



**THE ANTIBACTERIAL ACTIVITY OF THE METHANOLIC CRUDE EXTRACT FROM  
THE FRUIT OF *PYRUS COMMUNIS* (PEAR) FAMILY NAME *ROSACEAE***

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**ABSTRACT**

Antimicrobial Resistance (AMR) among infectious diseases is a growing public health challenge and poses a global health crisis. AMR threatens the effectiveness of prophylaxis and medication therapy of current antibiotic agents against pathogenic microorganisms. This hampers the control of infectious diseases resulting to increase in health care cost and risk of spreading resistant microorganisms in the community. The alarmingly prevalent health threat of Antimicrobial Resistance alters the therapeutic aid of antibacterial agents against deadly infections. One of the safe ways to combat and prevent the exacerbation of such threat is to provide further alternative substances with prospect of being more effective and powerful antibacterial agents. Health care in ancient times included the use of leaves, flowers, stems, berries and roots of herbs for their therapeutic or medicinal value. According to Indian Ayurvedic Medicine, the fruit of Pear is known to possess a lot of therapeutic activities such as anti-inflammatory, anti-hyperlipidemic, anti-diarrheal and anti-bacterial property. *The Pyrus Communis* of family Rosaceae and genus *Pyrus*, is a species of pear native and is commonly known as "white china pear" that can be mainly found in Asian countries, its origin is in Western China and is distributed all over the world. Pear fruit was procured in Sampaloc, Manila and about 100 g of Pear fruit were weighed and macerated with 80% methanol for 48 hours, filtered and evaporated to incipient dryness. The methanolic crude extract of *Pyrus Communis* (Pear) was investigated for its antibacterial potential against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus epidermidis* through bactericidal assay using agar well diffusion method. The methanolic crude extract of Pear showed minimum zone of inhibitions comparable to the standard, Cefepime, against the microorganisms.

**KEYWORDS:** Cefepime, Pear, Antimicrobial Resistance, Antibacterial.

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## CHAPTER 1

### THE PROBLEM AND ITS BACKGROUND

#### INTRODUCTION

The Antimicrobial Resistance (AMR) has become the widely monitored phenomenon that has infamously negated the efficacious effects of antibiotics, such resistance gives the ability of a microorganism to make some antimicrobial or antibiotic agent ineffective. The overgrowing cases of AMR pose a global threat to the healthcare community, another approach to combat such growing epidemic is to open and provide wide array of prospective antibacterial agents from various sources. It is therefore essential to give great priority in conducting studies that will contribute to the pool of effective antibacterial agents that are capable of ending the battle against the resistant bacteria. According to WHO in 2015, in the Philippine setting, over 14,000 cases of drug-resistant infections were detected in 2015 and by 2050, AMR could lead to 10 million deaths every year (WHO, 2015). One of the most abundant sources of discovering medicinal agents come from herbal plants and fruits. In the Indian Ayurvedic medicine, the fruit Pear is deemed to be the fruit of immortality, arising from the family Rosaceae which is known to possess antibacterial property. The *Pyrus Communis* (Pear) contains Arbutin, a powerful constituent exhibiting potent antibacterial property. The researchers have chosen to seek interest in the determination of the antibacterial property of *Pyrus Communis* (Pear) against common resistant pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* through bactericidal assay using agar well diffusion method.

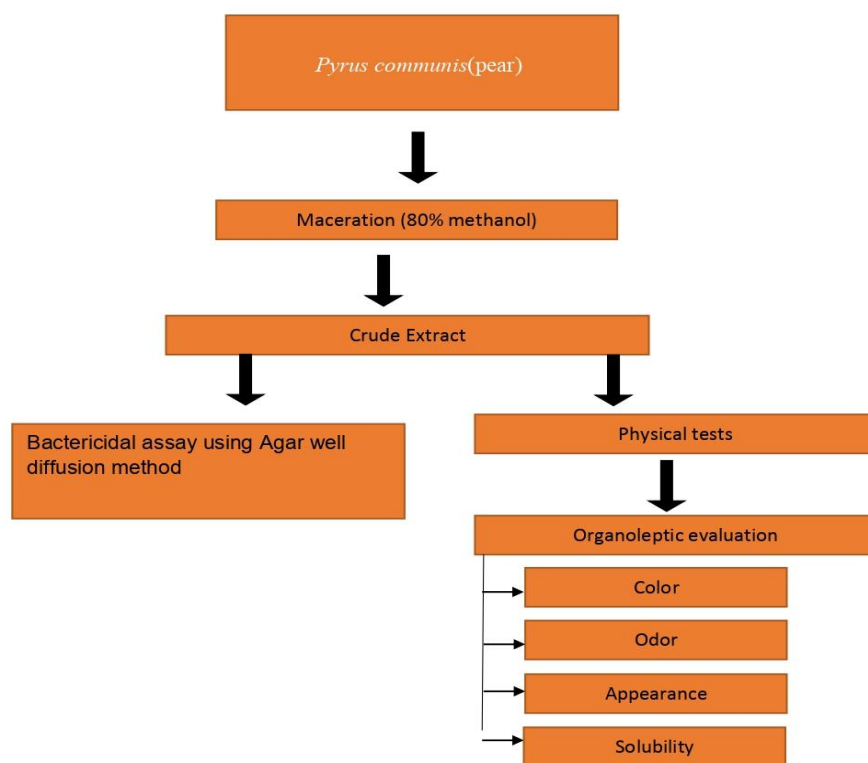
#### Background of the Study

In the worldwide setting, 490,000 people developed multi-drug resistant infections globally and drug

resistance is starting to complicate the fight against HIV and malaria, as well (WHO,2016). Furthermore, it is stated that “the most critical group includes multi-drug resistant bacteria that pose a particular threat in hospitals, nursing homes etc.” These bacteria have gone resistant to a large number of antibiotics, including carbapenems and third generation cephalosporins, the best available antibiotics for treating multi-drug resistant bacteria. However, in the Philippines, a country which currently have high malaria, HIV and TB cases, along with AMR, are expected to suffer more (DOH, 2017). The fruit Pear has been around for ages, hold 2<sup>nd</sup> rank after apple in nutrition amongst cultivated fruits. Its nutritional value does not end with that, further studies prove that it contains antibacterial activity because of the presence of phytoconstituent arbutin which is a bacteriostatic, when converted into hydroquinone in the body.

