Bassetti et al., 2013).

Another mechanism of resistance include a decreased drug entry into the bacterial cell due to a decline in the inner and outer membrane permeability or due to the quick ejection of the drug through active resistance pump system. Porin transmutations in resistant breeds can happen as a result of a decrease in the membrane permeability. For instance, a particular porin named OprD can result in mutations that lead to carbapenem resistance in Pseudomonas aeruginosa strains. In addition, resistance to quinolones and aminoglycosides may be significantly influenced by a

reduction in the permeability of the outer membrane. Tetracyclines, on the other hand, are eliminated via an active pumping system known as the P efflux system that depends on energy and therefore the drug cannot concentrate inside the cells. Beta-lactams, chloramphenicol, streptogramins, 14-membered macrolides, and quinolones can all be successfully resisted by active pumping systems (Yüce, 2001; Mayer et al., 1995; Bassetti et al., 2013; Nikaido, 1994).

Additional mechanism of resistance includes the usage of an alternative metabolic pathway like in the case of sulfonamide and trimethoprim antibiotics where bacteria can gain ready folate from the environment instead of synthesizing folate (Jawetz et al., 1995; Mayer et al., 1995).

Table 2-1: Resistance mechanism of bacteria to different antibiotics.

Antibiotic kind	**Resistance form**	**Resistance process**	**Familiar example**
Aminoglycoside	Decreased uptake Enzymatic modification	Changes in outer membrane AGEs	*P.aeruginosa* Gram-negative bacteria
Beta-lactams	Altered PBP Enzymatic Degradation	PBP 2a Penicillinase which are classified as per ambler classification	Mec A in *S.aureus*, CONS, *S.pneumoniae* Gram-negative bacteria
Macrolides	Reformed goal Efflux pumps	Methylation of the ribosome active site and lowered binding Mef type pump	Erm-encoded methylases in *Aureus, Pneumoniae*, and *Pyogenes* species. *S.pneumoniae*, and *S.pyogen*
Oxazolidinones	Altered goal	decreased binding to the dynamic site due to mutation	*E.faecium* and *S.aureus*

Quinolones	Altered target Efflux	Mutation leading to reduced binding to active spot (s). Membrane carriers	Mutations in gyr A in enteric gram-negative bacteria and *S.aureus*. Alterations in gyr A and par C in *S.pneumoniae*. Nor-A in *S.aureus*
Tetracyclines	Efflux Altered target	New membrane transporters Production of proteins that bind to the ribosome and alter the conformation of the active site	tet genes encoding efflux proteins in gram-positive and gram-negative bacteria tet (M) amd tet (O) in gram-positive and gram-negative bacteria species
Chloramphenicol	Antibiotic inactivation Efflux pump	Chloramphenicol acetyl transferase New membrane transporters	CAT in *S.pneumoniae* cm1 A gene and flo gene efflux in *E.coli*
Sulfa medications	Altered target	Mutation of genes encoding DHPS	*E.coli*, *S.aureus*, *S.pneumoniae*

2.4 Gram positive bacteria

Thick cell walls are a characteristic of gram-positive bacteria. These microbes give a positive result in a Gram stain test. The chemical dye used in the test causes the bacterium's cell wall to turn purple. Gram-positive bacteria are distinguished by their distinctive structure where they typically exhibit several traits. The lack of an outer membrane is considered the first characteristic of gram positive bacteria since in contrast to gram-negative bacteria, gram-positive bacteria do not have an outer membrane. In addition peptidoglycan, polysaccharides, teichoic acids, and proteins make up the cell wall, which encircles the cytoplasmic membrane of the bacteria, which is known as intricate cell wall. However, foreign substances are easily absorbed by this type of bacteria. The peptidoglycan in gram-positive bacteria is a thick layer forming up to 40 to 80 layers. Although, flagella which aid in movement, may be present on gram-positive bacteria, they hardly ever have pili, or hair-like structures (Sizar & Unakal, 2021).

2.4. 1 Staphylococci spp.

Gram-positive bacteria called staphylococci are characterized by their individual cocci, which split into numerous planes to create clusters a certain resemble grapes. Staphylococci have sizes between 0.5 and 1.5 micrometers. The genus Staphylococcus till now contains 32 species and 8 subspecies, many of which prefer to colonize and coexist in human skin of body (Kloos & Bannerman, 1994). Although Gram testing cannot distinguish Staphylococcus aureus from other staphylococcal species, clusters of gram-positive cocci often suggest Staphylococcus species. Other species that occasionally show up in clinical specimens and present as clusters of gram-positive cocci include Micrococcus, Dermacoccus, Alloiococcus, and Rothia species (Ruoff, 2002). Yet, S. aureus and Staphylococcus epidermidis are among commonly investigated strains. On the other hand, the staphylococci are immotile, doesn’t form dormant bacterium in the form of spores and optional anaerobes cells that usually survive in the presence of oxygen using aerobic respiration process, whereas in the absence of oxygen, the bacteria use anaerobic fermentation pathway. The majority of organisms have fairly complicated dietary needs. However, similar to other bacteria they necessitate an organic supply of nitrogen, up to more than 10 amino acids like arginine and valine that are considered essential to the bacteria, in

addition, to B vitamins, such as vit B1 known as thiamine and Vit B3 recognized as nicotinic acid (Kloos & Schleifer, 1986). Moreover, elements of this sort are catalase- affirmative with oxidase inactivity, setting them apart from members of the class Streptococci, which exhibit no catalase activity and differ from staphylococci in terms of their cell wall composition (Wilkinson, 1997). Furthermore, Staphylococci tolerates excessive salted medium (Wilkinson, 1997) and show a hotness endurance property (Kloos & Lambe, 1991). It is common practice to distinguish infectious staphylococci by their capacity to manufacture coagulase, and thus have the ability to form blood clotting (Kloos & Musselwhite, 1975). Therefore, S. aureus can be distinguished from other sort like the epidermidis one, which exhibit no action using the coagulase test (Ruoff, 2002).

2.4.2 Staphylococcus aureus

S. aureus is a facultative anaerobe, gram-positive, rounded shape, non-softening bacteria, while lacking spores yielding ability. The colonies have smooth, spherical, elevated, and sparkling colonies that are frequently golden or yellow. It is a component of the body's microbiota and is found on the skin and in the upper respiratory tract. Boils and shallots are examples of superficial skin lesions caused by S. aureus. It also causes localized abscesses, Deep seated infections such as osteomyelitis and endocarditis, severe skin infections furunculosis, infections of hospital origin (nosocomial), food poisoning by the release of enterotoxins into food, and sepsis by the release of superantigens into the circulation are all examples of such illnesses. Antibiotic resistance to numerous strains of S. aureus is gradually rising. In hospitals, methicillin resistance leads to epidemics (Baron, 1996). Penicillin, beta-lactam antibiotic, and vancomycin are the treatments for S. aureus infections (Kuroda et al., 2001). A difficult protective covering is thought to be the cell wall of *S. aureus*, which is somewhat shapeless in terms of display, and relatively thick. The cytoplasm, which is surrounded by the cytoplasmic membrane, is found below the cell wall. The fundamental constituent of the cell border is a protein linked to glucose that is known as peptidoglycan. In addition, dual kind of teichoic acids that represent a macromolecule that contains phosphate do exist, the first one is the teichoic acid of the cell wall while the second is membrane connected lipoteichoic acid of the cell that is hook up tightly to

the peptidoglycan through covalent bonds or imbedded in the lipid membrane of the organism. Teichoic acid equip the *staphylococcal* cell surface by forming an anionic charge and take a part in the acquirement and localization of metal ions, exceptionally cations with two double bonds, together with the performances of autolytic active sites (Wilkinson, 1997). Peptidoglycan and teichoiate cooperatively solitary count for about 90% of the unit wall weight, the surplus is made-up of exterior proteins, and macromolecules that act as hydrolases. Part of the mentioned elements are implicated in connecting the organisms to superficial layers and are pathogenicity contributing factor (Karakawa & Vann, 1982; Thakker et al., 1998). Ultimately, over 90% of S. aureus that affect humans which would expose to making capsular polysaccharides. Capsule assembly is reported to lower phagocytosis in vitro, and to amplify S. aureus virulence in a rats bacteremia model (Wilkinson & Holmes, 1979; Thakker et al., 1998).

The development and existence of the organism is reliant on the cells capability to acclimate to ecological fluctuations. To combat these changes, *S. aureus* has developed a variety of defenses, especially when it infects people. Three phases—lag, exponential, and stationary—can be identified on an expansion curve of S. aureus cultivated in perfect circumstances (Figure 2.1). Throughout the exponential stage, bacterium metabolism is quick and efficient to confirm persistent progress. As the bacteria mature and cease spreading (post-exponential) and cellular breakdown and building up reactions are re-organized for longstanding existence under unfavorable circumstances. Three globally acknowledged regulators of virulence determinant production exist in *S. aureus,* agr (Recsei et al., 1986), sar and sae that control the expression of extracellular protein, and other proteins indispensable for growth. Numerous exoproteins, like enterotoxins C and B, and protease, are produced more frequently as a result of the accessory gene regulator (agr), according to studies (Cheng et al., 1993; Giraudo et al., 1994). In addition, during the post-exponential and stationary growth phases, the same gene regulator inhibits the production of proteins related to cell walls, such as fibronectin binding proteins with fibrinogen binding proteins (Fosteer et al., 199o; Lindbeerg et al., 199o). A following governing domain entitled *staphylococcal* accompaniment controller was identified (sarA), yet, it was distinctive from the accessory gene regulator (agr) one. A sar A mutant enhances the exposition of proteases while decreasing the materialization of a number of exoproteins, including α-, β-, and δ – haemolysin (Cheunng et al., 1994; Chan & Fosterr, 1998). Study has indicated which sarA are

needed for agr reliant control (Heinrichs et al., 1996; Lindssay & Foster, 1999). Supplementary genes like sarH1 (also known as sarS) as well as sarT with resemblance to sarA have been found as a result of the publication of the S. aureus genome (Tegmarks et al., 2ooo; Chung et al., 2001) (Schmidts et al., 2oo1).

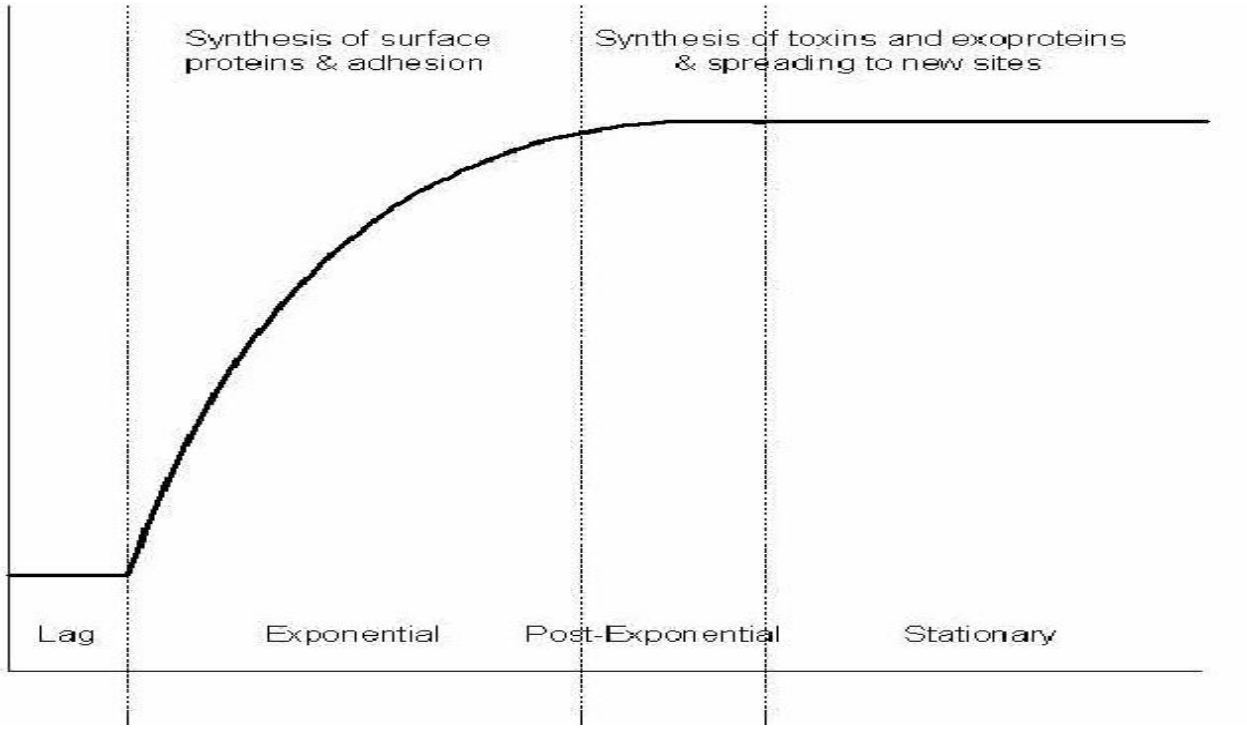


Figure 2.1: The three phases lag, exponential, and stationary of *staphylococcus aureus*.

Bacteria establish an infection in the lag phase, then move into the exponential phase where they proliferate and produce surface antigens as well as necessary components for growth, cell differentiation, and attachment. Gathering triggers a density mechanism that is driven by sensation during post-exponential, which leads to the creation of toxins and exterior proteins. As a result, the bacteria can leave the localized infection or abscess that has formed during the stationary phase and enter new areas in which the same operation can indeed be repeated.

The synthesis of haemolysin like α- and β, DNA breaker, coagulase, and proteinA can all be decreased as a result of a mutation in the sae gene, a loci demonstrated to play a part in the creation of pathogenicity contributing factor (Giraudo et al., 1994). However, no variances in d-haemolysin, protease, or lipase production levels were found. Northern blot clearly showed that sae influences exoprotein representation at the transcription point (Giraudo et al., 1997).
Sigma factors (σ) representing proteins that join to the core RNA polymerase to produce the holoenzyme that binds to particular promoters, may also be involved in the alteration of virulence determinants (Moran, 1993; De0ra & Misra, 1996). There are two sigma domains in *S. aureus*: σA, the principal sigma aspect responsible of the genes illustration in charge of cleaning the cell from toxins, therefore, maintaining cell progress (De0ra et al., 1997); and the B type, that represent an alternative factor and controls the expression of many genes important for biological processes. In addition, it has a role in virulence basis production, and stress response (Deora and Misra, 1996) (Horsburgh et al., 2002).

2.4.1.1 Infections related S. aureus

S. aureus is regarded as a significant pathogen that can attack both immunocompromised hospitalized patients and healthy community members, where infection can extend from minor skin diseases to potentially fatal heart infections like endocarditis. Because of this, the signs and symptoms of staphylococcal infections can vary widely depending on the location and extent of the infection. The nasopharynx region of the human body and the skin both contain this bacterium naturally (Wilson et al., 2007). A study revealed that about 4% and more of surgical patients treated in one of multiple hospitals located in U.K. developed a nosocomial infection that is outlined as an infection undetected before the admission to a health care center (Plowman et al., 2001). In addition, a hospital's atmosphere encourages the development of S. aureus strains that are resistant to treatment. According to the same previously mentioned study, S. aureus caused 81% of the detected infections, and 61% of these infections were methicillin-resistant.

Although the mucous membrane and skin serve as effective shields to prevent S. aureus from penetrating the underlying tissues, bacteria, however, can access the underlying tissue once this barrier is damaged by trauma or surgical incision, and with a deterioration in the immune system

caused by surgical stress, an infection can arise and can progress into subsequent local abscess (Elek, 1956), or sepsis succeeding its infiltration into the blood (Waldvogel, 1990). On the other hand, ingestion of enterotoxin containing food that represents one of the extracellular toxins synthesized by *S. aureus* can cause poisoning. Poisonous shock set of symptoms (TSS) is only brought on by bacterial breeds that carry the TSST-1 gene, responsible for TSST-1 toxin fabrication. (Waldvogel, 1990). Staphylococcal scalded skin syndrome is linked to the exfoliative toxins (SSSS) that are made up of the three conditions toxic necrolysis epidermal, scarlatiniform erythema, and bullous impetigo, all of which cause destruction to the skin's epidermis. (Howard & Kloos, 1987).

According to the literature, infection rates after orthopedics surgery are ranging from 2% up to 25% for accessible cracks (Gustilo et al., 1990) compared to 1 to 2 % incidence for sealed breakages (Boxma, 1995). In addition, S. aureus was discovered to frequently infect soft tissue (Petty et al., 1985), bone joints, and metal-biomaterials (Barth et al., 1989). It is recognized that the harm induced by the insertion of biomaterial into the human body increases the vulnerability to infection (Elek & Conen, 1957), it also stimulates the inflammatory processes, such as reactive oxygen and lysosomes protease, by triggering the host immune response (Merritt & Dowd, 1987; Gristina, 1994). After being inserted, biomaterial prostheses acquire a covering from host blood components, including ECM (Extracellular Matrix) (Baier et al., 1984) and bacteria are met with a living, integrated cellular surface when host cells, like fibroblasts, come in contact with the biomaterial surface and form strong connections. S. aureus attachment can be resisted by an incorporated viable cell layer with effective host defense mechanisms (Gristina, 1987). However, S. aureus has a range of binding pathways that let them adhere to biological materials and to the ECM amino acids sequence build on the surface of such materials, such as MSCRAMMs (Microbial surface constituents recognizing bonding matrix molecules) (Herrmann et al., 1993) where host defense cells are unable to move S. aureus once they have attached to a surface (Gristina, 1994) and therefore proving that Teichoic acids present on the cell enclose of S. aureus bacteria will being anionic, are essential for the initial step of biofilm construction, according to research by Gross and his colleagues (Gross et al., 2001).

The susceptibility to infection is also affected by a collection of implant-related parameters. These considerations encompass the implant's size and shape (Melcher et al., 1994), the implant's method and steadiness, the surface properties (Worlock et al., 1994), and the matter and its compatibility with biological components (Cordero et al., 1994), in addition to other factors (Gerber & Perren, 1980).

The negatively charged bacteria are first repelled by biomaterial surfaces because they typically contain a negative charge. However, van der Waals and hydrophobic forces are applied at a distance of about 15 nm, which surpasses repulsion (Pashley et al., 1985). Charged, hydrogen, and strong bonding take place between the biological material and the microbes or cell at distances of about 1 nm, representing the interaction between the receptors on the bacterial cell wall and those on the ECM (Gristina, 1987). Therefore, it can be said that the two most crucial elements of biocompatibility are those that affect how living cells and biomaterials interact and stick together as well as how bacteria adhere to biomaterials (Stickler & McLean, 1995).

