Evaluate the antibacterial properties of ethanolic extract and chloroform extract of Parsley (Petroselinum crispum) stem against selected pathogens, Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) using Disk Diffusion Assay

CHAPTER I
THE PROBLEM AND ITS SETTING
Background of the Study
The world is well renowned of its capacity to produce and reproduce every plant species, thus they are of
great importance in the life of man as they have abilities to serve as medications to heal and prevent diseases and to eradicate certain pathogens. As the earth revolves on its axis, there are many evolutions happening from time to time, from the environment, to human, to diseases, and to medication. Herbal medicine has greatly brought solutions in treating various diseases throughout the history, although it is yet unwritten in science, some already proved its efficacy without doubt.

Plants form an important part of life in many indigenous communities such as readily available and cheaper alternatives to medicines. In the study conducted by Somchit et al in 2003, plants have been found to cure bacterial infections including urinary tract infections, respiratory diseases, gastrointestinal disorders, and cutaneous infections, wherein the bacteria that cause infection are often known to become resistant to the proposed antibiotics. One example is the Oregano, which is a folk Philippine indigenous alternative medicine and is proved to have effects in treating urinary tract infections and as an antimicrobial agent. In 2006, Lewis and Ausubel found out that antimicrobial agents have substantially reduced the threat posed by infectious diseases since its discovery in the 1940s. The concept of antimicrobial therapy came from ancient times, when natural products are used for the treatment of different ailments, without any knowledge as a basis. It was during 19th century where many developments started in this field. Probable use of dyes as antimicrobial agents was first reported by Paul Ehrlich in early 1900s, which was then followed by Alexander Fleming who was then, honored Nobel Prize award for his great discovery of Penicillin.

According to Karl in 2011, over the last two decades, there is a worldwide increase of multi-drug resistant organisms that concerns the public health, as few therapeutic options remain available, making different diseases known to man difficult to treat. The impact of antibiotic resistance on health care costs and mortality is very alarming. Not only does it increase the severity of diseases but also adds to the financial burden of the sick. With the problems we are facing, there is an urgent need to search for cost-effective medications of plant origin that would eventually serve as a source of new antibiotics in the market. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.

The researchers would like to assess the antibacterial activities of ethanolic extract and chloroform extract of parsley stem against selected pathogens. This study aims to determine whether the extracts possess antibacterial property and have the potential to be an alternative antibacterial drug. This research will greatly affect the society by explicating, promoting and showing to the community its effectiveness and usefulness that even a raw and organic material can be a good, cheap and readily available antibacterial. This study shall be done through the investigation of the antibacterial activities of ethanol extract and chloroform extract of Petroselinum crispum stem against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).

Objectives of the Study

General Objective
The objective of the study is to evaluate the antibacterial properties of ethanolic extract and chloroform extract of Parsley (Petroselinum crispum) stem against selected pathogens, Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) using Disk Diffusion Assay emphasizing on the size of the zones of inhibition produced by the aforementioned stem extracts as compared to the positive control, Amoxicillin.

Specific Objectives
1. To identify the presence of flavonoids and tannins in the ethanolic extract and chloroform extract of Parsley.
2. To determine the mean zones of inhibition of ethanolic extract and chloroform extract of Parsley (Petroselinum crispum) stem against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).
3. To determine the mean zone of inhibition of Amoxicillin against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).
4. To compare the mean zones of inhibition of ethanolic extract and chloroform extract of Parsley (Petroselinum crispum) stem against the mean zone of inhibition exhibited by Amoxicillin.

Statement of the Problem
The study generally aims to evaluate which parsley stem extract possess significant antibacterial activity. Specifically, this study aims to answer the following research questions:
1. Are flavonoids and tannins present in ethanol extract and chloroform extract of Petroselinum crispum?
2. What are the mean zones of inhibition produced by the parsley ethanol extract against Staphylococcus aureus (a gram-positive bacteria) and Escherichia coli (a gram-negative bacteria) in the following concentrations?
2.1 125 g/disc
2.2 250 g/disc
2.3 500 g/disc
2.4 1000 g/disc

3. What are the mean zones of inhibition produced by the parsley chloroform extract against Staphylococcus aureus (a gram-positive bacteria) and Escherichia coli (a gram-negative bacteria) in the following concentrations?
3.1 125 g/disc
3.2 250 g/disc
3.3 500 g/disc
3.4 1000 g/disc

4. Is there a significant difference between the mean zones of inhibition produced by the ethanol extract and chloroform extract of parsley stem against Staphylococcus aureus (a gram-positive bacteria) and Escherichia coli (a gram-negative bacteria) in the following concentrations?
4.1 125 g/disc
4.2 250 g/disc
4.3 500 g/disc
4.4 1000 g/disc