#### Conceptual Framework

This study was conducted by following such procedures or steps: outsourcing of active raw materials, reagents, and chemicals, collecting and garbling of pear, maceration of pear by 80% methanol, crude extraction and organoleptic testing and determination of the antibacterial activity of pear through bactericidal assay using agar well diffusion assay.



**Figure 1: The Research Paradigm.**

### General Objective

This research aimed to determine the antibacterial property of the methanolic crude extract of the fruit *Pyrus Communis* (Pear) in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus epidermidis* through bactericidal assay using agar well diffusion method.

### Specific Objectives

This research aimed to specifically

1. Determine the percentage yield of the crude extract
2. Conduct physical and organoleptic evaluation
3. Determine the zone of inhibition of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus epidermidis* using agar well diffusion assay
4. Compare the anti-bacterial activity of the pear methanolic crude extract against Cefepime as positive drug control.

### Hypothesis

The researchers would like to determine that the antibacterial activity of the methanolic crude extract of *Pyrus Communis* (Pear) has no significant difference with the standard drugs, Cefepime.

### Significance of the Study

It is essential to further contribute to a large pool of prospective anti-bacterial studies as it is deemed to be the only guaranteed measure to solve the already existing threat of Anti-Microbial Resistance (AMR). Antibiotic resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill

them. That means the germs are not killed and continue to grow. Infections caused by antibiotic-resistant germs are difficult, and sometimes impossible, to treat. In most cases, antibiotic-resistant infections require extended hospital stays, additional follow-up doctor visits, and costly and toxic alternatives (CDC, 2018). That is why, it is essential to find a solution and combat this growing health threat and thus, provide a rational drug therapy that can improve the quality of life of those people who are prone to getting resistant infections. One of the first line treatments to fight infection is through the aid of anti-bacterial drugs, the anti-bacterial is the first line weapon in fighting bacterial infections and have greatly benefited the health-related quality of human life. However, over the past few decades the antibiotics were under threat as many commonly used anti-bacterial drugs have become less and less effective against certain illnesses. The fruit pear as favored by many people as a delightful fruit comparable to apple, contains essential substances such as Vitamin C, quercetin and arbutin. According to Ranjeet Kaur, the powerful constituent, arbutin, is correlated with biochemical processes that operate as defense mechanism against bacterial invasion. Therefore acts as antibacterial too (Ranjeet Kaur, 2012).

### Scope and Delimitation

1. The study focused on the determination of the fruit of *Pyrus Communis* (Pear) in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus epidermidis* through bactericidal assay using agar well diffusion method.

2. The fruits of pear were procured from Sampaloc Market, Manila during the months of June - September 2018.
3. The crude was extracted and subjected to physical test and biological test.
4. The study determined the zone of inhibition (ZOI) of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus epidermidis* using agar well diffusion assay.
5. It also included the comparison of the obtained ZOI of standard drug, Cefepime, against the crude extract with concentrations of 50%, 75% and 100%.
6. Furthermore, the researchers disclaimed that they did not cover the phytochemical investigation, pharmacodynamics and pharmacokinetics properties of the crude extract of Pear.

#### Definition of Terms

The following terms were defined for the convenience and maximum comprehension of the readers.

**Anti-Bacterial.** It is an agent destroys or inhibits the action of bacteria.

**Agar Well Diffusion Method.** It refers to the movement of molecules through the matrix that is formed by the gelling of agar. When performed under controlled conditions, the degree of the molecule's movement can be related to the concentration of the molecule. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an antibacterial agent.

**Anti-Microbial Resistance (AMR).** It is resistance of a microorganism to an antimicrobial medicine to which it was originally sensitive.

**Bacillus subtilis.** It is known also as the hay bacillus or grass bacillus, it is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans.

**Escherichia coli.** It is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms.

**Pseudomonas aeruginosa.** It is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans.

**Pyrus Communis.** This is also known as common pear, a species of pear native to central and eastern Europe and southwest Asia.

**Staphylococcus aureus.** It is a type of bacteria. It stains Gram positive and is non-moving small round shaped or non-motile cocci.

**Staphylococcus epidermidis.** It is a gram-positive, coagulase-negative cocci that is a part of our normal flora.

**Terebinthinate.** It is an odor relating or resembling turpentine or a viscous sweet liquid.

**Zone of Inhibition (ZOI).** It is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth.

## CHAPTER 2

### REVIEW OF RELATED LITERATURE AND STUDIES

This chapter includes related literature and studies that provide source of information with regard to the study. This summary served as basis of knowledge about the antibacterial property of the crude extract from *Pyrus Communis* (Pear), family Rosaceae against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*.

#### Antimicrobial Resistance

Overuse and unnecessary use of antimicrobials for humans and animals which also promote the development and spread of resistance, either directly or through the environment so the most commonly reported resistant bacteria were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, which causes tuberculosis (TB), as WHO has been tracking it since 1994 and providing annual

updates in the Global tuberculosis report. (World Health Organization, 2005).