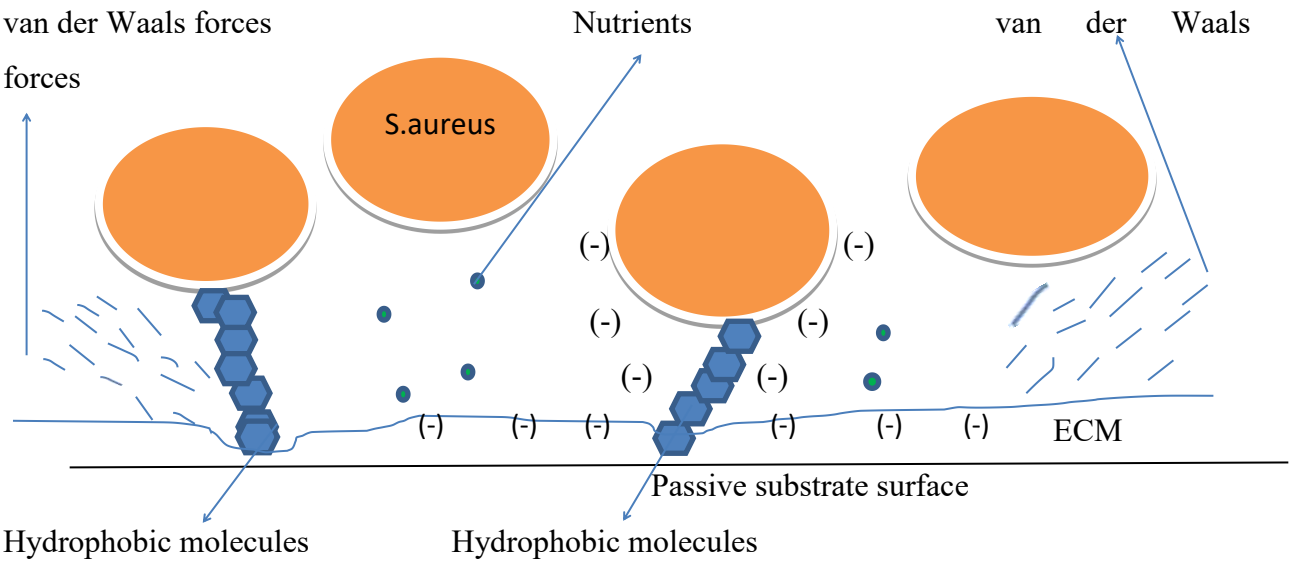


Figure 2.2: Diagrammatic representation of the interactions involved in bacterial adhesion to a substrate surface.

At some ranges, attractive van der Waals energies and the hydrophobic connections among molecules overcome the initial separating energies amid charges that are the same on the shells

of bacteria and substrate that are represented by circles colored in blue. In the right circumstances, the ECM is established, enabling ligand-receptor interaction and bacterial adsorption to the substrate (figure 2.2) **(**Gristina et al., 1985).

2.4.1.2 Treatment of *S. aureus* infections

Resistance to each new class of antibiotics against *S.aureus*, such as penicillins, sulfonamides, tetracyclines, glycopeptides, and others, has emerged throughout the history of treatment, making therapy more challenging (David & Daum, 2017). In the 1940s, penicillin was first used to treat S. aureus outbreaks, which significantly reduced morbidity and death. But by the late 1940s, penicillinase-related resistance had arisen. (Eickhoff, 1972). Most often, viral vector genes that code for antibiotic resistance are responsible for the fast expand of antibiotic-resilient bacteria (Morris et al., 1998). Later on, the antibiotic methicillin was created in the late 1950s to treat penicillin resistant organism. Yet, within two years of clinical usage, the first methicillin-resistant S. aureus (MRSA) forms have been identified, despite the fact that methicillin is effective in treating PRSA infections. In a hospital located in England, the primary instance of methicillin- unaffected MRSA was found in 1961 (Henderson & Nimo, 2018). The occurrence of MRSA increased globally throughout the years and nowadays it is a major cause of S. aureus infections in hospitals linked to a high fatality rate (McGuinness et al., 2017).

In many parts of the world, including Europe, the US, North Africa, the Middle East, and East Asia, MRSA is currently the most common resistant pathogen (Guo et al., 2020). MRSA was listed as a top worry for antibiotic resistance in the US based on the CDC research conducted in 2013. Recent research indicate that although MRSA isolation rates have decreased over time, further observation and prevention strategies would still be required to stop the spread of this infection. International rates for MRSA were 44.2% between 2005 and 2008. In 2014, MRSA was shown to be the primary cause of even higher than 80% of S. aureus infections in portions of Asia between the south and the east, the Western Pacific, and other branches of the globe. By 2016, this proportion had decreased to 39.0%, and MRSA frequencies had changed significantly (Schulte & Munson, 2019).

Membrane-bound proteins known as penicillin-binding proteins (PBPs) are present in methicillin-susceptible S. aureus (MSSA). Beta-lactam drugs bond to PBPs and damage peptidoglycan as a result. Other names for PBPs that the gene positioned in the chromosome mec of staphulococcus element of MRSA, expresses are PBP2a or PBP2′. Methicillin and oxacillin, two -lactam antibiotics, have a poor affection for the substitute protein. As long as the peptidoglycan remains unaltered, the MRSA can persist (Rai & Khairnar, 2021). Owing to the incorporation of numerous additional endurance fundamentals into the sequence of SCCmec, which enables it to function as a spot of incorporation for genetic transferable components such plasmids and transposons, MRSA strains are frequently referred to as multidrug-resistant (MDR) bacteria (Szweda et al., 2012). On the other hand, Vancomycin was first used in 1958 to treat penicillin-resistant S. aureus, but methicillin quickly took its place. Vancomycin has been the cornerstone for MRSA ever since it became popular due to the growth of MRSA. It is a glycopeptide that inhibits the growth of gram-positive illnesses and has antibacterial properties (Hiramatsu, 2001). Some MRSA bacteria frequently produce VISA strains known as hetero-VISA after being subjected to vancomycin. It appears that VISA resistance is linked to thickening of the peptidoglycan in the cell wall, which increases the focus for the glycopeptide in the cell wall and necessitates the use of more drug to stop bacterial growth (Hanaki et al., 1998). But in 2002, a patient in the United States developed the country's first case of vancomycin-resistant S. aureus (VRSA) infection. The presence of the van gene in this strain raises the possibility that *S. aureus* and vancomycin-resistant enterococci traded genetic material to develop the resistance determinant (Sievert et al., 2002).

There is presently no treatment recommendation due to the low number of VRSA infection patients and only a small number of the reported VRSA cases included comprehensive clinical information. However, excluding vancomycin endurance, most VRSA separates are still sensitive to a variety of antibiotics. According to a report, ceftaroline, daptomycin, linezolid, minocyline, tigecycline, rifampin, and trimethoprim/sulfamethoxazole were all effective against >90% of the 13 VRSA isolates (Saravolatz et al., 2012). Therefore, following a clinical laboratory's determination of VRSA isolation, a systemic antimicrobial therapy using potent antibiotics is typically implemented (Finks et al., 2009; Friães et al., 2015). In addition, the onset of an infection begins with bacterial colonization. The most frequent site for VRSA isolates is a

wound, which also offers a setting for MRSA and VRE co-infection and co-colonization. As a result, vigorous wound care is typically used in the treatment of VRSA infections In addition to aiding in the elimination of VRSA, wound treatment also prevents further plasmid delivery by removing the ideal conditions for co-colonization (CDC, 2002; Sievert et al., 2008).

2.4.2 The Staphylococcus epidermidis

The most common kind of coagulase-negative staphylococci is S. epidermidis, which also represent one of the main source of infections with implanted medical devices (IMDs) (Rupp & Archer, 1994). Its capacity to create multilayered, highly organized biofilms on artificial surfaces can be one of the reason for its pathogenicity (Figure 2.3).

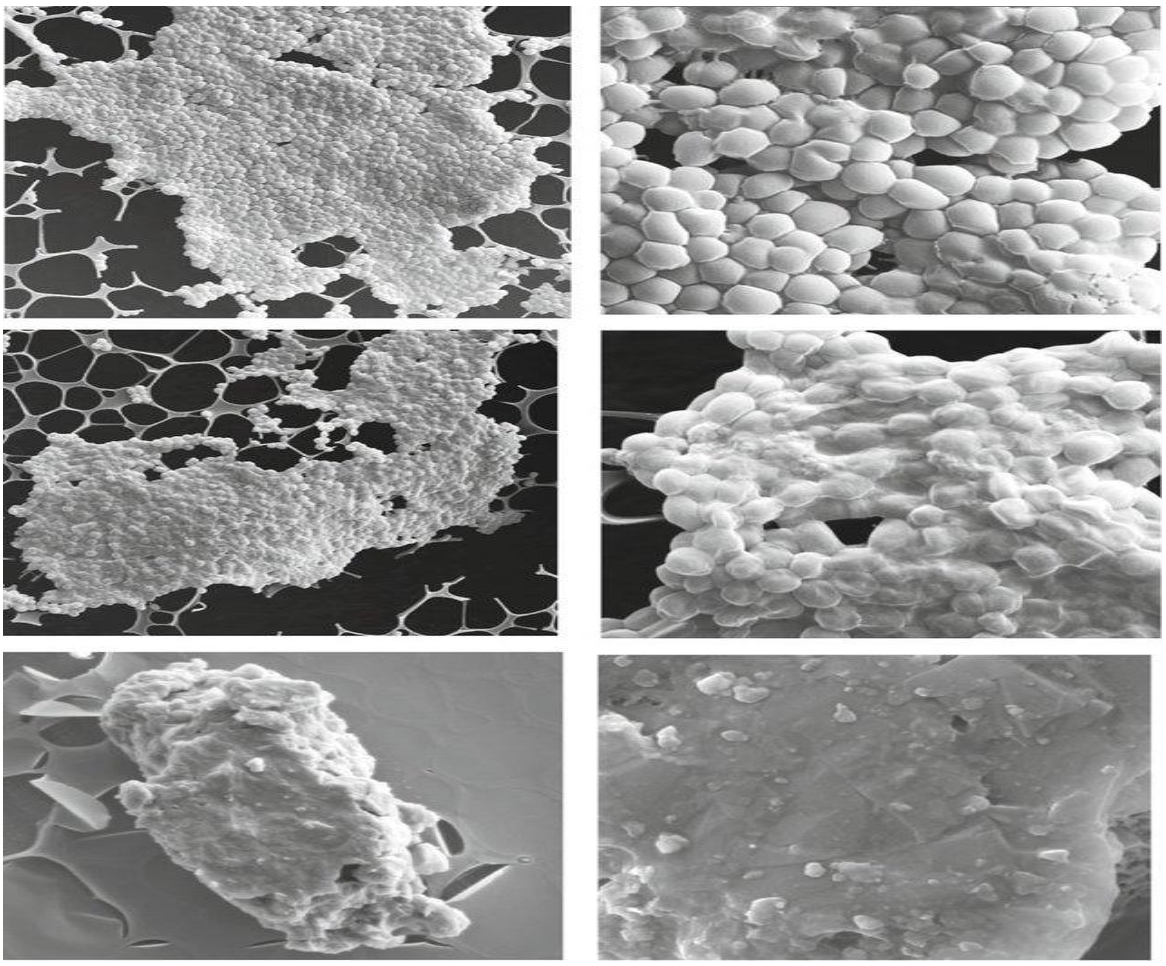


Figure 2.3: Scanning electron microscope image of *S. epidermidis* biofilm.

Multicellular populations of device-associated biofilms are up to 1000 times more resilient than their unrestricted equivalents, referred to as planktonic, and making them exceptionally tough to eliminate (Gilbert et al., 1997; Costerton et al., 1999). Limited penetration, a slower rate of growth, a unique genetic phenotype (O'Gara, 2007; Harison et al., 2004; Fitzpatrick et al., 2005), the appearance of resistance genes (Maira-Litra'n et al., 2000), and the existence of biofilm viable cells are all factors that contribute to a high resistance (Lewis, 2005; Roberts & Stewart, 2005).

The skin at the region of incision, the medical equipment used during the surgery, floating contamination, and bacteria shed by medical team and other healthcare professionals are only a few of the sources for potential infectious organisms. Long hospital stays, several surgeries performed at the moment of implant, distant infections in other body regions, the length of surgery, and the degree of tissue destruction are other factors that raise the risk of prosthesis infection (Choong & Whitfield, 2000). On the other hand, over the past several years, there have been more medical device-related infections due to their increase use beside the rise in the percentage of critically ill and immunosuppressed patients (Raad et al., 1998).

2.4.2.1 Spectrum of *S.epidermidis* Infection

S. epidermidis, which is primarily thought of as non-infective, typically inhabiting human epithelial cells and mucosal tissue, and rarely infects humans with well-functioning immunity (Ziebuhr et al., 2006*)* can sometimes initiate infection of the heart valve, a condition recognized as endocarditis (Caputo et al., 1987). Moreover, in latest periods, the bacteria became a significant contributor to nosocomial illnesses (Vuong & Otto, 2002). However, only when this staphylococcus from the coagulase-negative class penetrates the body's defensive epithelial layers, it can result in a serious infection (Kocianova et al., 2005). As previously mentioned, infections caused by medical devices are the primary kind of infection linked to *S. epidermidis* (Vuong & Otto, 2002). A good example of illnesses linked to devices include keratitis brought

on by contact lens usage as well as prosthetic valve endocarditis upon prosthetic valve grafting (Verhoef & Fleer, 1983; Elder et al., 1995), endophthalmitis with late onset following surgery and vision correction implant (Jansen et al., 1991), urinary tract infection after using a urinary catheter (Warren, 2001), in addition to infections connected to prostheses, such as the bacterial discharge of joint prostheses following joint replacement surgery (Gallo et al., 2003). All of the previously mentioned examples have the propensity to have disastrous effects.

2.4.2.2 Treatment of *S. epidermidis*

The ability of *S. epidermidis* to colonize the surface of IMDs through the production of extremely resistant biofilms distinguishes the pathophysiology of infections connected to medical devices. Reduced basic cell functions and the generation of protective features are features of S. epidermidis biofilms (Kong et al., 2006). By becoming less sensitive to harmful molecules like antimicrobials substances, cytokines, and antibacterial peptides, as well as by switching to a mild state that reduces inflammation and immune cells migration to the infection spot, physiological adaptations in S. epidermidis biofilms shield the bacteria from the host immune defense system and therefore enabling bacteria survival (Yao et al., 2005). (Yao et al., 2005b).

Alternatively, *S.epidermidis* is a prominent nosocomial pathogen that has a distinctive multidrug resistance profile, comprising antagonism to methicillin, glycopeptide and quinolone antibiotics, in addition to its capacity to form inherently challenging biofilms on the external part of materials (Raad et al., 1998; Ziebuhr et al., 2006). Furthermore, a perfect environment for the growth of resistant bacteria is provided by the extensive use of antimicrobials and antiseptics in hospitals settings. S. epidermidis' effectiveness as an infectious agent in the hospital setting is also attributed to its greatly adaptable character, innate genetic variety, and native genetic tractability, all of which allow it to survive in unfavorable exterior conditions (Yao et al., 2005a). The frequency of staphylococci strains resistant to methicillin, the antibiotic of first preference, has been rising for a long time (Rupp & Archer, 1994). Methicillin resistance affects over 80% of S. epidermidis strains resulting in nosocomial infections, and the majority also have resistance to other antibiotics (Wielders et al., 2001). The problematic situation of vancomycin being the

final viable antibiotic against many multi-resistant germs and the emergence of S. aureus and S. epidermidis strains with intermediate resistance to vancomycin have led to the development of novel medicines. Such antibiotics are the oxazolidinone family and quinupristin/dalfopristin (Raad et al., 1998).

2.4.3 Lactobacillus species

One of the many and evolutionarily diverse kingdoms of lactic acid-producing bacteria, including the type genus Lactobacillus, is Lactobacillales. They are rods or coccobacilli that are Gram-positive and do not produce spores. They have rigorous dietary requirements, such as those for carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins. They are also rigorously fermentative, aero-tolerant or anaerobic, aciduric or acidophilic (Goldstein et al., 2015). Since they don't produce porphyrinoids, they have no heme-dependent functions. In some strains of Lb. mali, pseudo-catalase is produced. When given glucose as a carbon source, lactobacilli can either be homofermentative, which results in the production of more than 85% lactic acid, or heterofermentative, which results in the equimolar production of lactic acid, carbon dioxide, alcohol such as ethanol, and in some cases acetic acid (Tannock, 2004).

Despite being a natural component of the commensal human flora, Lactobacillus species have also been isolated from a number of human diseases (Salminen et al., 2006). The most intriguing probiotic potential of lactobacilli, the capacity to immunomodulatory human cells to produce an anti-inflammatory response, in addition to the ability to produce antioxidant has also been discussed (Ljungh & Wadstrom, 2006). Despite the widespread use of probiotics, severe infections are uncommon in the literature and frequently entail comorbidities (Simkins et al., 2013). On the other hand, when compared to other research addressing the significance of probiotics, those examining the role of probiotics in skin conditions occur very frequently. Several studies have shown compelling evidence that live probiotics including Lactobacilli species or their used culture fluid can considerably improve wound healing by lowering infection (Markowiak, 2017).

It has only been possible to assess the in vitro susceptibility of human isolates of Lactobacillus species in a small number of studies that frequently used various approaches. In addition, Concerns have also been raised regarding the possible spread of lactobacilli-derived resistance to other organisms. However, other organisms' growth can be inhibited by strain-specific bacteriocins and bacteriocin-like substances produced by lactobacilli (Liévin-Le Moal & Servin, 2014). Studies and generalizations of this genus are challenging due to its taxonomic intricacy. Some lactobacilli species have built-in resistance to vancomycin and aminoglycosides (Gueimonde et al., 2013). Penicillin and ampicillin can, however, be used to treat several of the species.

2.6 Plant Extraction

According to the World Health Organization, plants are the primary source of health care system for 3.5 billion people living african and Asian countries Impoverished (Agyare et al., 2009). A sizable class of physiologically active secondary metabolites of plants is represented by plant polyphenols. They consist of tannins, lignans, lignins, anthocyanins, phenolic acids, stilbenes, coumarins, and lignans. These compounds provide protection from free radicals and poisons as well as having an essential role in resistance against different microbial infections. Plant polyphenols today experience of the ever degree of awareness not only through the scientific community but also, and most notably, general people due to their quantity and availability in fruits, seeds, agriculture, and other related foods and drinks, whose regular intake has been declared to be good for human health. They are useful in lowering the danger of some age-related deteriorating processes and diseases, which is supported by their ability to scavenge oxidation generated free radicals, which has frequently been noted (Quideau et al., 2011). Hence, phenolic compounds possess antioxidant, anti-inflammatory, anti-tumor as well as anti-bacterial properties (Majekodunmi, 2015). On the other hand, the extraction practices are highly significant for the analysis of phytochemicals. There are both conventional and cutting-edge extraction techniques. In phytochemical screening tests, maceration, percolation, and soxhlet extraction techniques that are considered as the traditional method, are the frequently utilized methods. However, there are also certain novel techniques, including rapid solvent extraction, microwave assisted extraction, ultrasound assisted extraction, and supercritical fluid extraction. (Azwanida, 2015; Raaman, 2006).