5. Is there a significant difference between the mean zones of inhibition produced by the ethanol extract and the chloroform extract from that of the Amoxicilin, the control, against Staphylococcus aureus (a gram-positive bacteria) and Escherichia coli (a gram-negative bacteria) in the following concentrations?
5.1 125 g/disc
5.2 250 g/disc
5.3 500 g/disc
5.4 1000 g/disc

Null Hypothesis
1. Flavonoids and tannins are absent in the ethanol extract and chloroform extract of Petroselinum crispum stem.
2. The Petroselinum crispum stem ethanol extract has no antibacterial effect against Staphylococcus aureus and Escherichia coli.
3. The Petroselinum crispum stem chloroform extract has no antibacterial effect against Staphylococcus aureus and Escherichia coli.
4. There is no significant difference between the antibacterial activities of the parsley stem extract using ethanol and the parsley stem extract using chloroform.
5. There is no significant difference between the mean zones of inhibition produced by the ethanol extract and the chloroform extract from that of the Amoxicilin, the control, against Staphylococcus aureus (a gram-positive bacteria) and Escherichia coli (a gram-negative bacteria).

Conceptual Framework

Figure 1.1 Conceptual Framework

Significance of the Study
This study serves as an evaluation of antibacterial activities of Parsley (Petroselinum crispum) stem extracts (i.e. ethanol and chloroform extracts) incorporated in MHA medium can inhibit growth of the test bacteria specifically, Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).

In this study, the researchers sought to determine if parsley stem extract using ethanol and the parsley stem extract using chloroform will yield the same result or whether the difference is statistically significant or not. This study is mainly focused in supplying enough information about the antibacterial activity of Petroselinum crispum, also known as Parsley. The use of Parsley is very common among Filipinos especially because Filipinos are fond of cuisines, that they use Parsley as a garnish or tool to make a dish more palatable. With
this study, parsley may not only serve as a garnish or an extra leaf on top of a mashed potato, but also as a potential source alternative treatment for diseases in the future.
Knowledge of the antibacterial activity of the test plants/extracts may provide valuable information, which would greatly help the community and society that intends to promote traditional medicinal herbs as cheaper source of alternative treatment.

The researchers also thought of accomplishing this comparative study to serve as a reference for the future researchers who would may replicate the study's methods, procedures and manipulations.

Scope and Delimitation
This study was limited only to the antibacterial properties of Petroselinum crispum stem. Other parts of the said plant were not used. Stock culture of Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922), which were available in the laboratory were used in the fulfillment of this study hence, clinical specimens were not collected from individuals for the isolation of the said microorganisms. Chloroform and ethanol were used as the extracting solvents with the following concentrations: 125 \( \mu \)g/disc, 250 \( \mu \)g/disc, 500 \( \mu \)g/disc, 1000 \( \mu \)g/disc. Concentrations other than those were not considered within the limits of this study. Parsley stem and its antibacterial activity will be the sole focus of this study. Other significant mechanisms or abilities of the said plant will not anymore be contained within the limits of this research.

Definition of Terms

Antibacterial. Anything that destroys bacteria or suppresses their growth or their ability to reproduce. Herbal medicine. The study or practice of the medicinal and therapeutic use of plants. Müller-Hinton agar. A microbiological growth medium that is commonly used for antibiotic susceptibility testing. Parsley. a species of Petroselinum in the family Apiaceae, native to the central Mediterranean region and widely cultivated as a herb, a spice, and a vegetable. Resistant. Implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistance mechanisms (e.g. beta-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies. (CLSI definition).
Susceptible. Implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection. (CLSI definition)
Zone of inhibition. A circular zone around a disc containing an antibiotic, for example, in which the growth of bacteria susceptible to the antibiotic is inhibited.

CHAPTER II
REVIEW OF RELATED LITERATURES AND STUDIES

Foreign Literature
All over the history, plants have been a source for new drug compounds, as plant-derived medicines have made great impact to human well-being and health. In a research done by El-Astal et al. (2003), these plants were developed as compounds with potentially significant therapeutic application against human pathogens including bacteria. Petroselinum crispum, commonly known as Parsley, has been utilized in the cosmetic, pharmaceutical and food industries. It is a member of Apiaceae family. The name “Petroselinum” is derived from the Greek word “petros” which means “stone,” denoting the plant’s habit of growing in stony places. Petroselinum crispum is a rigid, abundantly branched, biennial to perennial herb that can reach up to thirty to one hundred (30-100) centimeters tall, smooth and scented in all parts. The lean and fibrous root system is coupled with taproot that measures up to one (1) meters long. It is condensed with leaves in radical rosette when young. The stem is tubular, furrowed and deep. The leaves are arranged interchangeably, one to three pinnately compound, dark green, refined, curled or flat with cover at the base. It is in the lower leaves that the petiole is longest. The upper leaves are less segmented while the uppermost leaves consist of a limited acute divisions only (de Guzman and Siemonsma, 1999).
Parsley is known to be natural to the central Mediterranean region (Tunisia, Southern Italy and Algeria) and
adopted elsewhere in Europe, Africa and Asia. It is commonly used as a relish in soups, salads, sauces, vegetables and even meat (Maria, 2006).