The Philippines has reported an alarming number of cases of drug-resistant infection According to the World Health Organization, resistant infections currently claim at least 50,000 lives annually across Europe and in the United States. Its data also indicated that in the Philippines, over 14,000 cases of drug-resistant infections were detected in 2015, and by 2050, AMR could lead to 10 million deaths every year. (WHO, 2018)

#### Antimicrobial agents

Different types of antimicrobials exist: antibiotics, anti-viral, anti-fungal, anti-protozoan etc. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources. Examples of those with a natural origin are phenyl

propanoids (chloramphenicol), polyketides (tetracycline), aminoglycosides (streptomycin, gentamycin), macrolides (erythromycin), glycopeptides (vancomycin) and second-generation  $\beta$ -lactams (cephalosporins). Those from synthetic sources are sulphonamides, quinolones and oxazolidinones. Most antibiotics exert their action either by inhibition of the bacterial cell wall or protein synthesis. Exceptions are the quinolones that inhibit DNA synthesis, and the sulphonamides that inhibit the synthesis of metabolites used for the synthesis of deoxyribonucleic acid (DNA) (Singh and Barrett 2006). Most anti-viral, anti-fungal, anti-protozoa and anti-cancer drugs however are obtained from synthetic sources. Because of the re-occurring resistance of pathogenic microorganisms to antibiotics, as well as the side effects presented by these antibiotics, investigation of other sources of antimicrobials, such as medicinal plants, for their antimicrobial properties is gaining ground. Plants produce secondary metabolites (phytochemicals), which have demonstrated their potential as antibacterials when used alone and as synergists or potentiators of other antibacterial agents. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could therefore be of use in the treatment of resistant bacteria (Abreu *et al.* 2012).

In an attempt to combat the various forms of disease that have continued to plague humans from time immemorial to this day, different types of antimicrobials have been developed to fight the pathogens responsible for these diseases. Antimicrobials, which are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesized derivatives (Cowan 1999), antimicrobial peptides produced by complex organisms as well as some microbes (Jenssen *et al.* 2006) and medicinal plants, which appear to be the focus of mainstream medicine today (Cowan 1999).

#### **Pyrus Communis exhibiting Anti-Bacterial Activity**

Fresh pear juice and aqueous extract of leaves show antibacterial activity against *Staphylococcus* and *Escherichia coli* because of the presence of phytoconstituent arbutin (bacteriostatic), which gets further, converted into hydroquinone in body. This hydroquinone also possesses anti-bacterial activity, boosts biochemical processes and operates defense mechanisms against bacteria invasion. Aqueous extract of young shoots of Pear show antibacterial activity as it contains hydroquinone. Ethyl acetate extract shows strongest antibacterial activity than other extracts. (Milind Parle, 2016).

According to Kıymet Güven, Ersin, Yücel & Ferda Cetintaş the antimicrobial activity of the ethyl acetate extracts from fruits of *Pyrus communis* was investigated by the agar well diffusion assay, and the extracts exhibited antimicrobial effect against most of the bacteria and all of the yeasts tested. (Kıymet Güven, 2008).

#### **Staphylococcus Aureus**

*Staphylococcus aureus* is a gram-positive bacteria with the shape of cocci and can also be classified as “grape like” clusters. The infection of these bacteria is common in community acquired as well as hospital acquired and one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. (Tracey A. Taylor; Chandrashekhara G. Unakal, 2017) *Staphylococcus aureus* is as well as food poisoning and toxic shocks (Perez *et al.*, 2009). The rate of mortality associated with *Staphylococcus* Gram-positive bacteria that cause diseases such as skin and soft tissues infections *aureus* in developing 16 world exceeds one of the developed countries (Nickerson *et al.*, 2009). The increasing use of antimicrobials against *Staphylococcus aureus* has led to the development of resistance hence need to develop new antimicrobial agents (Kwon *et al.*, 2007). Medicinal plant extracts have shown a wide range of antimicrobial activity against both bacterial and fungal pathogens (Manvi *et al.*, 2010). Studies carried out have shown that some edible plants extracts also have antimicrobial activity against *Staphylococcus aureus* (Alzoreky *et al.*, 2003). Other studies carried out have shown a great synergistic activity of plant extracts and spices when used against not only pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and other bacteria organisms, both Gram positive and Gram negative (Das *et al.*, 2012).

#### **Escherichia coli**

*Escherichia coli* are members of a large group of bacterial germs that inhabit the intestinal tract of humans and other warm-blooded animals (mammals, birds). Newborns have a sterile alimentary tract, which within two days becomes colonized with *E. coli*. commonly live in our intestines of healthy people and animals and most of the subspecies of the *Escherichia coli* are not detrimental but sometimes caused brief diarrhea, the most common type of *Escherichia coli*, particularly *Escherichia coli* 0157:H7 can cause intestinal infection and other strains causing intestinal sickness are called Shiga toxin-producing (STEC) after the toxin that they produce. (Jill- Seladi Schulman, 2017).

*Escherichia coli* are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic (Kaper *et al.*, 2004). When pathogenic they usually cause urinary tract infections, systematic infections and enteric infections (Mandell *et al.*, 2005). The development of resistance by *Escherichia coli* due to increasing in the use of antimicrobial agents has led to the use of medicinal plants extracts against it (Akram *et*

al., 2007). Medicinal plant extracts have shown to have antimicrobial activity against enteropathogenic *Escherichia coli* found in food material (Fullerton et al., 2011). Traditional products used in food preserving (spices) have antimicrobial activity against multiple antibiotic resistant *Escherichia coli* isolated from water (Rahman et al., 2011). Other studies carried out on plants with a medicinal value such as *Allium sativum* has shown antimicrobial activity against *Escherichia coli* (Ziarlarimi et al., 2011).

### Staphylococcus Epidermidis

Staphylococci are known as clustering Gram-positive cocci, nonmotile, non-spore forming facultatively anaerobic that classified in two main groups, coagulase-positive and coagulase-negative. *Staphylococcus epidermidis* with the highest percentage has the prominent role among coagulase-negative Staphylococci that is the most important reason of clinical infections. Due to various virulence factors and unique features, this microorganism is respected as a common cause of nosocomial infections. Because of potential ability in biofilm formation and colonization in different surfaces, also using of medical implant devices in immunocompromised and hospitalized patients the related infections have been increased. In recent decades the clinical importance and the emergence of methicillin-resistant *Staphylococcus epidermidis* strains have created many challenges in the treatment process. (Amirmorteza Ebrahimngin zadeh Namvar et al., 2014).