2.6.1 Extraction methods

2.6.1.1 Maceration

This process involves adding the fluid to the complete batch of raw material, whether it is whole or coarsely ground, and letting the mixture rest at room temperature for a minimum of three days while stirring it periodically to dissolve the soluble components. The combination is then

separated after squeezing the wet solid material, and the mixed liquids are made clear by filtration or by sieving them after settling (Singh, 2008). Given that the active compounds cannot be completely removed, maceration is not regarded as a sophisticated approach (Raaman, 2006).

2.6.1.2 Percolation

In order to prepare tinctures and fluid extracts, this method is most usually utilized to extract the active components. Typically, a percolator, a slim, pointed jar that is accessible at both ends, is utilized (Majekodunmi, 2015). A suitable amount of the specified mixture is used to moisten the solid ingredients, they are then put into the percolator's top and left to rest for roughly four full hours in a firmly covered container. More menstrual fluid is added to the mixture to help form a slight sheet above the mass, which is next left to soften for one day in a sealed pot. The output of the percolator is then unsealed to enable the watery content to flow out gently. Menstruum is added as needed until the percolate has accumulated to roughly three to four equally sized portions of the end product's required volume. The percolate then is infused with the liquid obtained from the squeezed marc. There is enough fluid supplied to provide the required bulk. The mixed liquid is cleaned by filtering or standing, followed by emptying. (Singh, 2008).

2.6.1.3 Soxhlet extraction

The hot Continuous Extraction, where this technique involves putting the finely crushed crude drug into a leaky bag or "thimble" composed of sturdy filter paper, which is then positioned in chamber E of the Soxhlet device (Azwanida, 2015). Heat is applied to flask A's extracting solvent, which then vaporizes and condenses in condenser D. The crude medication is extracted by contact as the condensed extractant drips into the thimble containing it. Liquid in chamber E rises to the top of siphon tube C when that level is reached. E's contents are sucked into flask A. This operation is supported continuously till an evaporated drop of solvent from the siphon tube leaves no residue. Comparing this procedure to those previously mentioned, the advantage is that significantly less solvent is needed to extract huge amounts of medication. This has a significant impact on time, energy, and ultimately cost-effective. It is only used as a batch process at small

scales, but at medium or large scales, it can be transformed into a continuous extraction process, which is much more cost-effective and viable (Singh, 2008).

2.6.1.4 Supercritical fluid extraction

An alternate sample preparation technique called supercritical fluid extraction (SFE) aims to use fewer organic solvents and process samples more quickly. Temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors are some of the variables to take into account. For SFE, cylindrical extraction vessels are typically employed, and their performance is unquestionably excellent. Another crucial phase is the collection of the extracted analyte after SFE. During this maneuver, there could be a significant analyte shortage., giving the investigator the impression that the real efficacy was low (Singh, 2008). Contemptuous fumes such as carbon dioxide, nitrogen, and so many more are employed to obtain active constituents (Prabu et al., 2013). The use of CO2 as an extracting fluid has various benefits. Besides having good physical characteristics, carbon dioxide is also widely available, cheap, and safe. However, while being the favored fluid for this type of extraction, carbon dioxide has a number of polarity restrictions. Solvent polarity is critical for extracting polar solutes or when there are significant analyte-matrix interactions. To overcome such restrictions, organic solvents are commonly included to the fluid used to extract carbon dioxide. Lately, argon has replaced carbon dioxide since it is more passive and less expensive. The maximum retrieve rates for argon are gained at 150° C combined with 500 atm. Constituent recovery proportions typically rise with the intensification of pressure or temperature (Singh, 2008).

The extraction process has several obvious benefits:

- Low-temperature constituent extraction, which rigorously avoids heat and some organic solvents' damaging effects.
- No traces of solvent.
- An extraction method that is friendly to the environment.

The rapid rise of SFE's applications has been the area of development where growth has been most significant. Pesticides, environmental samples, foods and perfumes, essential oils, polymers, and natural goods all find extensive use for SFE in the extraction process. The prohibitive capital investment in the extraction process is the main barrier to its commercial adoption (Singh, 2008).

2.6.1.5 Microwave assisted extraction

In this procedure, microwave energy makes it easier to separate the active components from the plant material and transfer them into the solvent. Magnetic and electric fields in microwaves are perpendicular to one another. Through ionic conduction and dipolar rotation, the electric field produces heat. The ensuing heating is rapid and is equal to the solvent's dielectric constant. In contrast to traditional procedures, microwave aided extraction warms the entire sample at once. Heat during extraction destroys fragile hydrogen links due to molecular dipole rotary motion, and ion migration promotes permeation into the sample or structure (Kaufmann & Christen, 2002).

2.6.1.6 Ultrasound Extraction (Sonication)

Utilizing ultrasound at frequencies between 20 kHz to 2000 kHz throughout the procedure results in cavitation by increasing the permeability of cell barriers. The technique works well in certain circumstances, like when rauwolfia roots need to be extracted, but the greater costs prevent it from being used widely. One weakness of the process is the irregular, and good-known, destructive influence of ultrasonic power (more than 20 kHz) on the effective constituents of therapeutic floras, which ensues the creation of free radicals and consequently undesirable variations in the preparation particles. Yet, this technique is more suitable for the extraction of thermally unstable compounds (Maran et al., 2017).

2.6.1.7 Accelerated solvent extraction

Solvents are employed in accelerated solvent extraction techniques at high pressures and temperatures to maintain the solvent's fluid condition throughout the extraction process. As a result of the solvent's increased ability to solubilize the analyte at elevated temperatures, the diffusion rate also rises. A greater temperature further decreases the viscosity state, allowing the solvent to more readily permeate the material's pores. The pressurized diluent allows more adjacent interaction with the analyte. However, this technique has some advantages of using less time and solvent is needed for the withdrawal of active ingredients. Other advantage of this process includes the removals of 1 to 100g in minutes, the impressive solvent saving and varied extent of purposes as well as the handling of acidic and alkaline matrices (Mottaleb & Sarker, 2012).

2.7 Qualitative methods for identifying phytochemicals

2.7.1 Alkaloids

Wagner's test: A few drops of Wagner's reagent are added over a small amount from the plant extract and a reddish brown precipitate illustrates the presence of alkaloids (Banu & Cathrine, 2015).

Mayer's test: dual drips of chemical are combined laterally to the side of a test cylinder containing small sample from plant extract. The presence of alkaloids is indicated by the formation of a white creamy precipitate (Banu & Cathrine, 2015).

Dragendroff's test: The addition of few drops of Dragendroff's reagent to the extract gives red precipitate if alkaloids are present in the sample (Tiwari et al., 2011).

Hager's test: A small amount of Hager's reagent is added to the extract and the formation of a yellow precipitate indicates the presence of alkaloids (Tiwari et al., 2011).

2.8 Quantitative procedures

Chromatography methods is suitable for both quantitative and qualitative examination. Chromatography using Gas, simple liquid, high execution liquid and a thin layer with high performance specifically can be handled to perform a quantifiable analysis (Gusthinnadura et al., 2017).

2.8.1 Gas Chromatography (GC)

A fusion of volatile ingredients that evaporates while lacking decomposition can be employed in this practice. Both qualitative and quantitative measurements are performed when using Gas chromatography technique joined by mass spectrometry (Raaman, 2006). The herbal obtain can be liquefied using methanol as the solvent and filtered through the solid phase extraction support that is typically polymeric before proceeding into the analysis in order to detect the presence of different components (Prabu et al., 2013).

2.8.2 Liquid Chromatography (LC)

The word liquid chromatography (LC) applies to a variety of chromatographic systems, including size exclusion, ion exchange, liquid-liquid, and liquid-solid chromatography. Chromatography performed in glass column is an illustration of traditional LC, which involves the mobile phase evaporating through a glass column containing a finely separated fixed phase while moving through the column with the aid of gravity, where a sample can be divided into its component elements. However, based on how the sample interacts with the mobile and stationary phases, this separation happens (Singh, 2008). As the mobile phase, a liquid with low viscosity is employed. The immiscible liquid may be covered by a porous support, and the stationary phase bed may also include a fine layer of fluid stage adhered to the top of an absorbent material or a sorbent with regulated opening size (Raaman, 2006).

2.8.3 High Performance Liquid Chromatography (HPLC)

Due to a availability of several characteristics in greater performance liquid chromatography (HPLC) equipment, liquid chromatography has surpassed gas chromatography.

High resolving power, quick separation, ongoing column effluent monitoring, qualitative and quantitative measurements, and segregation, as well as programming of analytical processes and data organizing are all advantages offered by HPLC. Depending on their interactions with the solid particles of a closely packed column and the solvent of the mobile phase, the compounds or active components are separated. Mixtures sensitive to vaporization or that break down at extreme temperatures profit from HPLC. In a solitary process, this approach can offer both qualitative and quantitative measurements. More structural details about the chemicals are revealed using HPLC in conjunction with sensor that detect photons of ultraviolet light and mass spectrometer (He et al., 1997). In addition, chromatographic separation lessens the amount of an enantiomer in a racemic combination and can remove contaminants with various polarities. Crystallization can be utilized in both of these situations to create the pure result (Singh, 2008).

2.8.4 High Performance Thin Layer Chromatography (HPTLC)

As a very effective substitute for high-performance liquid chromatography (HPLC) and gas chromatography, the HPTLC methodology is an automated, sophisticated method of thin layer chromatography with better and enhanced separation efficiency and detection limits. Planar or flat-bed chromatography are other names for high-performance thin-layer chromatography (Singh, 2008). Similar operating principles to those of TLC, such as the adsorption principle of separation, are used by HPTLC. The mobile phase or solvent can flow thanks to capillary action. According to their affinities, or to put it another way, according to their adsorbent capacity, the analytes migrate toward the stationary phase. Stronger affinities cause the component to travel more slowly in the direction of stationary phase. Low affinity components travel swiftly in the stationary phase's direction. On a column chromatography plate, the components are then separated. This technique is reliable, quick, and effective for quantitative chemical analysis. Despite being based on TLC, one such method improves the resolution of the compounds in order to separate them or do a quantitative analysis. (Gusthinnadura et al., 2017).

2.9 Extraction of Cinnamon

One of the first herbal remedies known is cinnamonomum zeylanicum, which has been referenced in Chinese manuscripts since 4000 B.C (Elumalai et al., 2011). The Lauraceae family includes the tropical evergreen tree Cinnamomum zeylanicum. The bark and leaves of the cinnamon tree are employed in a variety of culinary preparations as well as in medicinal. (Schmidt et al., 2008).

Due to its distinctive qualities, cinnamon is frequently utilized in medicine. The essential oil from its bark is high in trans-cinnamaldehyde and has antibacterial properties that are effective against degenerative bacteria and fungus, animal and plant diseases, and food poisoning. (Faix et al., 2009). The bark and leaves of Cinnamomum spp. with majority apply in spices in home kitchens and their distilled essential oils are used as flavoring agent in the food and beverage industries (Elumalai et al., 2011). Cinnamaldehyde have antibacterial activity (Utchariyakiat et al., 2016; Rana et al., 2011). The primary constituent of cinnamon oil and the one that gives it its distinctive smell is the odorant trans-cinnamaldehyde (Zinn et al., 2015). Cinnamic acid is a significantly important fuctional group as an effective insuling releasing agent (Sharma, 2011). Cinnamaldehyde extraction have been done with sonication and soxhletation (Aprianto, 2001). The highest concentrations of cinnamaldehyde were obtained by sonication extraction with ethanol as the solvent, at 3.37%, while those from soxhletation were at 3.12%. By the mean of soxhletation procedure using ethanol and aquadest as solvents, other cinnnamaldehyde extraction investigations have been carried out (Araar, 2009).

The tree's bark is dried and used to make spices. However, cinnamon is a common ingredient in American cereals, foods made with grains, and fruits and it is one of the most popular and reasonably priced spices in the world. On the other hand, the spice also contains antioxidants and other active ingredients available in the hydrophilic portions of cinnamon, yet not the oil extracted from cinnamon. These ingredients are considered the driving power to how cinnamon produces the corresponding health effects (Roy et al., 2009).

More than 300 volatile composites have so far been identified as components of cinnamon essential oils. It was demonstrated that cinnamon's oils and extracts have a unique antioxidant function that is, in particular, credited to the existence of phenolic and polyphenolic ingredients (Schmidt et al., 2008). Because they didn’t required toxicity data owing to their relative safety, some plant essential oils or their constituents have been suggested as substitutes to the widely

used synthetic pediculicides. Hence, compared to plant extracts, plant essential oils are widely accessible and are reasonably priced (Elumalai et al., 2011).

Steam distillation and Soxhlet extraction techniques can be relied on to obtain the essential oil. However, the simplest way to obtain cinnamon essential oil is through steam distillation, resulting in its high application rates. It is less expensive than alternative extraction techniques since it doesn't call for solvent, besides being safer. The benefit of steam distillation is that the qualities of the oils produced by this process are not changed, and it is reasonably inexpensive to operate at a basic level. An element of the oil never decomposes in this way because steam lowers the boiling point of that element. In addition to being affordable, it is also comparatively quicker than alternative ways (Wong et al., 2014).

2.10 Extraction of Radish

Numerous essential vegetables that are significant economically are members of the Cruciferae plant family. Europe and Asia are the original home of Raphanus sativus L. It grows between 190 to 1240 meters above sea level in temperate areas and it is 30 to 90 cm tall, with strong roots that come in a variety of shapes, sizes, and colors. These plants have a strong flavor and are nutritious (Fig. 2-3). One of the staple foods of Japan is roots of radish that are salted, which are ingested in the country in an estimated 500,000 tons annually. The distinctive yellow coloration of salted radish roots develops during preservation (Rosa & Rosalinda, 2004). More recent research indicates a significant correlation between consumption of cruciferous vegetables, such as radish, and the risk for numerous malignancies (Verhoeven et al., 1996) where Epidemiological studies have shown a negative relationship between cruciferous plant consumption and the risk of developing stomach, colon, bladder, skin, and other cancers (Verhoeven et al., 1996). Therefore, researchers are progressively centering on traditional medicine in an effort to find fresh ideas for empowering treatments of viral and microbial diseases, as well as tumor (Srinivasan et al., 2001; Benkeblia, 2004).

This type is frequently used to treat respiratory and hepatic conditions. The antibacterial job of its extracts and its durability contribute to its efficacy in treating microbial illness, as claimed by conventional medicine (Paredes, 1984). Salmonella thyphosa, Pseudomonas aeruginosa, and

Bacillus subtilis were all susceptible to the root's juice's antibacterial properties. Moreover, Streptococcus mutans and Candida albicans were both sensitive to the ethanolic and aqueous tincture' effects while the entire plant's aqueous extract exhibits action against Sarcinia lutea and Staphylococcus epidermidis (Caceres, 1987). On the other hand, the leaves' aqueous extract demonstrated antiviral activity against the influenza virus. While in tests with Salmonella typhimurium TA98 and TA100, an aqueous extract of the roots displayed antimutagenic action.

Figure 2.4: Radish plant

2.10.1 Pigments

The characteristics yellow color of salted radish roots develops during preservation. The major pungent component of radish, 4-Methylthio-3-Butenyl-Glucosinolate (4-MTBG), is one of the key ingredients in the creation of the yellow color. Moreover, It is believed that the yellow compound 1-(2'-pyrrolidinethion3'-yl)-1, 2, 3, 4-tetrahydro—carboline-3-carboxilic acid was the condensation product of the breakdown of 4-methylthio-3-butenylisothiocyanate and L-

tryptophan. This carboline composite is thought to be crucial in the establishment of the yellow dye in radish roots that is salted (Ozawa et al., 1990).

2.10.2 Antimicrobial Activity of Radish

In vitro, Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi, and Bacillus subtilis growth was suppressed by the radish's crude juice. This common plant could provide a significant amount of antibacterial compounds (Abdou et al., 1972). When it came to suppressing Streptococcus mutans, radish plant watery extract performed much better than alcohol and antibiotic extract, but alcoholic extract than watery extract when it came to inhibiting Staph bacteria, according to a study that evaluated the composition of radish (Chiiad et al., 2018) to determine the interaction of the antibiotic (Gentamycin) and the watery and alcoholic extract of radish leaves on the suppression of microorganisms having caused gingivitis and dental caries. Tannins, carbohydrieds, glycosieds, resins, flavonoids, alkloids, and saponin are all present in the alcoholic extract, but only two compounds the coumarins and the terpines, are detected in the watery extract (Jawad, 1997).

Furthermore, with a minimum inhibitory concentration (MIC) of 30–60 g/ml, the cysteine-abundant peptides (Rs-AFP1 and Rs-AFP2) set apart from R. sativus demonstrated significant fungal killing activity against many fungal kinds. The loop connecting -strands 2 and 3 and the active portion of the antifungal protein appear to work together (Samblanx et al., 1996). Moreover, following breakdown of the seed coat, Rs-AFPs are particularly freed during seed germination (Terras et al., 1995). Two pure antifungal proteins, RAP-1 and RAP2, were discovered in the seeds of Korean radish (R. sativus), and they inhibited the growth of Candida albicans and Saccharomyces cerevisiae (Jong-Heum et al., 2001).

2.11 Extraction of Ginger

Zingiber officinale's rhizome, which is employed as a spice, medicine, and luxury, is known as ginger. It gives its genus and family its name (Zingiberaceae) that also include other prominent members of this plant family like galangal, cardamom, and turmeric. A phytochemical analysis

of several different varieties of ginger rhizomes revealed the existence of bioactive substances including shogaols, phenylbutenoids, diarylheptanoids, flavanoids, diterpenoids, and sesquiterpenoids as well as gingerols, all of which function against bacteria (Sivasothy et al., 2011). Additionally, numerous studies have demonstrated their positive effects against disease symptoms, their inflammation suppressing role, anticancer, antiallergic, neuronal cell protecting, as well as their anti-fungal, and anti-bacterial activities (Mesomo et al., 2012).