In 2008, Dr. John R. Christopher, one of the greatest master herbalists of the twentieth century, mentioned the uses parsley in his article. He used the herb not only for renal congestion, inflammation of the kidneys and bladder stones, and urine retention, but also for jaundice and venereal diseases. Dr. Christopher suggested that parsley root tea is good for stiff fingers and other joint issues. He said gallstones could be removed by drinking a pint of fresh parsley tea every day. The herb is also therapeutic to the adrenal glands and nerves and contributes to the tonicity of the blood vessels particularly the arterioles and capillaries. According to Dr. Christopher (2008), fresh parsley juice is a very effective in healing. Parsley juice is an excellent blood tonic, but it must be diluted with some kind of organic, fresh juice, such as lemon juice. Dr. Christopher recommended at least two quarts of strong parsley tea per day for the aforementioned issues in the previous paragraph, or even up to a cup of tea every hour.

The chosen bacterial isolates that will be used in this study to represent gram-positive and gram-negative organisms are Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922), respectively. Staphylococcus aureus, the most clinically noteworthy species of staphylococci, is accountable for various infections both minor and life-threatening. It can be recovered from almost all specimens and is a significant cause of nosocomial infections. The major concern with this organism is the increasing drug resistance. Microscopically, Staphylococcus aureus is a gram-positive cocci, which is arranged in ‘grape-like’ clusters. Based on colonial morphology, most strains of Staphylococcus aureus look as medium to large colonies, two (2) to three (3) millimeter in diameter with a convex, creamy appearance. The edge is whole and the colonies may be colored white to golden yellow. Most strains show a narrow zone of beta hemolysis whereas some strains are non-hemolytic. The only staphylococcal specie pathogenic to humans is also the same specie that is able to produce coagulase and that is, Staphylococcus aureus. Other tests to identify Staphylococcus aureus include growth and fermentation on Mannitol Salt Agar as designated by a yellow color in the medium as the pH becomes acidic and the phenol red indicator takes on its acidic color. This organism can grow in 6.5% to 10% sodium chloride and ferment the carbohydrate-alcohol, mannitol (Delost, 1997).

A study by Nouwen et al. (2004) showed data that up to one third of the population are colonized with Staphylococcus aureus in a constant pattern and carry the same strain all the time, up to one third of the population carry different strains of Staphylococcus aureus occasionally and about one-third of the population are resistant to colonization by Staphylococcus aureus.

In an expert report commissioned by the International Scientific Forum on Home Hygiene (2004), it was mentioned that usually, the individual is quite unaffected by colonization. Staphylococcus aureus can hastily colonize broken skin, such as shallow wounds, psoriasis, eczema and ulcers. It may not yield any symptoms, but sometimes, it causes boils or can go in the bladder or the blood stream causing bacteremia. The pathogenicity of Staphylococcus aureus is caused either by toxin production leading to tissue destruction or by direct invasion of tissue. Anyone with broken skin due to cut, wound or abrasion is at risk of getting Staphylococcus aureus infection from another source or carrier. Patients who are carriers of S. aureus can also self-colonize through their own infected wounds. The different strains of Staphylococcus aureus are unique with different characteristics. The strains are constantly present in the community and can potentially evolve into new strains. Most Staphylococcus aureus infections resolve naturally or in reaction to antibiotic treatment, but in previous years there has been amassed concern about the occurrence of Staphylococcus aureus strains that have developed resistance to multiple antibiotics. Methicillin resistant Staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that is resistant to many antibiotics, such as vancomycin, gentamicin, erythromycin and trimethoprim. The two groups of MRSA strains are healthcare-associated MRSA (HCA-MRSA) strains and community-acquired MRSA (CA-MRSA) strains. HCA-MRSA is a chief cause of nosocomial infection. Although most HCA-MRSA infections occur in a hospital setting, the organism is likely to affect the immunocompromised and geriatrics in a home care setting. In the home setting, the only at risk of contracting MRSA by family members is in situations when there is another family member or a pet infected with MRSA.