### Bacillus Subtilis

*Bacillus subtilis* can be isolated from myriad environments – terrestrial and aquatic – making it appear as though this species is ubiquitous and broadly adapted to grow in diverse settings within the biosphere. However, like all members of the genus *Bacillus*, *B. subtilis* is capable of forming highly resistant dormant endospores in response to nutrient deprivation and other environmental stresses<sup>1</sup>. These spores are easily made airborne and dispersed by wind<sup>2,3</sup>. Thus, spores might migrate long distances, land in a given environment but never germinate there. Considering that the traditional methods for isolating *B. subtilis* require that the organism be in its spore form, there is no guarantee that when a strain is isolated from a particular environment it was actually growing at that location. Thus, to date, the question of where *B. subtilis* grows remains largely unanswered. (Ashlee M. Earl et al., 2008).

### Pseudomonas Aeruginosa

*P. aeruginosa* is an aerobic gram-negative bacterium and *P. aeruginosa* is typified by motile, non-spore forming rods that are oxidase positive and lactose nonfermenters. *P. aeruginosa* is a member of the genus *Pseudomonas*, colloquially called the pseudomonads. The water-soluble pigments, pyocyanin and pyoverdine, give *P. aeruginosa* its distinctive blue-green color on solid media. *P. aeruginosa* produces indophenol oxidase, an enzyme that renders them positive in the “oxidase”

test, which distinguishes them from other gram-negative bacteria. The presence of polar flagella and pili gives *P. aeruginosa* motility.

Like many environmental bacteria, *P. aeruginosa* live in slime-enclosed biofilms which allow for survival and replication within human tissues and medical devices. Associated with the production of a biofilm protects *P. aeruginosa* from host-produced antibodies and phagocytes contributing to antibiotic resistance of this organism.

The *P. aeruginosa* organism thrives in moist environments such as soil and water. It can be found in large numbers on fresh fruits and vegetables. Human colonization begins within the gastrointestinal tract, with subsequent spread to moist cutaneous sites such as the perineum and axilla. It forms smooth fluorescent green colonies at 42°C, with a characteristic sweet (grape-like) odor, making it easy to recognize on solid media in the laboratory.

As a group, pseudomonads have minimal nutritional requirements. Many are capable of using a wide variety of environmental sources for nutrition; *P. aeruginosa* often only needs acetate and ammonia as the source of carbon and nitrogen, respectively. In addition *P. aeruginosa* can grow anaerobically, and does not carry out fermentation, rather obtaining energy from the oxidation of sugars. The flexible nutritional requirement permits its growth in marginal environments. They are difficult organisms to eradicate from areas that become contaminated, such as operating rooms, hospital rooms, clinics, and medical equipment. (Shigeki Fujitani, M.D. et al., 2015)

### Bacterial Infections in Filipinos

Of the 873 enrolled infants, 81 died (91%). After exclusion of presumed contaminants, positive bacterial culture from blood and/or cerebrospinal fluid was obtained in 35 infants (5.8%; 95% confidence interval 4%, 8%), 9 of whom died. The organisms responsible for meningitis were *Acinetobacter* spp. (4), *Streptococcus pneumoniae* (2), *Escherichia coli* (2), *Enterobacter* spp. (1), *Pseudomonas aeruginosa* (1), *Haemophilus influenzae* (1) and *Staphylococcus aureus* (1); those responsible for the other clinical diagnoses were *Salmonella* spp. (6), *Enterobacter* spp. (3), *Streptococcus pyogenes* (3), other Gram-negative organisms (8), *S. pneumoniae* (1) and *Staphylococcus aureus* (2). In 685 infants examined for viral causes of their illness, 223 viruses were isolated from 219 infants (32%; 95% confidence interval 28%, 36%). Enteroviruses were the most common potential pathogens identified (22% of infants studied), followed by respiratory syncytial virus (17%), rhinovirus (10%) and adenovirus (4%). Concomitant virus identification occurred in 10 of those with positive bacterial culture (29%; 95% confidence interval, 15%, 46%), with enterovirus being found in 7 of these cases. (Gatchalian SR et al., 2008).

### Plants as Antimicrobial Agents

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and “leads” which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. This review attempts to summarize the current status of botanical screening efforts, as well as *in vivo* studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations. (Marjorie Murphy Cowan, 2008).

Indiscriminate and irrational use of antibiotics has created an unprecedented challenge for human civilization due to microbe’s development of antimicrobial resistance. It is difficult to treat bacterial infection due to bacteria’s ability to develop resistance against antimicrobial agents. Antimicrobial agents are categorized according to their mechanism of action, i.e., interference with cell wall synthesis, DNA and RNA synthesis, lysis of the bacterial membrane, inhibition of protein synthesis, inhibition of metabolic pathways, etc. Bacteria may become resistant by antibiotic inactivation, target modification, efflux pump and plasmidic efflux. Currently, the clinically available treatment is not effective against the antibiotic resistance developed by some bacterial species. However, plant-based antimicrobials have immense potential to combat bacterial, fungal, protozoal and viral diseases without any known side effects. Such plant metabolites include quinines, alkaloids, lectins, polypeptides, flavones, flavonoids, flavonols, coumarin, terpenoids, essential oils and tannins. The present review focuses on antibiotic resistance, the resistance mechanism in bacteria against antibiotics and the role of plant-active secondary metabolites against microorganisms, which might be useful as an alternative and effective strategy to break the resistance among microbes. (Harish Chandra *et al.*, 2017).

### Botanical Description

*Pyrus* is a medium size flowering plant, It belongs to the family of Rosaceae that can grow over 19 to 60 feet in height depending on the tree species. Also, it is commonly known as “white china pear” that can be found mainly in Asian countries. The *Pyrus* is originated in Western China and is widely distributed all over the

world. Furthermore, it is branched into two major groups: European and Asian pears. It has an elongated and full-bodied texture and being one of the most generally produced goods in China (Silva, Medeiros Souza, Barbieri, & Costa de Oliveira, 2014).

The characteristics, which is readily distinguish genus of *Pyrus* are elongated and full-bodied texture and has a medium-sized tree reaching 19-60 ft in height and the leaves are alternately arranged in 2-12 centimeter long, glossy and greenish in some species, densely silvery-hairy in some others their shapes varies from oval to narrow lance like shape. The flowers are white with 2-4 centimeters in diameter with five petals. It is usually harvested during summer and autumn.