The use of plant-based herb and spice extracts for food preservation and flavoring is currently gaining popularity (Hasan et al., 2012). Products made from ginger, such as ginger oil, are sold all over the world for use in food and drug manufacturing. The plant grows up to 2 feet tall and bears greenish yellow, orchid-like blossoms with a pungent perfume. It is a tropical plant, and both food and medicine are made from its underground stem (Mishra et al., 2013). Although ginger is primarily known as a digestive aid for treating indigestion and nausea, studies have been done on its common influence on joint health. To ease nausea, ginger essential oil can also be ingested or applied topically (Offei-Oknye et al., 2015).

Ginger is employed as a raw material, and a water steam distillation process is used to extract the volatile oil of the ginger's scent component. Centrifugal separation is used to obtain cold-pressed ginger oil, which has a strong flavor. The creation of the yellowish solid ginger extract involves the use of an excipient. The present invention's approach benefits from easy manufacturing technology and a high ginger usage rate (Mishra et al., 2013).

2.11.1 Ginger's Chemical Components

The abiotic stresses, which include environmental stress factors like light, moisture, temperature, soil nutrients, and ozone, as well as biotic stress factors like herbivores, insects, microorganisms, and human factors like pollution, could change the quality and quantity of the bioactive phytochemical compounds in the ginger rhizome, which could explain variations in antibacterial results. However, based on the mentioned factors, ginger comprises, approximately 50% carbs, 9% protein and free amino acids, 68% fatty acids and triglycerides, are found in the dry form of ginger (Leung, 1984; Tang, 1992).

2.11.2 Antithrombotic Activity of Ginger

Due to its ability to reduce thromboxane generation and platelet aggregation in vitro, ginger has been proven to have antithrombotic properties. In addition, gingerdione has been demonstrated to prevent arachidonic acid from generating 5-hydroxyeicosatetraenoic acid and prostaglandin. On the other hand, while dehydroparadol and gingerol appeared to promote the inhibition of cyclooxygenase, shogoal seemed to be a superior blocker of 5- HETE production (Thomson et al., 2002).

2.11.3 Antimicrobial and Antifungal Properties of Ginger

The volatile oil obtained from the ginger root is called ginger essential oil (GEO). It has a very wide range of development prospects in the pharmaceutical, culinary, and cosmetics industries because of its distinctive aroma and biological activity. In particular, GEO is frequently regarded as a safe natural substance that has potential use in the treatment of respiratory and gastrointestinal conditions. On the other hand, GEOs have been thoroughly studied, with an emphasis on their antibacterial, antifungal, and antioxidant properties (Ju et al., 2019). Disruption of the bacterial cell membrane, which results in the release of macromolecules like bacterial proteins and nucleic acids, is one way to define the antibacterial action of GEOs. This, in turn, causes a drop in bacterial metabolic activity and finally leads to bacterial cell death. In addition, through altering the expression of a few genes that code for important cell-lysing enzymes, the GEO therapy may have an impact on the physiological activities of organisms (Wang et al., 2020).

Additionally, P. microspora's in vitro fungal growth and spore germination were successfully suppressed by ginger oleoresin, which also had antifungal effects by altering membrane permeability, weakening membrane structure, and oxidizing membrane lipids. The pathogen is usually killed by the GO treatments because they changed the shape of the mycelia and increased the leakage of intercellular electrolytes, proteins, carbohydrates, and nuclein from P. microspore (Chen et al., 2018).

2.11.4 Cancer

Following ginger in order of anti-oxidant potency were turmeric, dry garlic, and fresh garlic. Because of its high antioxidant capacity, ginger is a well-known effective again to prevent cancer. As anti - carcinogenic dietary medicines, ginger components are thought to slow the activities exerted by cyclooxygenase and lypoxygenase, provoke cell death, and have an effect against tumor. Also, it was hypothesized that ginger suppresses enzymes like lypoxygenase, the only supply of energy for cells cancer of the prostate where these tissues die after approximately two hours in absence of 5-LO enzyme. The presence of specific components, including gingerol beside other components, incorporating shogaols, is believed to be the cause of ginger's anticancer effects. (Park et al., 2006).

According to Cancer Deterrence Examination, the primary functioning ingredients in ginger, gingerols, slow down the development of human colorectal cancer cells (Bode, 2003). Scientists discovered that its cytotoxicity was connected to activator protein inhibition or apoptosis induction. DNA pieces of fragmented single and multiple nucleosome sizes that shape a characteristic DNA hierarchy through gel electrophoresis are known as apoptosis.

Chapter Three

3. Material and Methods

3.1 Study Population

The study was done at the Department of Biology in Cihan University located in Erbil city. Samples which collected from patients presenting to Rzgari hospital, starting June 2022 till October 2022. Samples were taken randomly from 50 patients including males, females, and children from different age groups presenting with signs of surgical wound infection.

3.2 Inclusion Criteria

- A surgical lesion presenting purulent exude.
- Wounds with serous discharge and a negative culture of the sample but signs of infection are present concurrently such as erythema, warmth and pain at the site.
- Physician diagnosing the wound as a surgical site infection (SSIs) (Oslon, M., et al., 1984).

3.3 Relevant History

Information regarding patient's gender, concomitant diseases, diagnosis as well as the type of operation previously performed and the prescribed antibiotics were collected.

3.4 Specimen Collection

Samples from the secreted pus or exudate were collected from each patient's surgical wound by the mean of dual sterile swabs underneath aseptic settings, where one of the swabs was handled for smear guidance while the second was utilized for culture purposes.

3.4.1 Specimen Conveyance

The swabs (swab with corresponding media) were brought to the Department of Biology, Cihan University in Erbil. The specimens were kept at 4°c up to 3 days in the sample tube. During this time, the culture media was prepared and autoclaved in order to be ready for the inoculation process of the previously collected samples.

3.5 Identification

The proliferated bacteria following culture in the incubated plates were identified based on previously set characteristics including cultural, morphological and biochemical feature (Holt, et al., 1986).

3.5.1 Identification techniques

The well-established method for manual microorganism identification to the species level, bioMérieux's API identification products are test kits for identification of Gram positive and Gram-negative bacteria and yeast. API 20 is a standardized identification system uses 21 miniaturized biochemical tests and a database.

3.6 Antibiotics type

List of used antibiotics is illustrated in table 3.1.

Table 3.1: Antibiotics type.

NO	Name	Dose
1	Oxacillin	10 mcg
2	Tetracycline	10 mcg
3	Gentamicin	10 mcg
4	Ampicillin\Cloxacillin	25/5 mcg
5	Amoxicillin	25 mcg
6	Ceftazidime	30 mcg
7	Rifampin	5 mcg
8	Netilmicin	30 mcg

9	Amikacin	10 mcg
10	Chloramphenicol	10 mcg
11	Imipenem	10 mcg
12	Ciprofloxacin	10 mcg
13	Ceftriaxone	10 mcg

3.7 Sample processing

The First step was to isolate the organisms from pus samples and then to evaluate the susceptibility of detected bacteria to different antibiotics through culture. In order to identify the bacteria, gram staining procedure was used. All pus samples were directly inoculated on McConkey agar, and blood agar plates and incubated aerobically at 37ºC for 24-48 hours with CO2 concentration between 7 to 10%. After 24 to 48 hours of incubation, the resulting microorganisms were identified using standard tests employed to identify bacteria type such as Gram stain, catalase, and visualization of the slide under microscope, as well as tube and coagulase tests. Lastly, each sample was inoculated on plates using the four flame method. Inoculated culture plates were kept in the incubator at 37° C for 24-48 h. (Bannerman, 2003; Oslon et al., 1984).

3.7.1 Catalase test procedure:

A small amount of cultured specimen from the plate is placed on a decontaminated microscope slide. If using colonies from a blood agar plate, it is advised to proceed carefully while not scraping up any of the blood agar since blood cells are catalase positive and any contaminating agar could give a false positive results. Few drops of H2O2 onto the smear is added. If needed, mixing using a toothpick is possible. The use of metal loop or needles with H2O2 is not recommended because it will give a false positive following metal degradation. A positive result is the rapid generation of O2 evidenced by bubbles formation. A negative result is translated as the nonappearance of bubbles or the presence of only few scattered bubbles. Noting that the slide

should be disposed in the biohazard glass disposal container. In addition, any used toothpicks should be placed in the Pipet Keeper (Diagram 3-1).

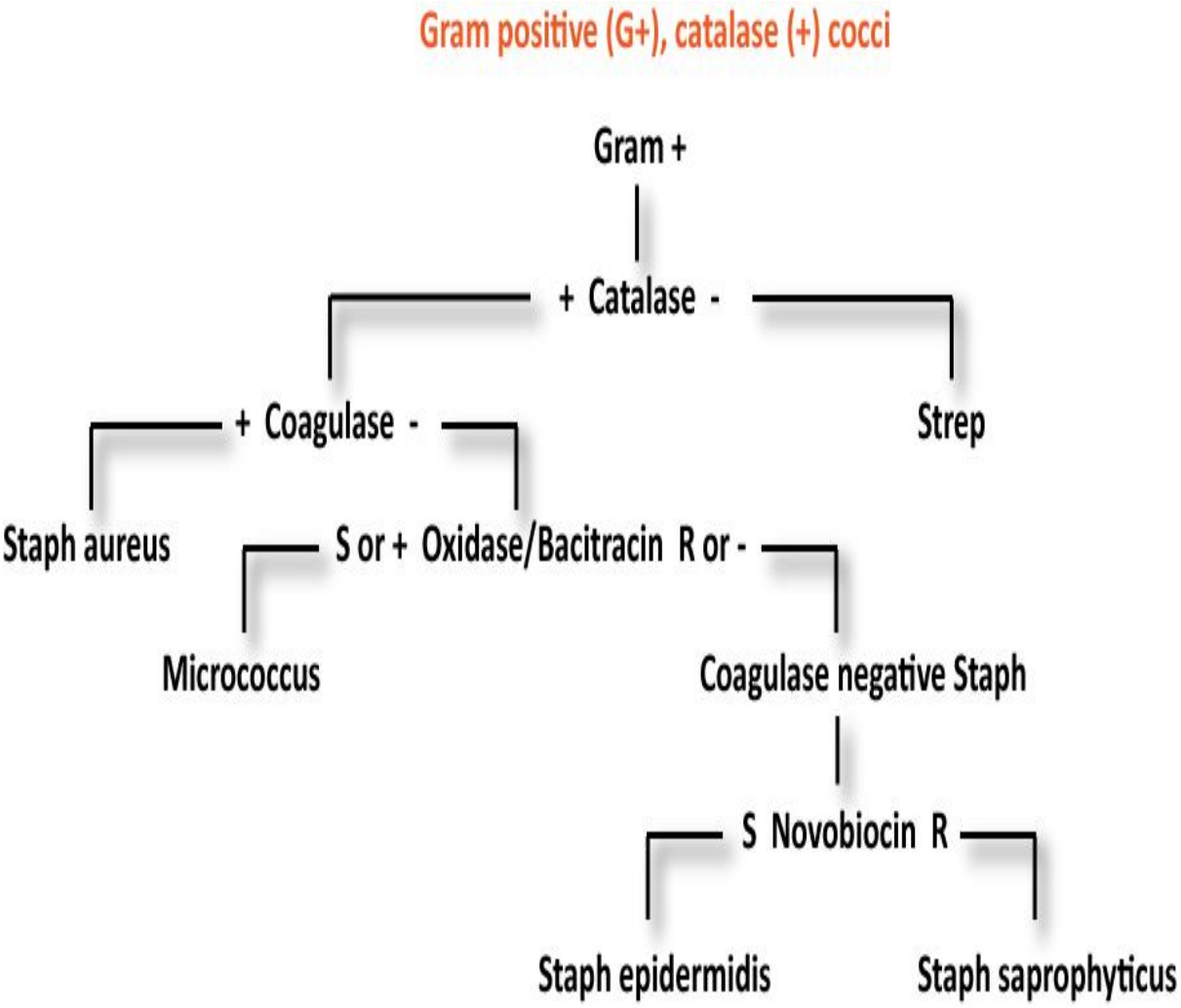


Diagram 3-1: Catalase test.

3.7.2 Gram Staining procedure:

The slide is first saturated with crystal (or gentian) violet for 60 seconds, followed by Gram's iodine flooding for 180 seconds. Carefully decolorizing step is performed Wash the smear with water after soaking it in 95% ethanol until the thinnest portions are colorless. The third stage is the most crucial step and the step that is frequently impacted by methodological variances in timing and reagents. Safranin recognized as the pink color stain (10% Fuchsine) is added to the slide for 60 seconds, followed by a washing step with water. The slide is then air dried, or placed on absorbent paper (Figure 3-1).

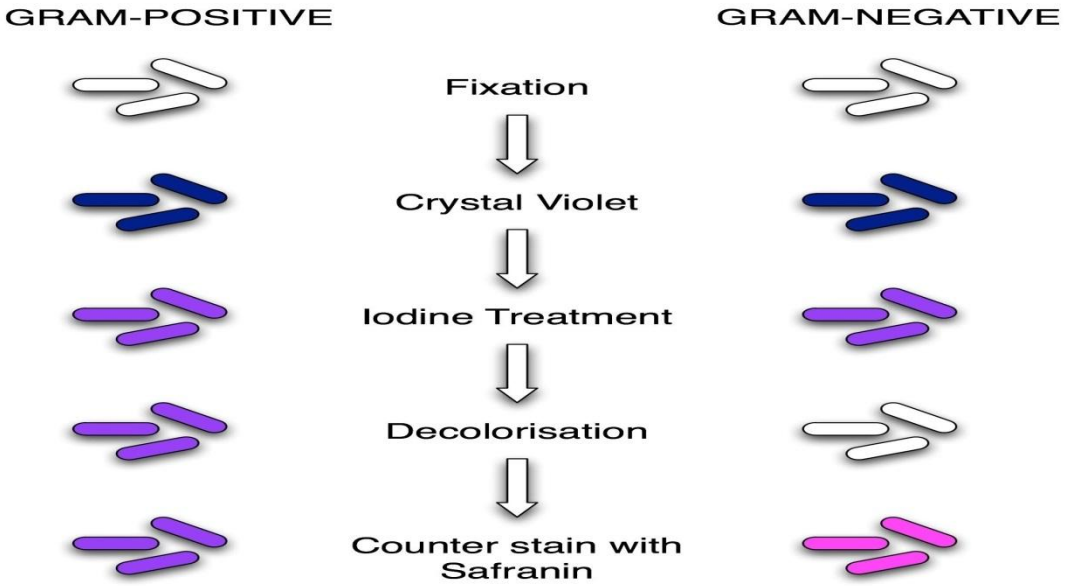


Figure 3-1: gram staining procedure.

3.7.3 Slide coagulase test

Two drops of distilled water are allocated on a clean glass slide. By labelling the slide, test strain (T) should be distinguished from the control (C). A clearly labeled slide is required for the control strains. The positive and negative control organisms are set on the uniform slide in order

to be examined simultaneously. Using emulsification, a homogeneous thick suspension of the test strain is produced. Insufficient viscosity will cause falsely negative responses in the suspension. Autoagglutination will be then observed. Strains which autoagglutination must be tested using an alternative procedure. A straight wire or loop is dipped in the plasma and then gently stirring process of the homogenous suspension is conducted. When using a reusable loop, sterilization of the loop before usage is required. It is important to note that plasma is added only to the test strain containing tested organisms but not to the control (C) as it serves as an autoagglutination control. Observation while waiting immediate white clumps is carried on. Positive result is considered as visible clumping within 10s, whereas negative result is non visible clumping within 10s (MacFaddin, 2000; Barrow & Feltham, 2003) (Figure 3-2).

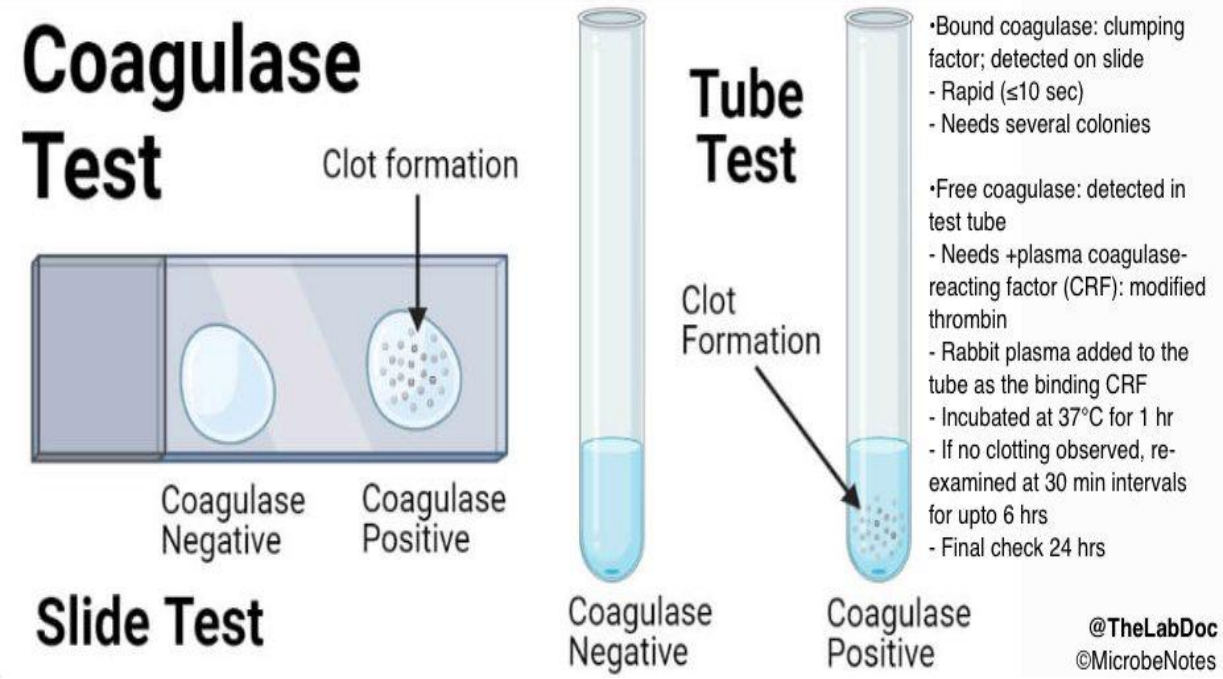


Figure 3-2: Slide coagulase test principle.

3.8 Antimicrobial Susceptibility Testing

Antibiotic vulnerability was assessed using the disks diffusion test, explained from Kirby Bauer technique. Antimicrobial containing discs were positioned on the agar plate within 15 minutes of inoculation using a sterilized forceps that were pushed tightly against the plate and then left at room temperature for 1 h to allow diffusion of the antibiotics into the agar medium. The plates were inverted and incubated for 18 to 24 hours at 35°C, with a CO2 concentration of 7 to 10%. The drugs were chosen, based on their action on a particular organism and also based on the antibiotic policy of the hospitals (Howard, 1994).