### Economic Importance

Overall the Pear fruit consumed naturally or can be incorporated in other dishes as a form of an ingredient such as in pies, cakes, strong cheese or carpaccio, risotto, jams, and ice creams. Studies show that it is one of the fruits of choice to be included in a diet due to its low caloric value and high fiber content. The pear can be a source of various vitamins and minerals including Vit A, B1, B2, B3 and C, along with sodium, potassium, phosphorus, calcium, magnesium, and iron. *Pyrus* is known to be excellent in the treatment of intestine inflammation and constipation along with curing anomalies including cystitis and kidney stones. (Silva *et al.*, 2014).

### Medicinal plants

Antibiotics which are developed in the middle of 20th century and modified there after have been successfully used in limiting major bacterial illnesses. But the emergence of resistance against commonly used antibiotics has possessed challenge to clinical in treating mixed infections caused by predominantly antibiotics resistant organism. Continuous race between development of newer antibiotics and emergence and selection of resistance may result in to multiple antibiotics resistant (Frost and O’Boyle, 1981) super infection of bacteria which might be very difficult to kill. This has renewed the interest of researchers the world over to empirically determine the potential antimicrobial activity of many indigenous plants not explored. Plants and their products are used for the treatment of many illnesses in human and animals in all 12 parts of the world. Purified active principles of many indigenous plants are still practiced in modern medicine.

Medicinal plants have been identified and used throughout human history (Lichterman, 2004). The use of medicinal plants to treat diseases is almost universal among non-industrialized societies and is often more affordable than purchasing expensive conventional drugs (Fabricant and Farnsworth, 2001). The World Health Organization (WHO) estimates that 80% of the world population especially Asian and African countries use herbal medicine for some aspect of primary health care

(<http://www.traffic.org/medicinal-plants>, 30th march 2014). Over 120 active compounds currently isolated from the higher plants are widely used in modern medicine and 80% of these show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001).

Human use of plants as medicines could be dated back to the Middle Paleolithic Age, which is about 60 000 years ago, according to fossil records (Fabricant and Farnsworth 2001). The first records written on clay tablets in cuneiform are from Mesopotamia and date from about 2 600 BC. Some of the substances that were used were oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), most of which are still in use today for treating ailments ranging from coughs and colds to parasitic infections and inflammation (Gurib-Fakim 2006).

Health care in ancient times included the use of leaves, flowers, stems, berries and roots of herbs for their therapeutic or medicinal value. These medicines initially

took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1996; Samuelsson 2004). Knowledge of the specific plants to be used and the methods of application for particular ailments were passed down through oral history and information regarding medicinal plants was eventually recorded in herbals (Balunasa and Kinghorn 2005).

#### Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. (Moulay Sadiki, 2015).

## CHAPTER 3

### METHODS AND PROCEDURES

This chapter presents the methods and procedures utilized by the researchers which includes the collection and preparation of plant sample, method of extraction and microbiological assays from crude extract from the fruit of Pear scientifically known as *Pyrus communis* family *Rosaceae*.

#### Research Design

This study is a prospective experimental that seeks to optimize and characterize the methanolic crude extract of Pear (*Pyrus communis*) family *Rosaceae*. It uses quantitative parameters to define the technical specification of the optimized crude extract as well as qualitative data to describe or characterize the property of Pear methanolic crude extract. The methods utilized to address the objectives of the study are illustrated in the schematic diagram in Figure 1.

#### 1.1 Collection and Preparation of Pear fruit

Pear fruit was bought from Bustillos wet market in Sampaloc, Manila during the month of August 2018. The fruits were washed with distilled water and were subsequently shredded into smaller pieces.

#### 1.2. Preparation of the Plant Extract

About 100 g of Pear fruits were weighed and placed in a 250 mL Erlenmeyer flask, macerated with sufficient amount of 80% methanol for 48 hours and were filtered afterward. The extract is evaporated to incipient dryness

through water bath. It was weighed and the percentage yield was computed using the formula.

$$\% \text{ Yield} = \frac{\text{Weight of the Crude Extract}}{\text{Weight of the Plant Sample}} \times 100$$

#### 1.3 Preparation of water based crude extract concentration

500mg was dissolved in 0.50 mL distilled water. This is equivalent to a 100.00% concentration.

### 2 Characteristic Evaluation of Crude Extract from the Pear fruit

#### 2.1 Physical Evaluation

##### 2.1.1 Organoleptic Test

The color, odor, and physical appearance of the crude extract were observed.

##### 2.1.2 Solubility Test

Two test tubes were prepared containing different solvents namely: 95% Ethanol and distilled water. Then a few grams of crude extract were dissolved in each of the test tube containing the solvent.

### 3. Microbiological Assay

The antibacterial activity of methanolic extract against clinical isolates of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* were determined using agar

well diffusion assay. Equidistant wells were made on the agar plate using cork borer (4mm diameter). 40 µl of crude extract and the positive control were placed in each well. Plates were incubated at 37°C for 24 hours, and the resulting inhibition zones were measured using vernier calipers. This experiment was done in triplicate and the antimicrobial activity was expressed as mean zones of inhibition.

The following numerical scale was used to assess the zone of inhibition.

≥20 mm	Susceptible
10-19 mm	Intermediate
<10mm	Resistant

## CHAPTER 4

### PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA

This chapter deals with the presentation, analysis and interpretation of the data obtained by the researchers. The data gathered from the results of the physical tests, phytochemical screening, instrumental analysis and biological testing, were analyzed and interpreted to determine the antibacterial activity of the methanolic crude extract from the fruit of *Pyrus communis* (pear) family name *Rosaceae*”

**Table 1: Percentage Yield of the Crude Extract.**

DATA	RESULTS
Weight of the Pear fruits	100g
Weight of the crude extract	11.90g
Percentage Yield	11.90%

Table 1 shows the computed percentage yield of the crude extract from fruits of Pear fruit. The percentage yield shows that in 100 grams of the cut pears; there is 11.90% of crude extract.

#### Physical Test

The color, odor and physical state were determined using actual observations perceived by the senses of the researchers.

**Table 2: Organoleptic Test Result.**

Organoleptic Test	Result/s
1. Color	Brown
2. Odor	Terebinthinate
3. Physical state	Semi-solid

Table 2 shows that the crude extract was brown in color, sweet odor, and semi-solid.

#### Solubility

The solubility test for the crude extract from Pear fruit was done using water, 80% methanol, chloroform, ether and hexane.

**Table 3: Solubility Test Result.**

Solvent	Result/s
1. Water	Very Soluble
2. 80% Methanol	Soluble
3. Chloroform	Soluble
4. Ether	Insoluble
5. Hexane	Insoluble
6. Benzene	Insoluble
7. Ethanol	Soluble
8. Ethyl Acetate	Insoluble

Table 3 shows that the crude extract from Pear fruit was very soluble in water, soluble in 80% methanol, ethanol and chloroform and insoluble in ether, benzene, ethyl acetate and hexane.

**Table 4: Result of Biological Test.**

Bacteria	Concentration 100%	Concentration 75%	Concentration 50%	Positive CFP	Negative Distilled water
Pear	Pear	0.00	Pear	29.40	10.00
Pseudomonas aeruginosa	15.10	10.00	10.00	27.93	10.00
Staphylococcus aureus	19.03	10.00	10.00	36.87	10.00
Escherichia coli	18.23	10.00	10.00	40.80	10.00
Staphylococcus epidermidis	14.20	10.00	10.00	24.33	10.00

Table 4 shows the mean results of the antimicrobial assay of the Pear crude extract, positive control Cefepime, and negative control Distilled water against

the microorganisms in 50%, 75% and 100% concentrations.

**Table 5: Comparison of Zone of Inhibition of Different Concentrations of Pear crude extract against *B. Subtilis***

Treatment group	Mean	Sd	Significance	Interpretation
Positive (CFP)	29.40	0.10	p<0.05 Means are significantly different.	Highly active
Negative	10.00	0.00	p<0.05 Means are significantly different.	Inactive
50% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
75% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
100% Extract	18.37	0.40	p<0.05 Means are significantly different.	Very active

Table 5 shows that the crude extract of Pear fruit in 50%,75 %, and 100% concentrations has a significant difference with the Positive control. This shows that only the crude extract of Pear in 100% concentration shows potential antimicrobial activity.

**Table 6: Comparison of Zone of Inhibition of Different Concentrations of Pear crude extract against *E. Coli*.**

Treatment group	Mean	Sd	Significance	Interpretation
Positive (CFP)	40.80	0.30	p<0.05 Means are significantly different.	Highly active
Negative	10.00	0.00	p<0.05 Means are significantly different.	Inactive
50% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
75% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
100% Extract	18.23	0.31	p<0.05 Means are significantly different.	Very active

Table 6 shows that the crude extract of Pear fruit in 50%, 75 %, and 100% concentrations has a significant difference with the Positive control. This shows that only

the crude extract of Pear in 100% concentration shows potential antimicrobial activity.

**Table 7: Comparison of Zone of Inhibition of Different Concentrations of Pear crude extract against *S. Aureus*.**

Treatment group	Mean	Sd	Significance	Interpretation
Positive (CFP)	36.87	0.21	p<0.05 Means are significantly different.	Highly active
Negative	10.00	0.00	p<0.05 Means are significantly different.	Inactive
50% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
75% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
100% Extract	19.03	0.25	p<0.05 Means are significantly different.	Very active

Table 7 shows that the crude extract of Pear fruit in 50%, 75 %, and 100% concentrations has a significant difference with the Positive control. This shows that only

the crude extract of Pear in 100% concentration shows potential antimicrobial activity.

**Table 8: Comparison of Zone of Inhibition of Different Concentrations of Pear crude extract against *S. Epidermidis*.**

Treatment group	Mean	Sd	Significance	Interpretation
Positive (CFP)	24.33	0.25	p<0.05 Means are significantly different.	Highly active
Negative	10.00	0.00	p<0.05 Means are significantly different.	Inactive
50% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
75% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
100% Extract	14.20	0.30	p<0.05 Means are significantly different.	Very active

Table 8 shows that the crude extract of Pear fruit in 50%,75 %, and 100% concentrations has a significant difference with the Positive control. This shows that only

the crude extract of Pear in 100% concentration shows potential antimicrobial activity.

**Table 9: Comparison of Zone of Inhibition of Different Concentrations of Pear crude extract against *P. Aeruginosa*.**

Treatment group	Mean	Sd	Significance	Interpretation
Positive (CFP)	27.93	0.55	p<0.05 Means are significantly different.	Highly active
Negative	10.00	0.00	p<0.05 Means are significantly different.	Inactive
50% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
75% Extract	10.00	0.00	p<0.05 Means are significantly different.	Inactive
100% Extract	15.10	0.30	p<0.05 Means are significantly different.	Very active

Table 9 shows that the crude extract of Pear fruit in 50%, 75%, and 100% concentrations has a significant difference with the Positive control. This shows that only

the crude extract of Pear in 100% concentration shows potential antimicrobial activity.

## CHAPTER 5

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter covers the outline of the entire research and the recommendations made by the researchers for further development of studies about the Pear fruit.

#### Summary of Findings

Based on the procedures and methods performed with the entire research, the following findings are included:

1. The percentage yield obtained from the methanolic crude extract of pear fruit was 11.90%.

2. The crude extract from the Pear fruit was soluble in water, ethanol and chloroform and insoluble in ether, ethyl acetate, benzene, and hexane.
3. The methanolic crude extract obtained from *Pyrus communis* has showed potential antimicrobial activity against all of the microorganisms utilized in the study.

#### APPENDIX A

##### PREPARATION OF SAMPLE



Cut pieces of Pear fruit and weighed.