For *Staphylococcus aureus* the antimicrobial agents were: Oxacillin (10 mcg), Tetracycline (10 mcg), Chloramphenicol (10 mcg), Ceftazidime (30 mcg), Rifampin (5 mcg), Netilmicin (30 mcg) Ampicillin\Cloxacillin (25\5 mcg), Imipenem (10mcg), Ciprofloxacin (10 mcg), and Ceftriaxone (10 mcg). For *Lactobacilli* agents were Oxacillin (10 mcg), Tetracycline (10 mcg), Gentamicin (10 mcg), Amikacin (10 mcg), Amoxicillin (25 mcg), Ceftazidime (30 mcg), Rifampin (5 mcg), Netilmicin (30 mcg) Ampicillin\Cloxacillin (25\5 mcg). Lastly for *Epidermidis* the used agents were Netilmicin (30 mcg), Ampicillin\Cloxacillin (25\5mcg), Tetracycline (10 mcg), Chloramphenicol (10 mcg) and Ceftriaxone (10 mcg).

The diameter of the inhibitory zone was measured using a millimeter scale in accordance with the Kirby Baur interpretation chart after 18–24 hours of incubation. According to The National Committee for Clinical Laboratory Standards (NCCLS), now titled the Clinical and Laboratory Standards Institute (CLSI) standards, the zone size around each antimicrobial disc was classified as sensitive, moderate, or resistant. The test of diffusion in the agar was directed according to the CLSI recommendations (CLSI, 2006), by using Mueller-Hinton Agar and antibiotic discs (Howard, 1994).

3.9 ginger extraction

Ginger worked on in this experiment were gotten from the Erbil town markets. In order to identify the antimicrobial activity of the ginger against *Staphylococcus aureus*, *Staphylococcus Epidermidis* and *Lactobacillus*, ginger extracts were used (figure 3.3).

Figure 3.3: ginger extraction machine

3.9.1 Preparation of ginger extracts

The mixture of 40 cc of soybean oil and ten grams of ginger powder was cooked in a water bath for half hour, allowed to cool to room temperature, and then purified using Whatman filter paper, and lastly saved in a sterile container in order to be used later on. Before using extracts in a disc diffusion assay, they were stored at 4°C to retain their antibacterial property, using a concentration of 1.25 mg/ml (Diagram 3.2).

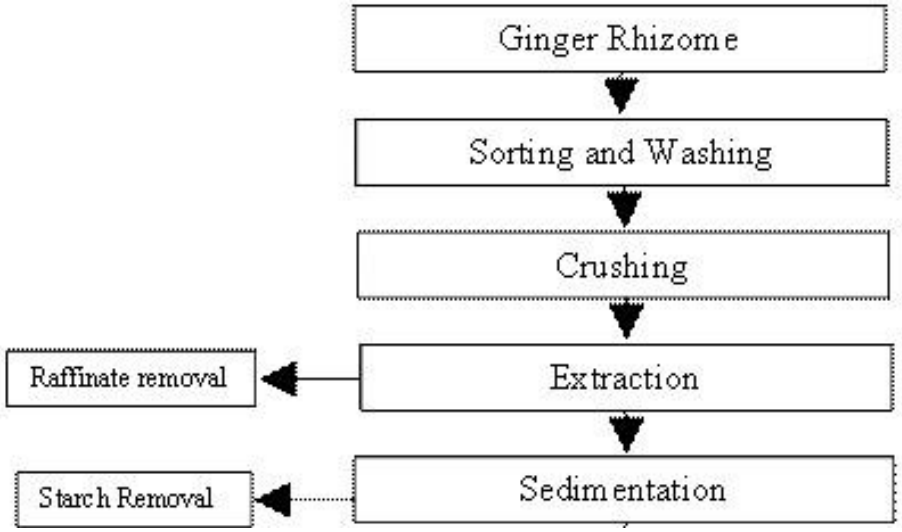


Diagram 3.2: Steam distillation method of ginger oil extraction.

3.9.2 Antibacterial assay

A punch machine was employed to create 6mm-diameter filter paper discs. In order to be used later, in a dry heat sterilizer, filter paper discs were disinfected, then kept in the fridge. With the aid of a clean cotton swab from the inoculum showing development of the 0.5 MacFerland standard, a uniform and uninterrupted layer of each organism isolate was created on MHA plates. In the laminar air flow cabinet, MHA plates were dried for 15 minutes and on these dried MHA plates, three to four discs of filter paper were stacked over one another, and ginger extract (twenty µl) was applied to each disc separately. Those plates were left at 37C° between 18 and 24 hours, and the areas of inhibition (defined as a distance from the top of the agar in millimeters) were then measured (Kamrul et al., 2014).

3.10 Radish extraction

R. sativus a white category of radish, was obtained from a local shop, lyophilized and crushed to a delicate powder preceding the active ingredient withdrawal using ethanol. A 300 mL solution of ethanol was stirred into approximately 10 g of powder form *R. sativus* root over the course of

24 hours at room temperature and 150 rpm speed. The extract was filtered through Whatman No. 4 paper. The residue was then re-extracted for a second time with additional 300 mL of ethanol. The combined extracts were then vaporized at 35 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to eliminate ethanol (Figure 3.4).

Figure 3.4: Radish root

3.10.1 Disc-diffusion assay

The disc diffusion experiment was also applied to determine the extract's activity against bacteria (Sokovic et al., 2008). Inoculums of the tested bacteria were arranged, equivalent to McFarland Standard 0.5. Uniform bacterial lawns were made using 100 µL inoculums on a Muller Hinton agar plate. Filter paper (Whatman) discs (5.0 mm) soaked with the tested concentrate was positioned over seeded plates. The plates were kept for 24 hours at 37 °C. Inhibitory zone (mm) was used to measure activity and then by deducting the disc diameter (5.0 mm) from the overall zone of inhibition displayed by the test disc in terms of clear zone all the way around the disc, the net zone of inhibition was calculated (Dejan et al., 2018) (Diagram 3.3).

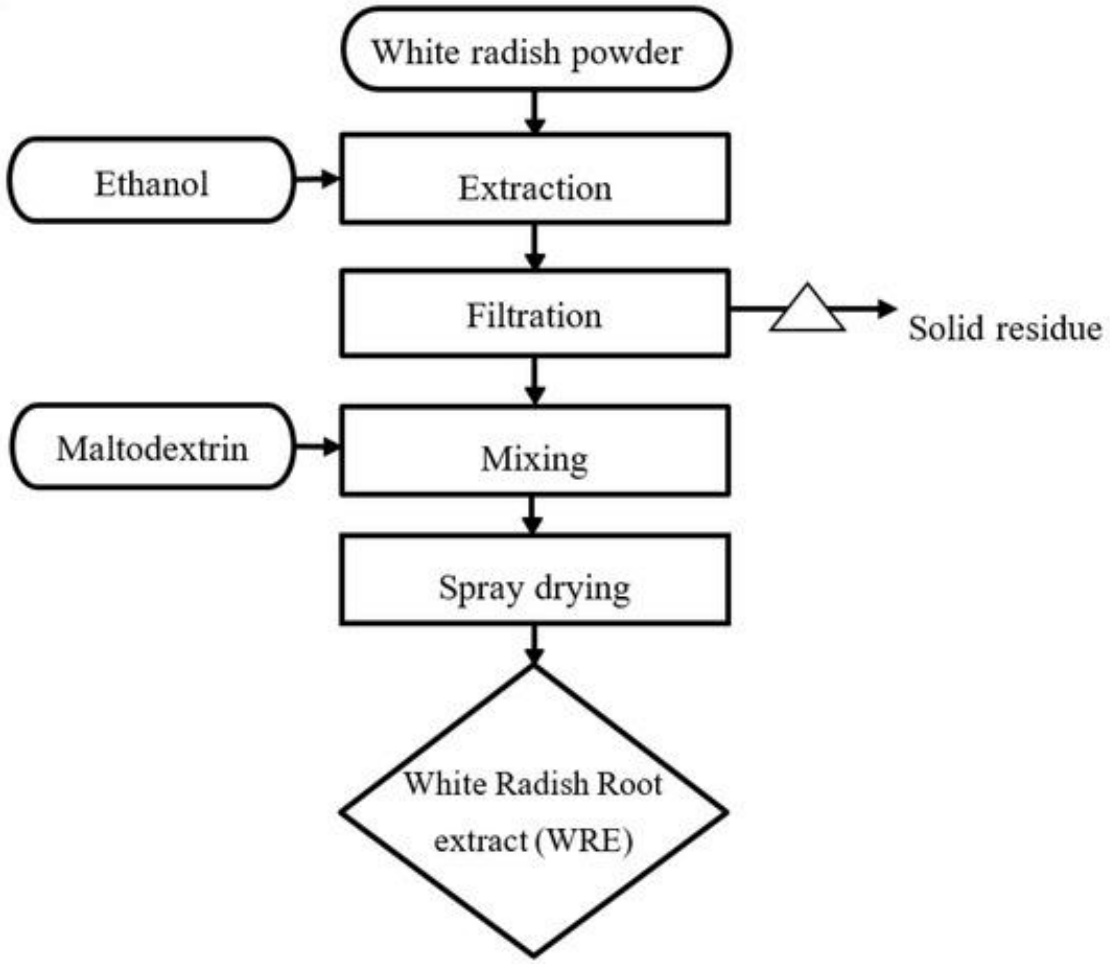


Diagram 3.3: Process of radish extraction.

3.11 Cinnamon Extraction

After cleaning the cinnamon spice with deionized water, it was dehydrated in sunlight for two consecutive days. A further dryness process was held in the oven at 400 C˚ for about an entire day. The dried material was subsequently pulverized into fine powdered matter by a grinder. Twenty grams of Cinnamon powder (weighed by the mean of an electronic scale of measure)

was moved into a 100 ml narrowed flask and supplemented by 40 ml of ethanol. A foil paper was applied to seal the conical flask, and then placed in a dark site for a maximum of seven days. Whatman No. 1 filter paper was used to filter the crude ethanol that had been extracted. Then it was concentrated under a 400 C˚ vacuum pressure by means of a rotating evaporator. The powder of (C.Z) was then solubilized with DMSO (dimethyl Sulfoxide) and several concentrations of the extract were formulated. The residual extracts were deposited in refrigerator at 4C° (Figure 3.5).

Figure 3.5: cinnamon bark

Using dimethyl Sulfoxide as the extractor, cinnamon crude extract was constituted in concentrations of 2.5%, 5%, and 10%. Bacterial solution was prepared in 0.90% of NaCl and equalized to 0.5 Mc, then spread over the complete surface of Muller Hinton agar using a pasteurized cotton. Paper discs soaked in various concentrations of cinnamon extract were placed on the bacterial lawn in a petri dish that was incubated at 37 ° C overnight. The diameter of the

clearing zone around the paper disk, plus a 0.5 cm subtraction, was used to measure the inhibition zone (Syahdiana et al., 2018) (Diagram 3.4).

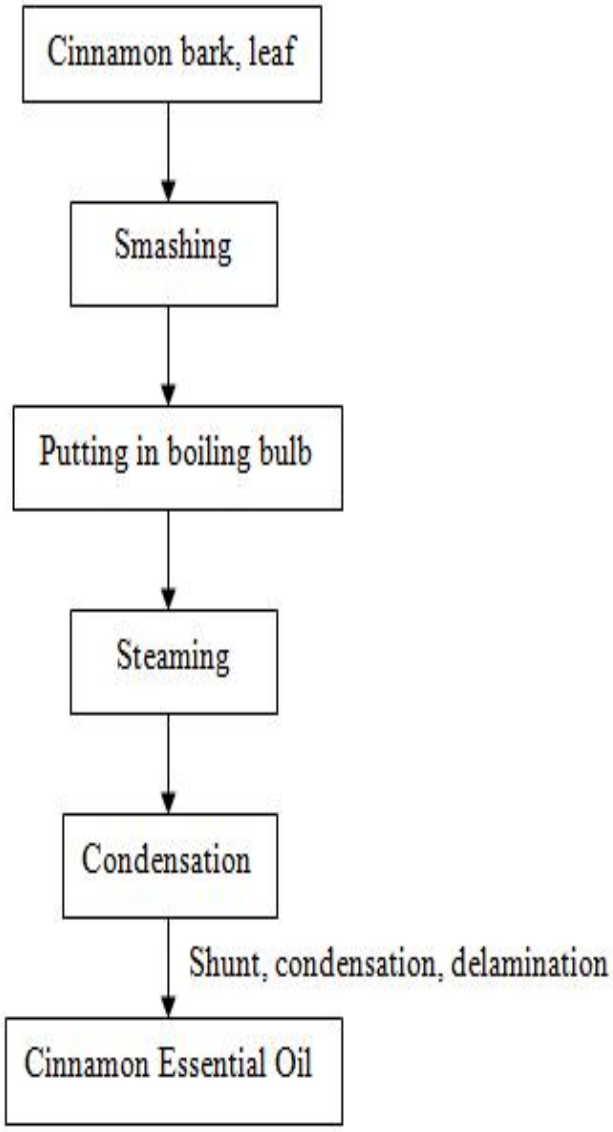


Diagram 3.4: Cinnamon essential oil process.

Chapter Four

4.1 Results

4.1.1 Samples of surgical wound infection

Out of the 50 samples processed for culture testing, bacteria was detected in 29 sample (58%), while 21 sample (42. %) were negative for the presence of bacterial infection (Table 4.1) (Table 4.2) (Figure 4.1). although 52% of the patients were females and 48% were male patients, positive results were more common in man where 52% of sample identified as positive belonged to male patients, whereas 48% of positive results belonged to female patients.

Table 4.1: Number of patients and cultured media for the sample of surgical site infection with the name of isolated organisms and the hospital as well as the patient's gender.

Serial No. Of patient	Media	Result and name of organism	Hospital	Sex	Age
1.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	42
2.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	35
3.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	18

4.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	25
5.	Macconky	Staphylococcousvsp.	Rzgai	Male	16
6.	Macconky	Staphylococcousvsp.	Rzgari	Female	10
7.	Macconky	Staphylococcousvsp.	Rzgari	Female	25
8.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	22
9.	Macconky& Blood	Staphylococcousvsp.	Rzgari	Female	34
10.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	23
11.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	27
12.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	12
13.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	18
14.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	41
15.	Macconky & Blood	Lactobacillus sp.	Rzgari	Male	34
16.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	37
17.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	18
18.	Macconky &	Staphylococcousvsp.	Rzgari	Male	25

	Blood				
19.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	22
20.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	17
21.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	13
22.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	15
23.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	18
24.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	25
25.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Female	23
26.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	21
27.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	37
28.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	34
29.	Macconky & Blood	Staphylococcousvsp.	Rzgari	Male	40
30.	No Growth	(-)ve	Rzgari	Male	51
31.	No	(-)ve	Rzgari	Female	26

	Growth				
32.	No Growth	(-)ve	Rzgari	Female	38
33.	No Growth	(-)ve	Rzgari	Female	46
34.	No Growth	(-)ve	Rzgari	Female	25
35.	No Growth	(-)ve	Rzgari	Male	24
36.	No Growth	(-)ve	Rzgari	Male	19
37.	No Growth	(-)ve	Rzgari	Female	16
38.	No Growth	(-)ve	Rzgari	Female	47
39.	No Growth	(-)ve	Rzgari	Female	34
40.	No Growth	(-)ve	Rzgari	Male	12
41.	No Growth	(-)ve	Rzgari	Male	29
42.	No Growth	(-)ve	Rzgari	Female	32
43.	No Growth	(-)ve	Rzgari	Female	41
44.	No	(-)ve	Rzgari	Female	53

	Growth				
45.	No Growth	(-)ve	Rzgari	Male	11
46.	No Growth	(-)ve	Rzgari	Male	33
47.	No Growth	(-)ve	Rzgari	Male	27
48.	No Growth	(-)ve	Rzgari	Male	21
49.	No Growth	(-)ve	Rzgari	Female	39
50.	No Growth	(-)ve	Rzgari	Female	31

Table 4.2: The frequency of wound infections at surgical sites

Number of patients	Positive case %	Negative case
50	58%	42%

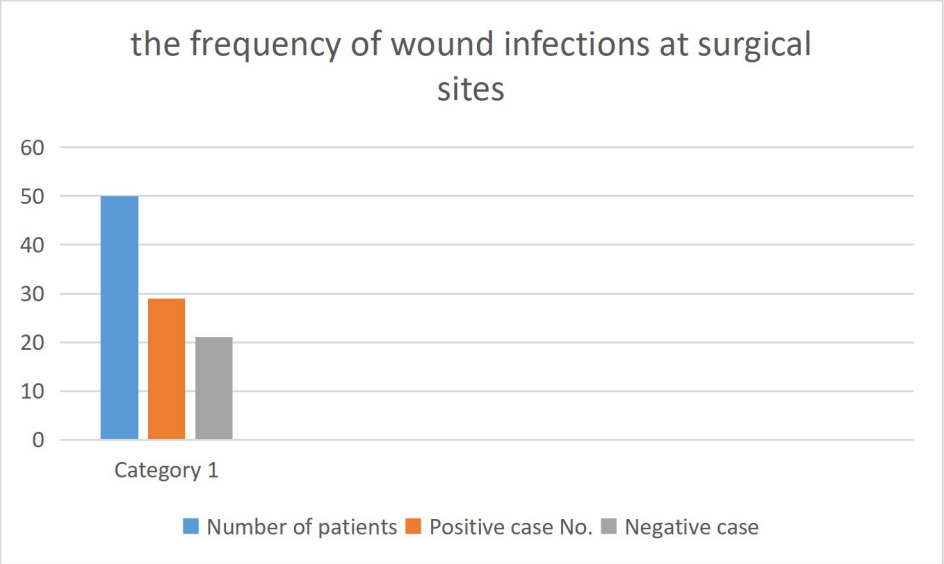


Figure 4.1: the frequency of wound infections at surgical sites

Table 4.3: Isolation result according to gender.

Isolation result	Number	Percentage	Positive	Percentage
Female	26	52. %	14	48%
Male	24	48 %	15	52%

In all of the detected 29 positive sample, infections were gram positive bacteria. However, 28 sample (96. 5%) were Staphylococcus spp. bacteria while only one (3.45%) Lactobacillus sp., was detected. However, the 28 bacteria of Staphylococcus spp. were identified using the API 20 kit method after checking the morphological, cultural characteristics and some of the biochemical tests which eventually ended up by giving 28 positive hits on Staphylococcus spp. 96% while Lactobacillus spp. showed only 1 positive hit respectively, that was probably due to the concentration of bacteria that was found in every 1ml counted which had a high activity to inhibit the growth *in-vitro* of all pathogenic gram-positive and gram-negative bacteria that was isolated from the wounds, which cause surgical wound infections. This indicated the therapeutic efficacy of bacteria (Table 4.4).