Pear fruit



Cut pear fruits  
macerated in 80%  
Methanol

**APPENDIX B**  
CRUDE EXTRACTION






Filtered juice and subjected to water bath



Crude extract

APPENDIX C  
AUTHENTICATION

	<p>UNIVERSITY OF SANTO TOMAS RESEARCH CENTER FOR THE NATURAL &amp; APPLIED SCIENCES UST HERBARIUM</p>	
<b>Certificate of Identification and Authentication of Botanical Specimens</b>		
<p>This is to certify that the BOTANICAL specimen/s herein listed and presented by the persons/s herein noted were identified and authenticated by this office.</p>		
<p>Name/s: John Oswald Lacanilao, Michael Angelo Nones, Gransley Clyve Virtusio, Russel Tones, Harold Tobias, John Raymart Raymundo</p>		
<p>SCHOOL/INSTITUTION: Centro Escolar University Manila</p>		
<p>Local Name Pear (Eng.)</p>	<p>Scientific Name <i>Pyrus communis</i> L.</p>	<p>Family Rosaceae</p>
<p>Collected from Manila Date of Collection: October 8, 2018</p>		
<p> Assoc. Prof. Cecilia B. Moran, Dr. Rer. Nat. CURATOR</p>		
<p>ISO: S037-00-F047</p>		

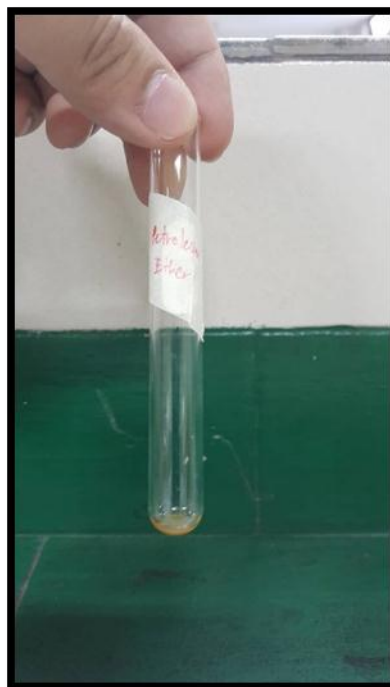
**APPENDIX D**  
**SOLUBILITY TESTS**



Methanol  
Petroleum Ether

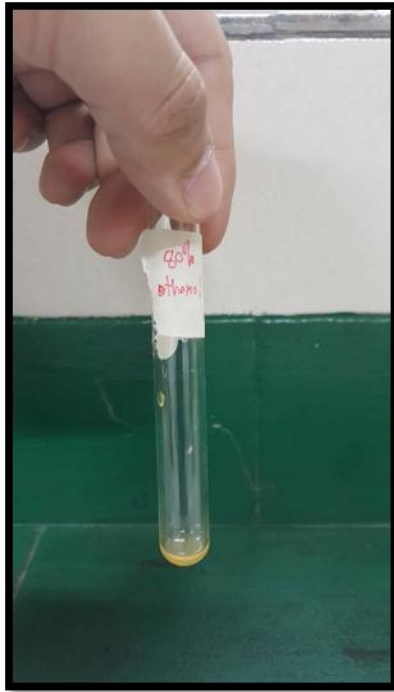


Distilled Water

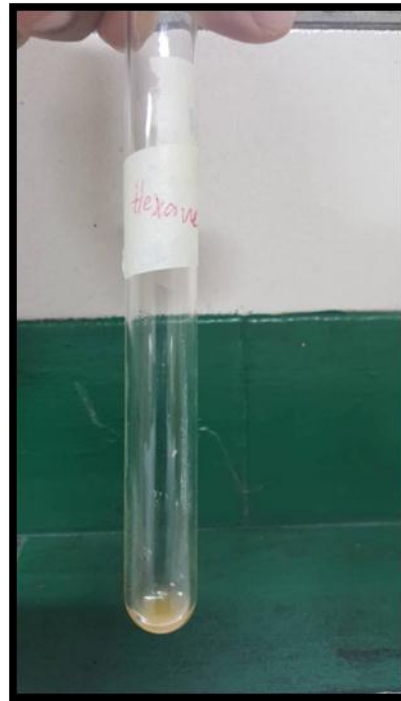


Petroleum Ether

**APPENDIX E**  
**SOLUBILITY TESTS**



80%



Hexane

Petroleum Ether  
Petroleum Ether



Ethyl Acetate

**APPENDIX F**

Zone of Inhibition of Pear Crude Extract in 50%, 75%, and 100% concentrations against *Bacillus subtilis*

(50% concentration crude extract)



(75% concentration crude extract)



(100% concentration crude extract)

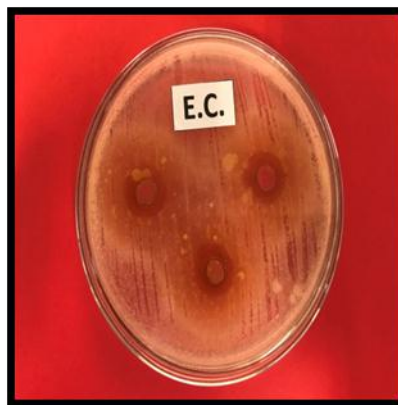


**APPENDIX G**Zone of Inhibition of Pear Crude Extract in 50%, 75%, and 100% concentrations against *Escherichia coli*

(50% concentration crude extract)



(75% concentration crude extract)



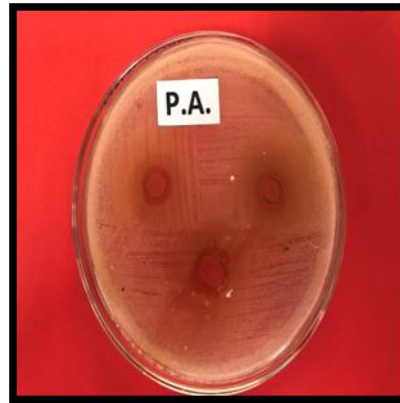
(100% concentration crude extract)