Table 4.4: Number of gram-positive and gram-negative bacteria that have been isolated.

Organisms	No. of isolates	Percentage %
Staphylococcus species	28	96,5%
Lactobacillus species	1	3,45 %

4.1.2 Plant extracts' antimicrobial properties and antibiotic susceptibility of *Staphylococcus species.*

The sensitivity pattern of *S. aureus* to the tested antibiotics was as following: Rifampin (20 mm), Tetracycline (16 mm), Netilmicin (34 mm) as well as Ampicillin/Cloxacillin (28 mm). However, the bacteria exhibited a pattern of resistance towards chloramphenicol, Oxacillin, Ciprofloxacin, Imipenem, Ceftazidime, and Ceftriaxone. (Figure 4.4, A, and B). Illustration in (Figure 4.3) represents *Staphylococcus aureus* control organism.

Each plant extract was tested against the isolated *Staphylococcus species* from infected samples. The results revealed that cinnamon lacks antibacterial activity against *Staphylococcus aureus*. Under the concentrations of (2.5%), (5%), and (10%) no inhibition zone was detected with any of the tested concentrations (Figure 4.5). on the other hand, the result presented in (figure 4.5) revealed the minimum inhibitory concentration (MIC) of ginger extract against *Staphylococcus species*, where dried ginger extracts showed some inhibitory effect on *S.* at a concentration of 1.25 mg/ml. Oil extract had no inhibitory effect on organism even at concentration of 10 mg/ml. Different concentrations of the radish root extract also had no inhibitory effect against *Staphylococcus species* (Figure 4.5).

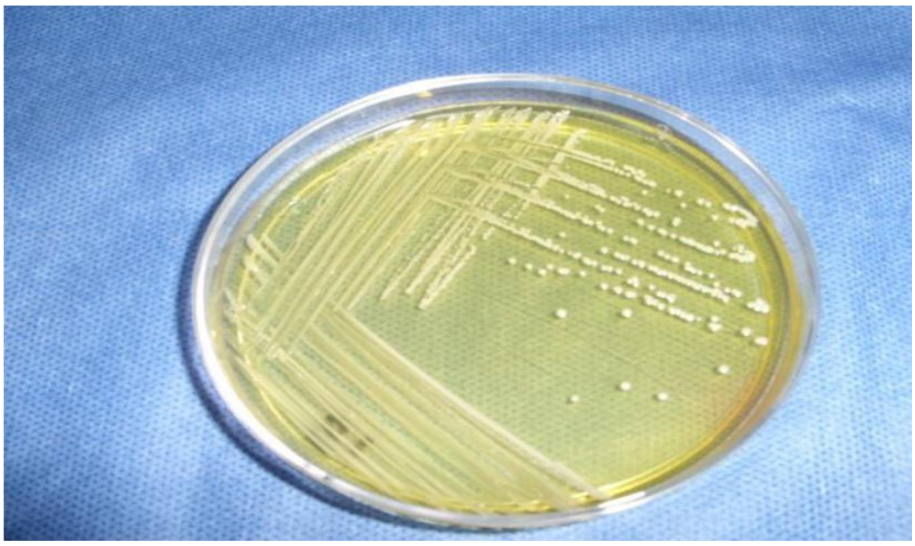


Figure 4.3: Staphylococcus species control

Figure 4.4: Antibiotic inhibition zone with staphylococcus species (A)

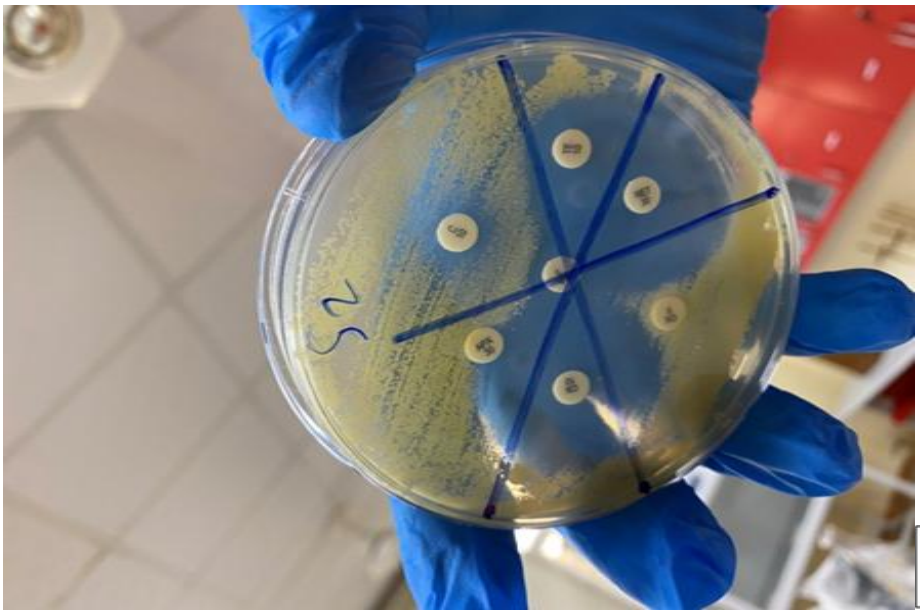
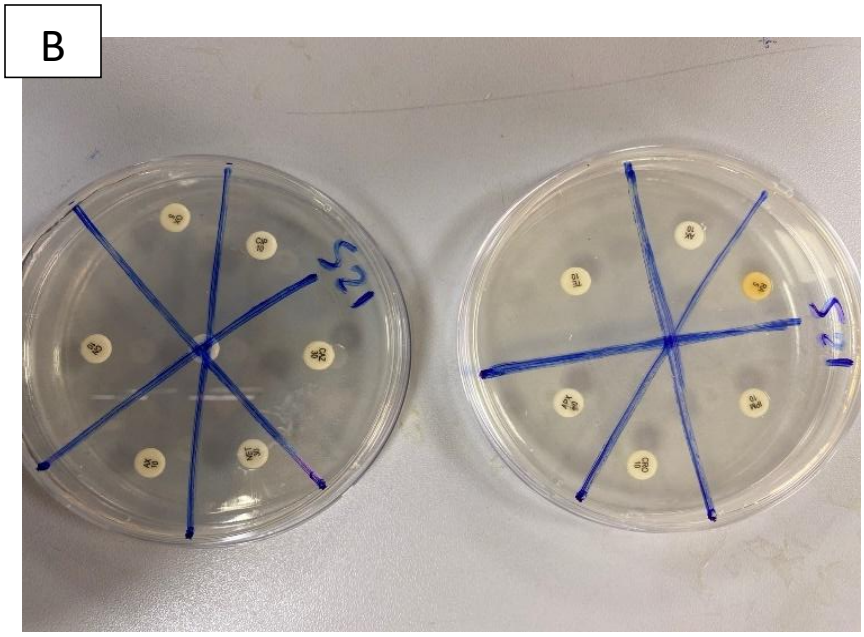


Figure 4.4: Antibiotic inhibition zone with Staphylococcus species (B)



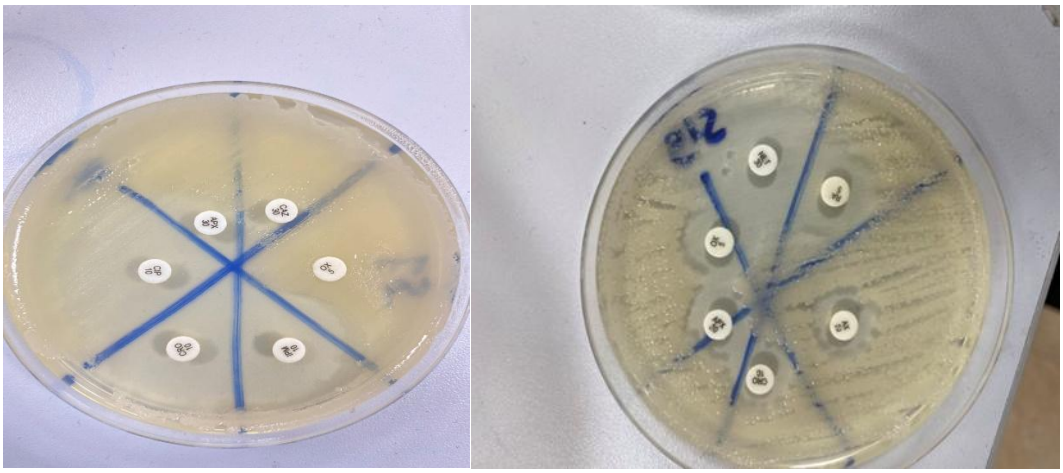
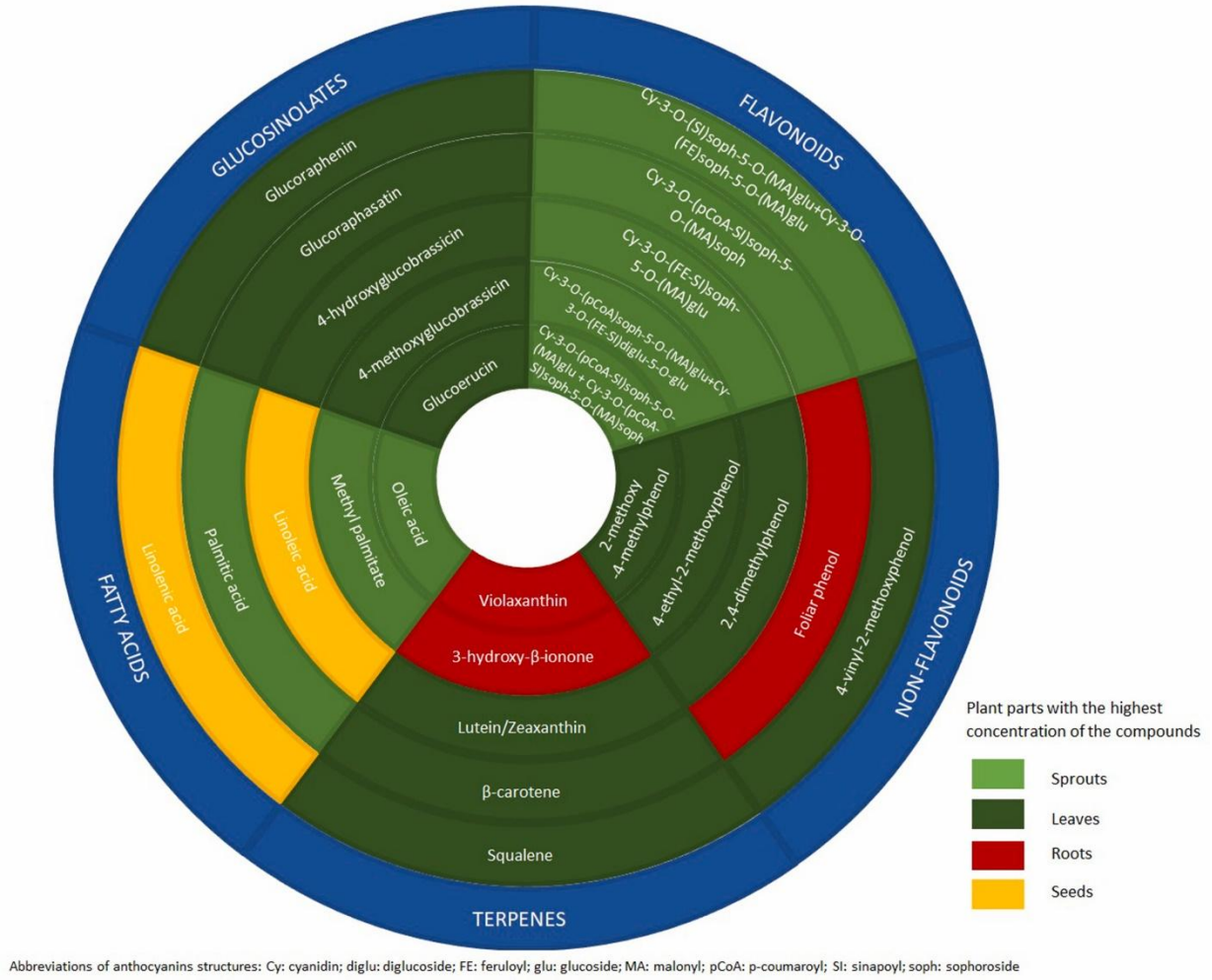


Figure 4.5: Inhibition zone of plants on *Staphylococcus species*

4.2 Gas Chromatography GC-MS of the most active compounds present in plant extract.

Phenolic compound present in the radish root extract were obtained by a combination of hexamethyldisiloxane and dimethylchlorosilane in pyridine, and showed a positive result for 3-Hydroxybenzoic acid, benzoic acid, gallic acid, syringic acid, isovanillic acid, protocatechuic acid, catechin, caffeine, epicatechin, quercetin found in the Radish root extracts in a 24% respectively.

Figure (4.6) shows the most active compounds of the roots of the extracted planted which was indicated by the Red color. While other parts were indicated by other colors like green and yellow.



Abbreviations of anthocyanins structures: Cy: cyanidin; diglu: diglucoside; FE: feruloyl; glu: glucoside; MA: malonyl; pCoA: p-coumaroyl; Sl: sinapoyl; soph: sophoroside

4.2.1 Gas Chromatography to detect molecules belonging to the phenolic class.

A GC-MS based on the Line#:1 R.Time:17.720(Scan#:2845) MassPeaks:303 RawMode:Averaged 17.715-17.725(2844-2846) BasePeak:131.05(7674) BG Mode:Calc. from Peak Group 1 - Event 1 Scan reported Compounds Names where (Z)-3-Phenylacrylaldehyde 2-Propenal, 3-phenyl-, (2Z)- 2-Propenal, 3-phenyl-, (Z)- (Z)-3-Phenyl-2-propenal (Z)-Cinnamaldehyde was confirmed as one of the most active compound presented in the extracted plant that was used against *Staphylococcus aureus* and other extracted bacterial (Figure 4.6).

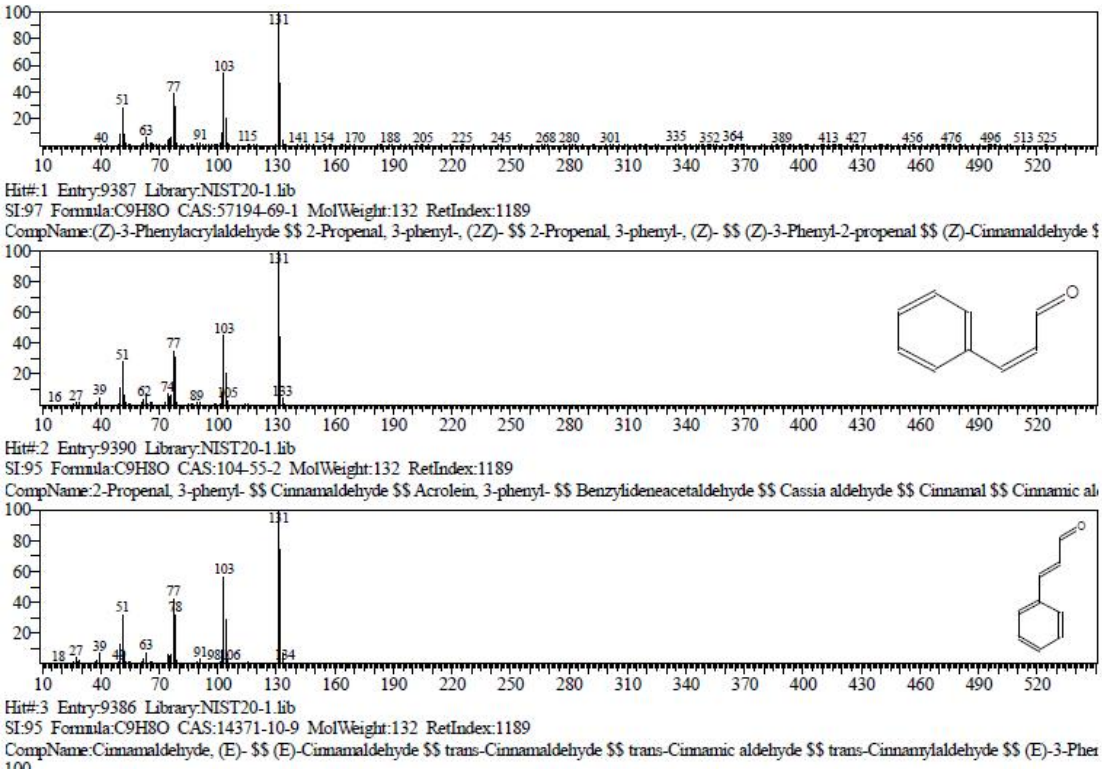


Figure 4.6: A chromatogram taken from the examination of a radish root sample is depicted in the upper picture (vegetative tissue). The MS ranges of frequently occurring TMS outcomes of catechin and other polyphenolic compounds.

At light Spectrum of 760 nm, Ascorbic acid, aromatic amines, and sugars are just a few of the other molecules with which the reagents for both processes react in addition to phenols.

Total phenolic quantification, total flavonoids, proanthocyanidin (condensed tannin) and hydrolysable tannin can also be performed by colorimetric approaches illustrated in appendix 2 for Cinnamon, Radish and ginger extract. Methanolic or ethanolic phenols removes of the plant combined with AlCl3 permit estimation of whole flavonoids concentration in the scale 410–423 nm. Proanthocyanidin levels are assessed using vanillin and dimethylaminocinnamaldehyde (DMCA) tests. (Naczk and Shahidi, 2004). The molecular mass of each extracted compound is provided in appendix 1.

Chapter V

Discussion

Wound infection continues to be a significant cause of morbidity and mortality in surgical patients specifically with the emergence of antibiotics resistance. At this time, bacterial infections that can fight multiple agents are seen as a universal health threat. As of 2019, the World Health Organization (WHO) and the CDC both have proclaimed that the humankind is at this time living in the “antibiotic apocalypse” period. Therefore, the dilemma of antibiotic resistance is now a significant healthcare issue. Gram positive aerobic cocci are the most frequent pathogens responsible for the infection of wound. However Gram negative bacilli and anaerobic bacteria are commonly involved in complicated diseases (Lakhundi & Zhang, 2018). As a result and in order to combat the threat of antibiotic resistance, efforts have been made while also looking into alternate sources of antimicrobial compounds, such as medicinal plants (Anand, et al., 2019).

Zhao and his colleagues, recently looked into the antibacterial properties of a polyphenol compound derived from sugarcane, with gallic acid being its main constituent and ferulic, coumaric, and chlorogenic acids following. With a MIC of 0.625 mg/mL, the tested mixture exhibited antibacterial activity against S. aureus strains. To test for conductivity changes and determine whether there is a correlation between the extract's antibacterial potential and the tested bacteria's membrane permeability, the scientists subjected S. aureus cell suspensions to sugarcane bagasse extract. In contrast to the control strains, they found increased conductivities in the extract-exposed cultures, suggesting that the separated components may impair bacterial covering unity and result in cellular electrolyte outflow. Furthermore, the scientists demonstrated that after an incubation process using a sub- repressive concentration of non-flavonoid polyphenols, phenolic acids also alter the shape of bacterial cells. The morphology of S. aureus after treatment with sugarcane bagasse extract was examined by the authors using scanning and communication electron microscopy. S. aureus cells that had been exposed to the extract displayed uneven surface wrinkles as well as breakdown, connection, and cluster of cellular

fragments or injured cells. All the modifications showed that the tested extract severely harmed S. aureus cells' exterior structure, causing cytoplasmic components to flow out of the cells (Zhao et al., 2015). As a result, the findings from scanning and transmission electron microscopy demonstrated that the residue from treating sugar cane has the potential to alter the geometry of staphylococcal cells and subsequently their structural properties.

Furthermore, Borges and his associates studied the antibacterial properties of ferulic and caffeic acids. With MIC values of 1250 _g/mL for caffeic acid and 1750 _g/mL for gallic acid, the instigators demonstrated that the two tested acids have antibacterial action towards the S. aureus sort. For both substances, MBC varied from 2500 to 5500 _g/mL. Similar to the previously noted study, they connected the bacterial cell wall destruction with subsequent cellular material leaking to the antibiotic effect of gallic and caffeic acids. (Borges et al., 2013).

The capacity of phenolic acids to interact with antibiotics is crucial to their antibacterial effects. In earlier research, reference and clinical S. aureus strains obtained from infected wounds were tested for the activity of protocatechuic acid ethyl ester (ethyl 3, 4-dihydroxybenzoate, EDHB) and caffeic acid (CA) against bacteria separately and combined together. It has been proven that EDHB has antibacterial action against strains of S. aureus used in clinical settings. The minimum inhibitory concentration range of EDHB for S. aureus type's alteration was 64 to 1024 _g/mL. Data achieved demonstrated strong interactions between clindamycin and EDHB. Since cefoxitin and EDHB appear to share the same binding site in the targeted bacterial cells, an interesting antagonistic tendency between the two drugs was seen. This can be explained by competitive interaction (Miklasin et al., 2015).

In our study, the prevalence of SSIs (surgical site infections) was 58%, with staphylococcus species being the most frequent causative agents and only one Lactobacillus sp. was detected. In India, surgical site infection rates range from 4 to 30%, according to studies (Agarwal, 1972; Rao and Harssha, 1975; Kowli, et al., 1985; Harbarth et al., 2008) with Methicillin- challenging S. aureus alone accounting for 5.1% infections affecting surgical wounds. Our results shows a higher rate of wound infection compared to the mentioned studies.

Our study also revealed that surgical wound infection are more common in males (52%) compared to females (48%), although more female were participating in the study. Similarly, an analysis of 438,050 surgical procedures from the German National Nosocomial Infections Surveillance System revealed gender-specific differences in surgical site infections where women had a lower SSI rate than males did (Langelotz, et al., 2014).

Moreover, our findings are in line with observations from the literature that S. aureus was the most prevalent isolate from postoperative wound infection (Nichols, 1998; Cruse & Foord, 1980). 71.42% of infections affecting surgical wounds contained S.aureus, the highly familiar isolated bacteria among the gram-positive bacteria, tailed by S.epidermidis (21.42%).

Over time, Staphylococcus aureus develops a relatively quick and effective resistance to several antibiotics. The low susceptibility of S. aureus to the following drugs, Tetracycline and Chloramphenicol, observed in the current investigation was consistent with studies conducted in Nigeria (Ndip, et al., 1997; Obiazi, et al., 2007) and Eritrean researchers (Naik & Teclu, 2009). S. aureus was sensitive to tetracycline and resistant to ceftriaxone, chloramphenicol, and imipenem. Additionally, S. aureus was resistant to ceftazidime, and ciprofloxacin, which is not in accordance with the report published by Lucia and her colleagues (Lucia et al., 2015).

S.epidermidis in detected samples exhibited resistance to Tetracycline, Ceftriaxone, and Chloramphenicol, which is consistent with data from previous literature (Roya & Hassan, 2019; Xiao, et al., 2011). Additionally, S. epidermidis was susceptible to Ampicillin, Cloxacillin, and Netilmicin (Sabath et al., 1976; Claus et al., 2004). Moreover, patient-isolated Proteus spp. were responsive to antibiotics like Netilmicin, Rifampin, Amikacin, Tetracycline, and Gentamicin and resistant to antibiotics like Oxacillin, Ampicillin/Cloxacillin, Ceftazidime, and Amoxicillin (Girlich, et al., 2020). In contrast, one study found that Proteae were the third utmost frequent UTI pathogen group in a Chinese hospital and these isolates had resistance rates of 8.6–9.8% to amikacin and 12.1–14.6% to levofloxacin (Yang, et al., 2018).

According to our findings, ginger, radish, and cinnamon exhibited high microbiological activities against S. aureus respectively. Similar to a previous study assessing the antimicrobial effect of cinnamon oil where Cinnamon essential oil exhibited the strongest action against staphylococcus aureus followed by E. coli among the three studied essential oils (Khaldoon, et al., 2016).

Addition, a recent study revealed that Radish leaves' alcoholic extract has a powerful inhibitory effect on *S. aureus* (Jaafar, et al., 2020).

Lastly although our findings indicate that ginger extract is ineffective in treating S. epidermidis, according to an investigation, ginger extracts were demonstrated to have therapeutic benefits, antibacterial action, and that the inhibition of bacterial growth was dose dependent (Malu, et al., 2009). Additionally, ginger ethanol and methanol extracts were more effective than ginger aqueous extracts against Shigella spp., S. epidermidis, E. coli, and S. aureus (Gull, et al., 2012). Our findings indicate that ginger extract is ineffective also in combating Proteus species. The growth of Proteus mirabilis was unaffected by ginger extract concentrations in any concentration.

The antibacterial properties of cinnamon have been linked to the existence of some active components, which may have diminished following grinding, exposure to air, or prolonged storage. (Simic, et al., 2014). According to past studies, cinnamon's antibacterial properties were presumably a combination of its oil and pure cinnamaldehyde.

Different biochemical mixtures can be separated, detected, and estimated using the instrumental approach known as gas chromatography mass spectrometry (GC/MS), which consists of a gas chromatograph (GC) connected to a mass spectrometer (MS). In this study several phenolic compound has been separated including tannin, flavonoids, syringic acid, caffeine, as well as protocatechuic acid, catechin, and quercetin and epicatechin. This finding were similar to the findings of a recent examination where at least five phenolic compounds were detected including syringic acid and gallic acid (Aissani & Sebai, 2022).

This study has some limitation to be mentioned, starting with small number of collected sample and the collection of samples from a single center hindering the ability to generalize the results.

In addition, the presence or long-lasting preservation of particular active ingredients, which have been diminished by melting and display to the atmosphere, might have attributed to the diminished cinnamon's antibacterial effects. Lastly, due to the transfer of the swabs, the pus specimens' negative results may not have been accurate and the bacteria may have already been lifeless. It might be a particular bacterial kind that doesn't thrive in these conditions. Taking into account that in the incubator, anaerobic bacteria cannot grow aerobically without specific media.

Hospitals must recognize the growing threat associated with infections and take quick action to control them because hospital infections are preventable.

Chapter IV
Conclusion and Recommendations

Despite the fact that this study showed a limited or no antimicrobial activities of ginger, cinnamon or radish extract against bacteria isolated from infected surgical wound, the results of this study provide a better evaluation of the microbiological origin of surgical site infections (SSIs) presenting to hospitals, which may have consequences on the management and treatment, since choosing the most effective empirical treatment for each patient requires an understanding of the microbiological epidemiology at each institution. More studies should be conducted owing to the fact that natural compounds are considered cheaper antibacterial alternative compounds and the antimicrobial ability of natural assortments suggests a broad scale of chances for novel antibacterial cures. In addition, to determine the utility of these antibacterial drugs in the clinical setting, additional research should concentrate on in vivo experiments and clinical studies. Staphylococcus aureus was the most frequent surgical site infection isolated, and these results can be validated due to the fact that S. aureus is a typical normal flora colonizing the skin.

Based on its ability to isolate and distinguish relevant chemical components, Gas C-MS is commonly used to assess the substance complication of analytical tasters. The methods for identifying biological activities and perturbations of biological systems have been made easier by recent advancements in GC-MS technology, which has also increased its significance for diagnostics and quality evaluation objectives. However, since handling, systematized sample management, and analysis settings prerequisite to be precisely established and disciplined, working to lessen data fluctuation and enable quantitative results, the drawbacks of global GChromatography-MS metabolite sketching should be understood by personnel.

Recommendations

1 – It is better to analyze the phenolic groups quantitatively.

2 – The use of LCMS (Liquid Chromatography Mass Spectrometry) as a technique is suggested.

3 – Further plant extract from other countries or regions are recommended to be tested.

4 –Statistical analysis are also recommended in order to assess the presence of any significance.

5– *Staphylococcus aureus* accounts for a diversity of complaints, most commonly skin and soft tissue septicity (SSTIs), in addition to bacteremia, sepsis, peritonitis, pneumonia, and endocarditis, *Staphylococcus aureus*, due to the fact that staphylococcus species are normally colonization the human skin. Therefore, it should be highly suspected in patient presenting with symptoms of infection.

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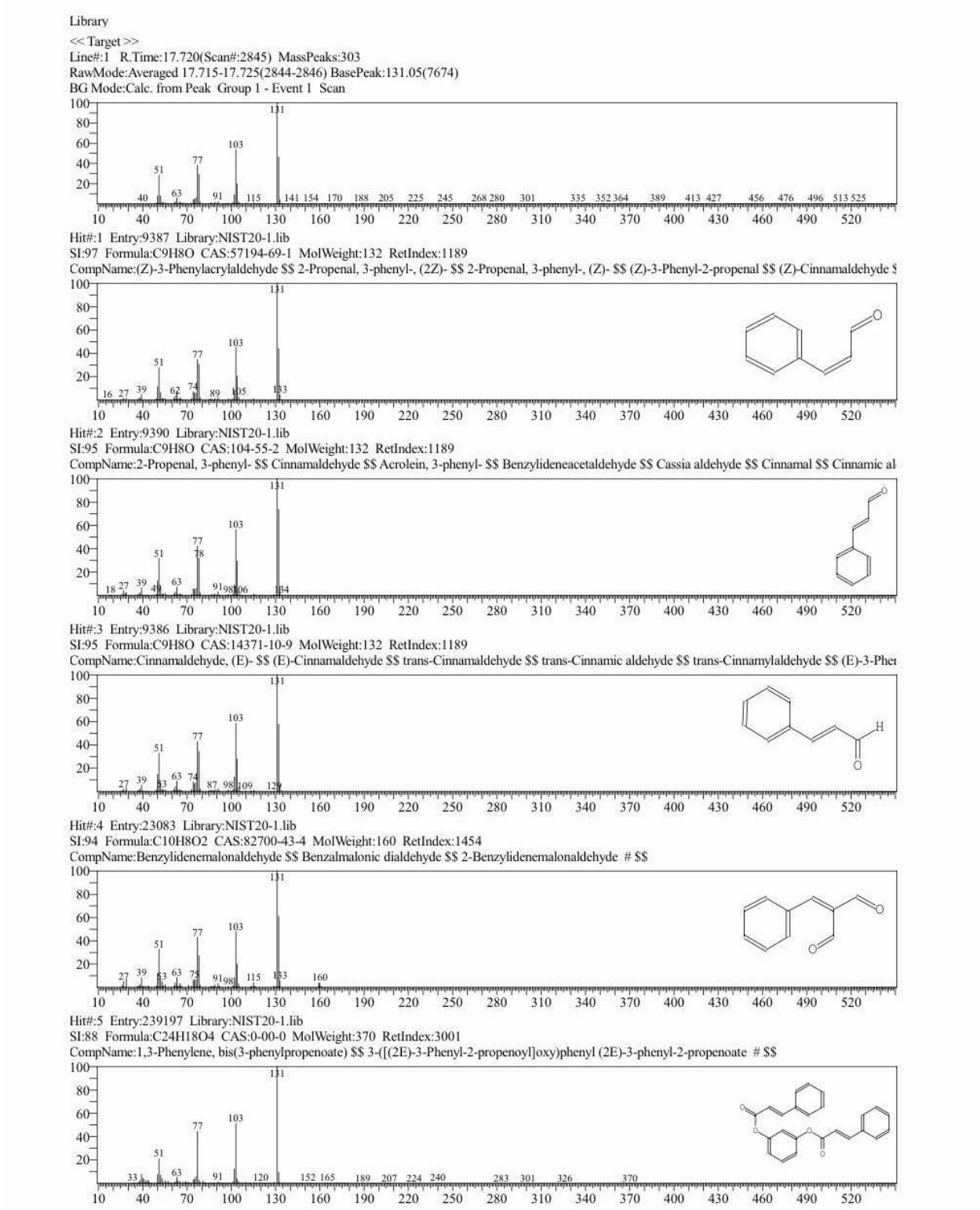
Appendixes

Appendix 1

Phenol GC-MS



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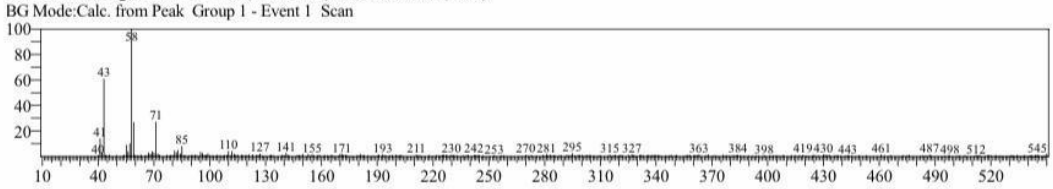

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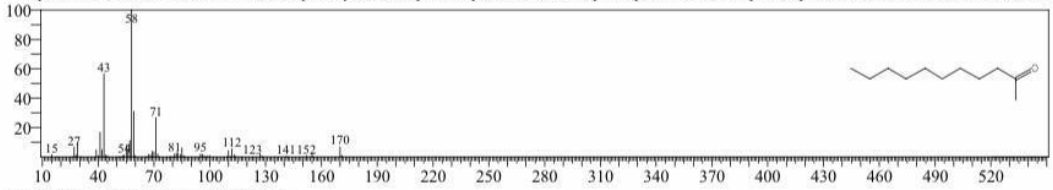

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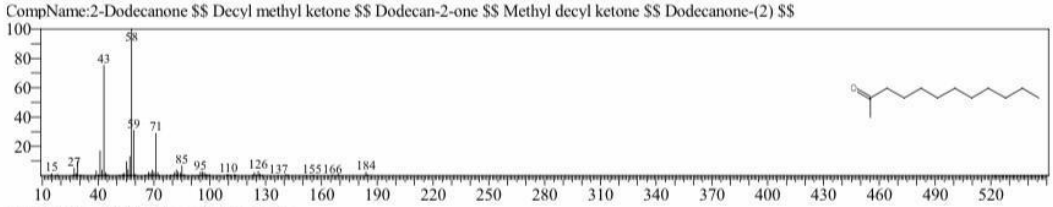

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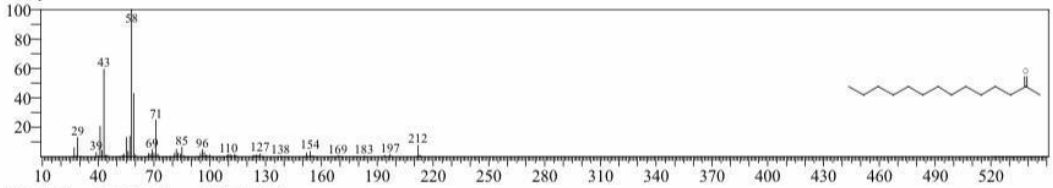

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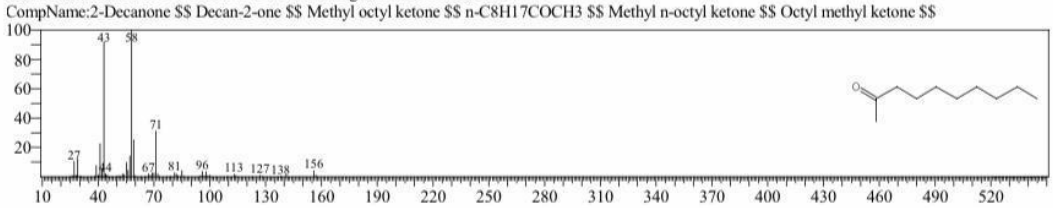

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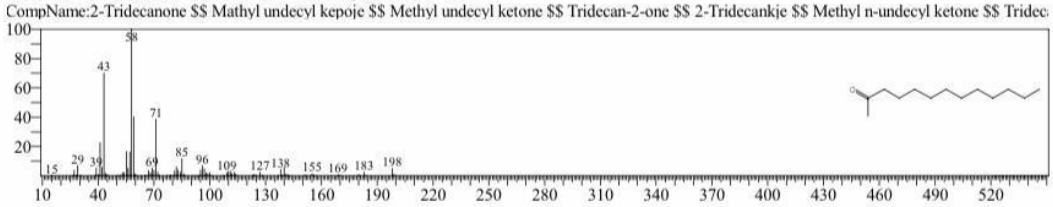

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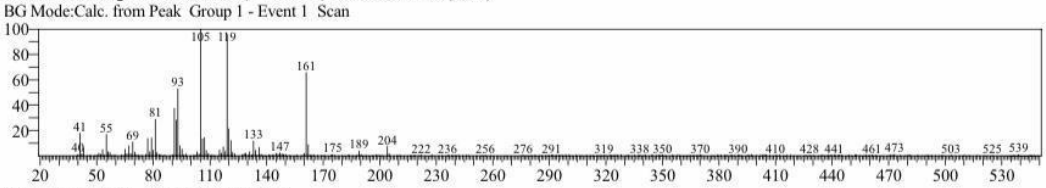

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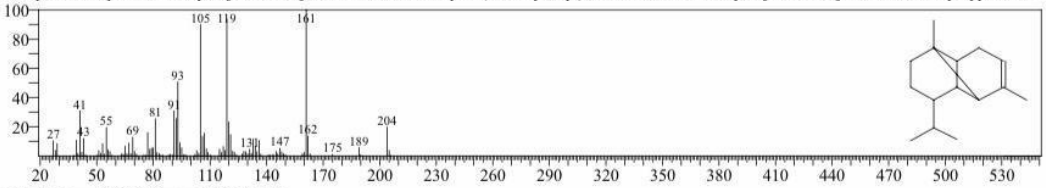