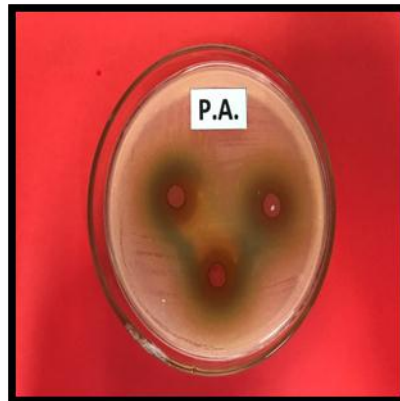
**APPENDIX H**

Zone of Inhibition of Pear Crude Extract in 50%, 75%, and 100% concentrations against *Pseudomonas aeruginosa*

(50% concentration crude extract)



(75% concentration crude extract)

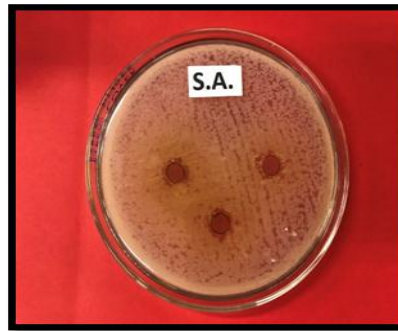


(100% concentration crude extract)

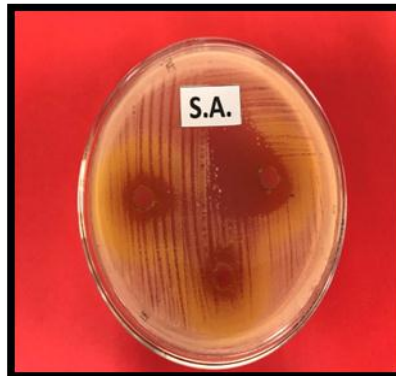


**APPENDIX I**Zone of Inhibition of Pear Crude Extract in 50%, 75%, and 100% concentrations against *Staphylococcus aureus*

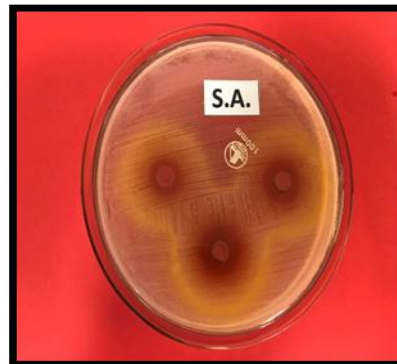
(50% concentration crude extract)



(75% concentration crude extract)



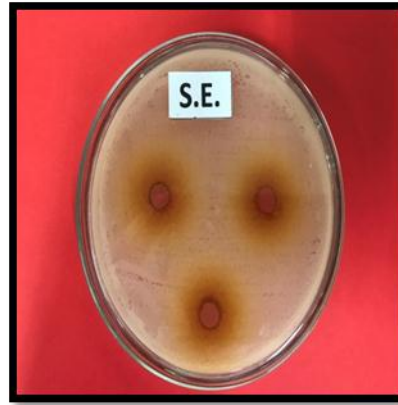
(100% concentration crude extract)

**APPENDIX J**Zone of Inhibition of Pear Crude Extract in 50%, 75%, and 100% concentrations against *Staphylococcus epidermidis*

(50% concentration crude extract)



(75% concentration crude extract)



(100% concentration crude extract)

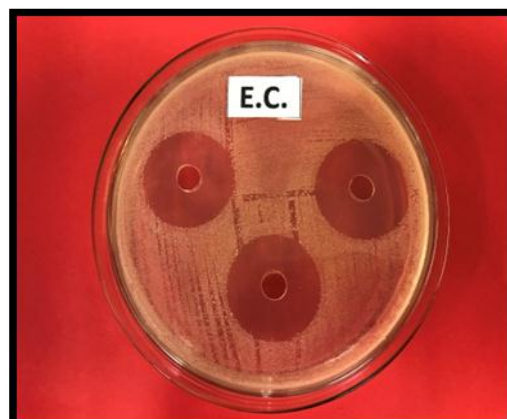


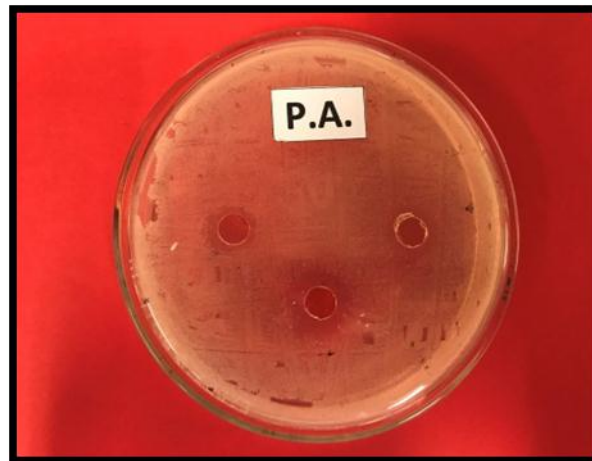
**APPENDIX K**

Zone of Inhibition of Positive Control, Cefepime, against *Staphylococcus aureus*



Zone of Inhibition of Positive Control, Cefepime, against *Escherichia coli*



**APPENDIX L**Zone of Inhibition of Positive Control, Cefepime, against *Pseudomonas aeruginosa*Zone of Inhibition of Positive Control, Cefepime, against *Staphylococcus epidermidis***APPENDIX M**Zone of Inhibition of Positive Control, Cefepime, against *Bacillus subtilis*

## CONCLUSION

Based on the results gathered, the researchers concluded that the methanolic crude extract of *Pyrus Communis* in 100% concentration has potential antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*.

## Recommendations

Based on the study conducted, the following are recommended:

1. To determine the antimicrobial activity using minimum inhibitory concentration.
2. To determine the antimicrobial activity of the flavonoid extract of Pear.
3. To perform antimicrobial assay using resistant microorganisms.
4. To make a formulation of appropriate dosage form.
5. To perform instrumental method of analysis to further identify the sample.
6. To continuously cultivate and propagate *Pyrus communis* for further scientific studies.

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