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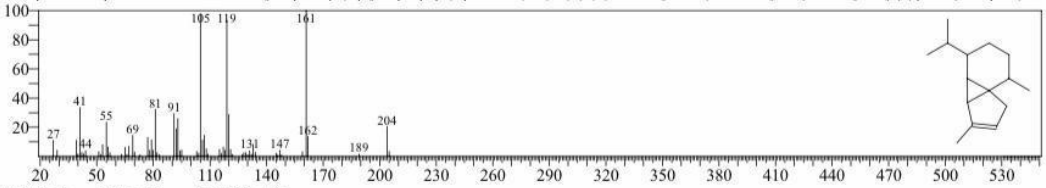

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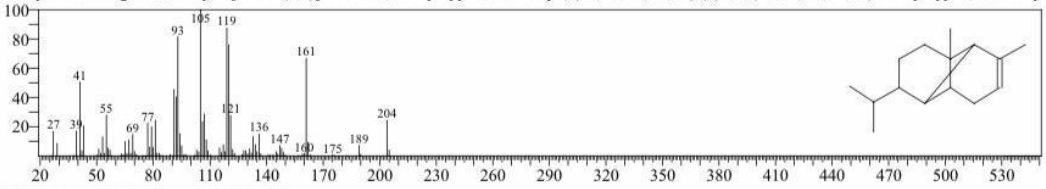
CompName:Ylangene $$ Tricyclo[4.4.0.0(2,7)]dec-3-ene, 8-isopropyl-1,3-dimethyl-, (1S,2R,6R,7R,8S)-(+)- $$ (1S,2R,6R,7R,8S)-8-Isopropyl-1,3-dimethy



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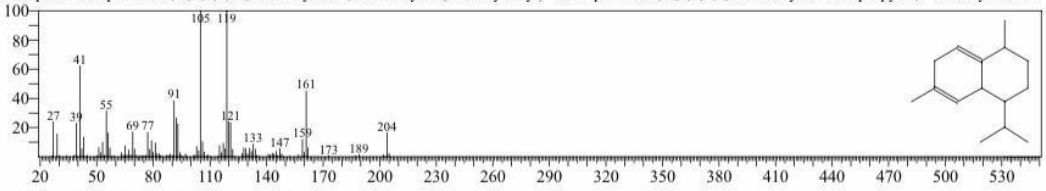

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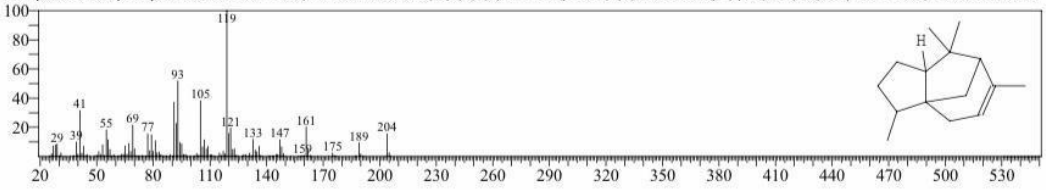

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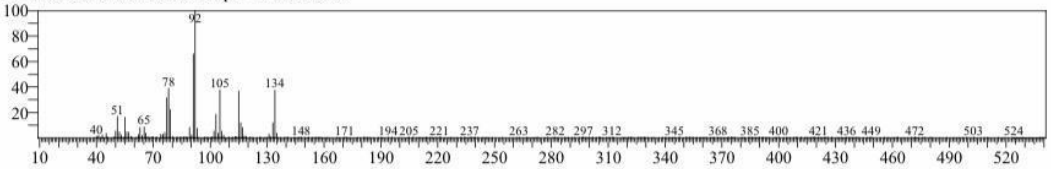
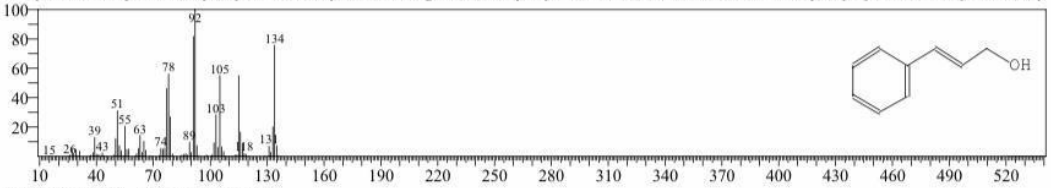
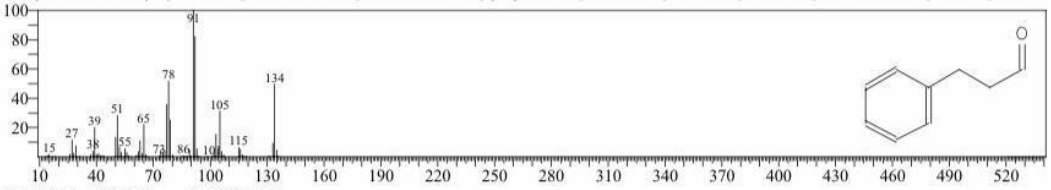


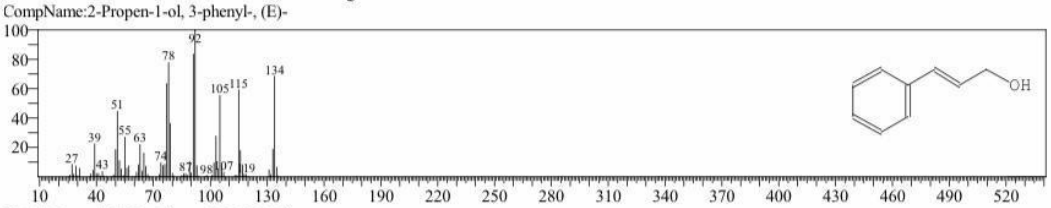
Hit#:1 Entry:9911 Library:NIST20-1.lib
SI:91 Formula:C9H10O CAS:104-54-1 MolWeight:134 RetIndex:1243
CompName:2-Propen-1-ol, 3-phenyl- $$ Cinnamyl alcohol $$ .gamma.-Phenylallyl alcohol $$ Cinnamic alcohol $$ Phenyl-2-propen-1-ol $$ Styrone $$ Sty



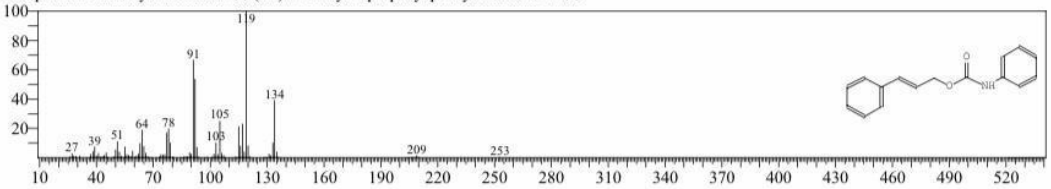
Hit#:2 Entry:9901 Library:NIST20-1.lib
SI:89 Formula:C9H10O CAS:104-53-0 MolWeight:134 RetIndex:1181
CompName:Benzenepropanal $$ Hydrocinnamaldehyde $$ .beta.-Phenylpropionaldehyde $$ Benzylacetaldehyde $$ Dihydrocinnamaldehyde $$ Hydrocinn



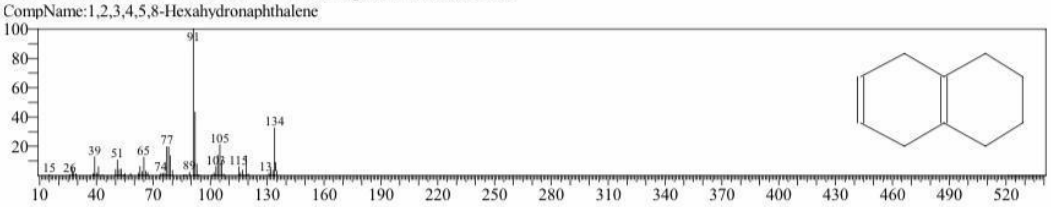
Hit#:3 Entry:9910 Library:NIST20-1.lib
SI:88 Formula:C9H10O CAS:4407-36-7 MolWeight:134 RetIndex:1243
CompName:2-Propen-1-ol, 3-phenyl-, (E)-



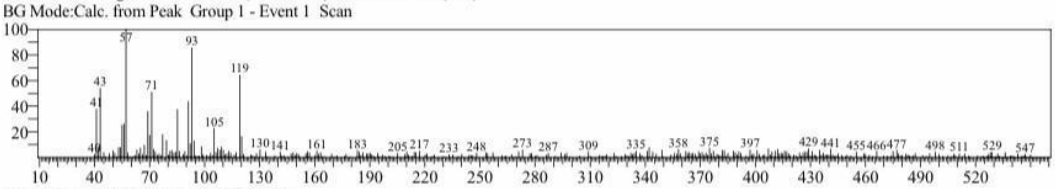
Hit#:4 Entry:108339 Library:NIST20-1.lib
SI:84 Formula:C16H15NO2 CAS:25076-44-2 MolWeight:253 RetIndex:2139
CompName:Cinnamyl carbanilate $$ (2E)-3-Phenyl-2-propenyl phenylcarbamate # $$



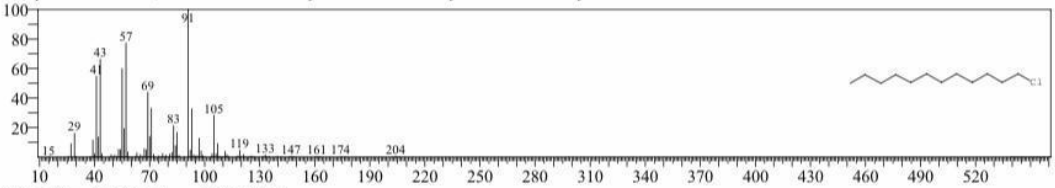
Hit#:5 Entry:9977 Library:NIST20-1.lib
SI:82 Formula:C10H14 CAS:36231-13-7 MolWeight:134 RetIndex:1122
CompName:1,2,3,4,5,8-Hexahydronaphthalene



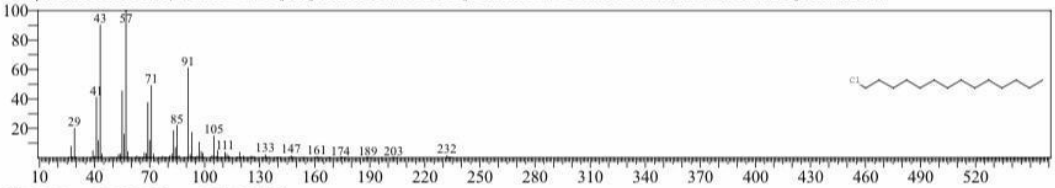
<< Target >>
Line#:5 R.Time:23.400(Scan#:3981) MassPeaks:327
RawMode:Averaged 23.395-23.405(3980-3982) BasePeak:57.05(632)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



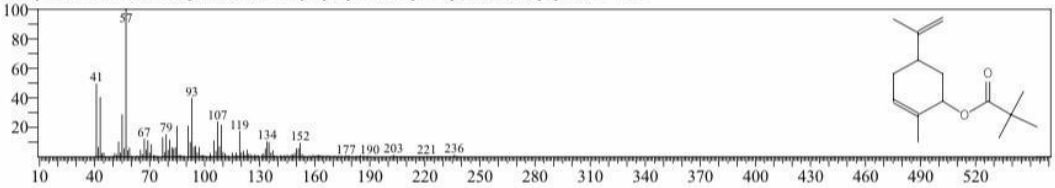
Hit#:1 Entry:56834 Library:NIST20-1.lib
SI:73 Formula:C12H25Cl CAS:112-52-7 MolWeight:204 RetIndex:1439
CompName:Dodecane, 1-chloro- $$ n-Dodecyl chloride $$ Dodecyl chloride $$ Lauryl chloride $$ 1-Chlorododecane $$



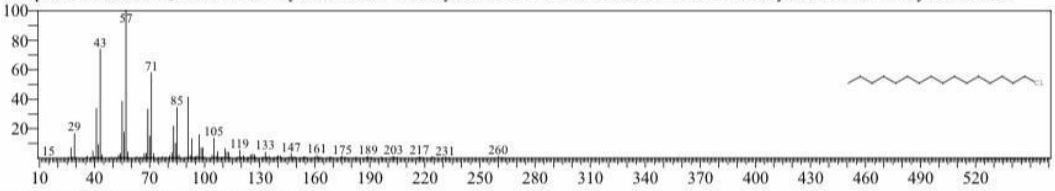
Hit#:2 Entry:85643 Library:NIST20-1.lib
SI:73 Formula:C14H29Cl CAS:2425-54-9 MolWeight:232 RetIndex:1638
CompName:Tetradecane, 1-chloro- $$ Myristyl chloride $$ Tetradecyl chloride $$ 1-Chlorotetradecane $$ n-Tetradecyl chloride $$



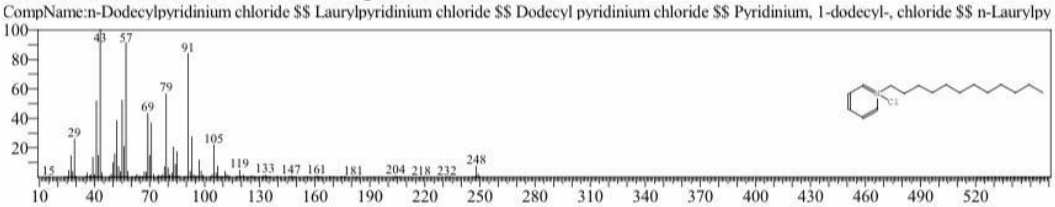
Hit#:3 Entry:89837 Library:NIST20-1.lib
SI:71 Formula:C15H24O2 CAS:0-00-0 MolWeight:236 RetIndex:1560
CompName:Limonen-6-ol, pivalate $$ 5-Isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate # $$



Hit#:4 Entry:116538 Library:NIST20-1.lib
SI:71 Formula:C16H33Cl CAS:4860-03-1 MolWeight:260 RetIndex:1837
CompName:Hexadecane, 1-chloro- $$ Cetyl chloride $$ Hexadecyl chloride $$ 1-Chlorohexadecane $$ n-Hexadecyl chloride $$ Palmityl chloride $$

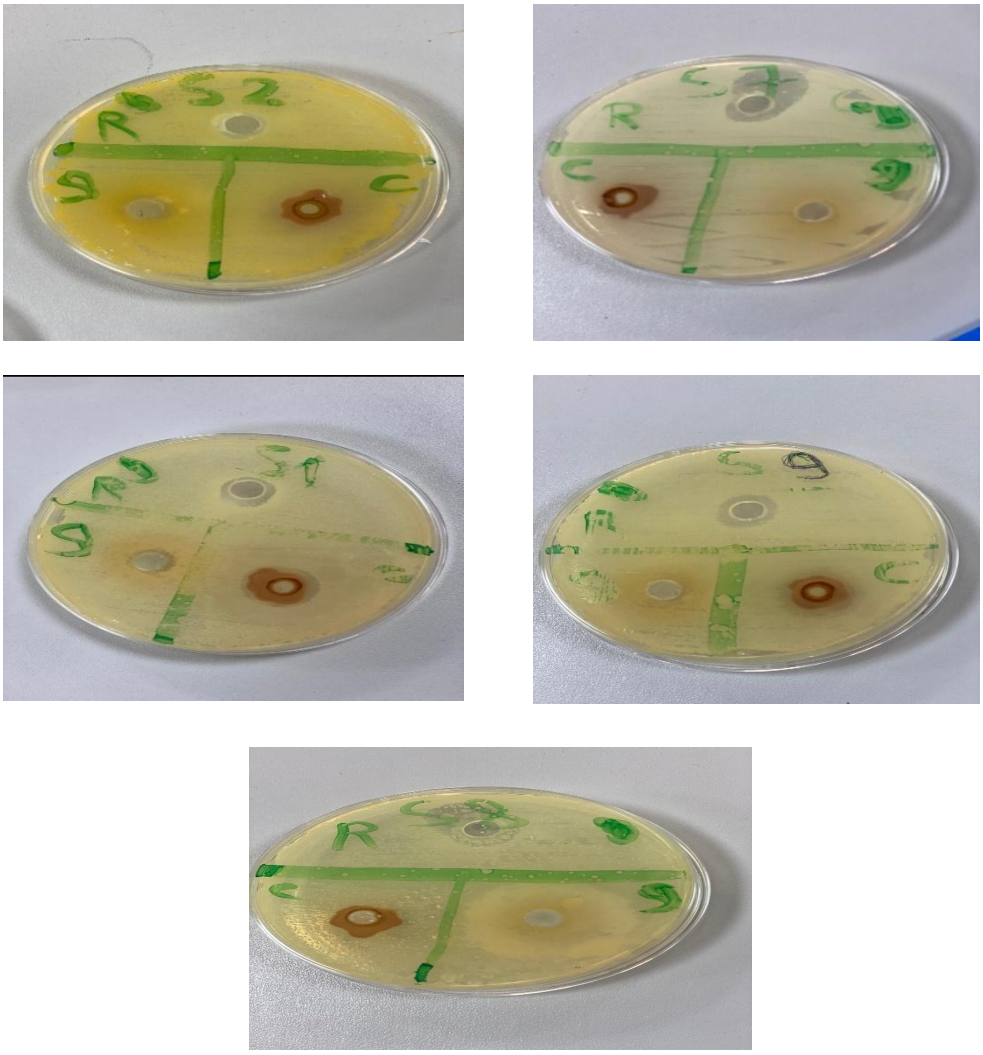


Hit#:5 Entry:143133 Library:NIST20-1.lib
SI:71 Formula:C17H30ClN CAS:104-74-5 MolWeight:283 RetIndex:0
CompName:n-Dodecylpyridinium chloride $$ Laurylpyridinium chloride $$ Dodecyl pyridinium chloride $$ Pyridinium, 1-dodecyl-, chloride $$ n-Laurylpy



Appendix 2

Well Diffusion of Plant Extraction



(Cinnamon, Radish, ginger)

Thesis

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2023	Near East University	Master in Medical Microbiology and Clinical Microbiology

Date	**TITLE**
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2020	Lab assistant european medical centre
2021	Medical microbiology lab of Cihan university

Foreign Languages	Reading comprehension	Speaking*	Writing*
ENGLISH	GOOD	GOOD	EXCELLENT
TURKISH	GOOD	GOOD	GOOD
Arabic	EXCELLENT	EXCELLENT	EXCELLENT
Kurdish	EXCELLENT	EXCELLENT	EXCELLENT
Persian	GOOD	GOOD	GOOD