Kniehlet al. (2005) described a recent study in Germany, of healthcare workers (HCWs) who had nearby and consistent contact with MRSA-colonized patients. MRSA was acknowledged from nasal swabs of eighty-seven 87 workers treated with topical antimicrobials. They were instructed to sterilize their bathrooms and personal
hygiene articles, and wash bed linen and pillows. Seventy-three out of 87 workers (or 84%) of HCWs lost their carrier status when tested after three days, and this was upheld after further sampling over three (3) months. Extended sampling allowed detection of eleven (11) cases of MRSA indicating decolonization. In eight (8) of these eleven (11) cases, screening identified colonization of close household contacts. Environmental sampling identified contamination in seven of the eight home environments. Contaminated surfaces included cosmetics, brushes, bed linen, pillows, and hand contact surfaces. Contamination in home environments cleared in most cases within a few weeks after eradication treatment, which included cleaning and disinfection, was applied to household contacts and surfaces. However, eradication took up to two years in heavily contaminated home environments, regardless of adequate medical treatment.

A normal flora of the human lower gastrointestinal tract, Escherichia coli, was first described by Theodore Escherich in 1885. It is the most common clinical isolate in the family Enterobacteriaceae (Delost, 1997). It is initially considered as a harmless member of the colon resident biota, it is now known as a noteworthy human pathogen associated with UTI (Mahon, 2010). Escherichia coli is a gram-negative, non-spore forming bacilli that can be easily recognized by the manifestation of pink-red colonies on MacConkey agar or by its characteristic ‘green metallic sheen’ on Eosin-methylene blue medium (Delost, 1997). It lacks cytochrome oxidase, can produce indole from tryptophan, can ferment glucose, lactose, trehalose and xylose but cannot use citrate as sole carbon source. Thus, it is oxidase negative, indole positive, Methyl-red positive, Vogues-Proskauer negative and citrate negative (Mahon, 2010).

Escherichia coli is present in myriad as a member of normal intestinal flora of humans and animals, where it is generally harmless. However, when misplaced in other parts of the body, Escherichia coli can cause severe diseases, such as urinary tract infections, meningitis and bacteremia. The several classes of enteropathogenic Escherichia coli have been identified on the basis of different virulence factors, including enterohemorrhagic Escherichia coli (EHEC), enterotoxigenic Escherichia coli (ETEC), enteropathogenic Escherichia coli (EPEC), enteroinvasive Escherichia coli (EIEC), enteroaggregative Escherichia coli (EAEC) and diffusely adherent Escherichia coli (DAEC). Escherichia coli O157:H7 and E. coli O111 are human health effects EHEC serotypes that can cause diarrhea ranging from mild and non-bloody to major and highly bloody, which cannot be distinguished from hemorrhagic colitis. Possibly fatal hemolytic uremic syndrome (HUS) can develop in between two percent (2%) and seven percent (7%) of cases. HUS characterized by severe renal failure and hemolytic anemia. As few as 100 EHEC organisms can cause infection making the infectivity of EHEC strains extensively higher than that of the other strains. ETEC produces heat-labile or heat-stable E. coli enterotoxin, and is a significant cause of diarrhea, especially in young children. Watery diarrhea, abdominal cramps, nausea and headache fall under symptoms of ETEC infection while infection with EPEC has been related with chronic non-bloody diarrhea, vomiting and fever in infants. EIEC usually causes watery and occasionally bloody diarrhea where strains attack colon cells in a mechanism similar to that of Shigella (Nataro and Kaper, 1998).

Amoxicillin will be used as a positive control drug in the study. Amoxicillin is an acid-stable, semi-synthetic drug that belongs to a class of antibiotics called the lactam antibiotics. It is revealed to be effective against a wide range of infections caused by various Gram’positive and Gram’negative bacteria in both human and animals. It is a variety of ampicillin with a different parent drug altered by hydroxylation of the phenyl side chain (Simaret al., 2011).

Amoxicillin is kills microorganisms through the inhibition of biosynthesis of cell wall mucopeptide during bacterial multiplication (Amin et al., 1994; Nagaralliet al., 2002). It effectively carries out its job by binding to penicillin-binding protein 1A (PBP’1A) inside the bacterial cell wall (Dousa and Hosmanova, 2005). The lactam antibiotics including amoxicillin open the lactam ring inactivation of the penicillin’sensitive transpeptidase by acylating the enzyme C-terminal domain thus, preventing the formation of a cross-link of two linear peptidoglycan strands, which in turn inhibits the third and last stage of bacterial cell wall synthesis (Amin et al., 1994; Nagaralliet al., 2002; Dousa and Hosmanova, 2005; Torres et al., 2010; Imoisili, 2008). Said process is essential for cell division other vital processes; and thus, the bactericidal property of lactam antibiotics including amoxicillin for microorganisms involves both lytic and non-lytic mechanisms. Cell lysis is then facilitated by bacterial cell wall autolytic enzymes such as autolysins (Imoisili, 2008).

Local Literature
An article in Biofarmer’s Digest featured a local farm in Laguna that cultivates flat-leaf parsley in a
greenhouse. With a greenhouse, parsley production is year-round. Ronald Costales, the farm owner, says that
the flat-leaf parsley is good for the health of the kidney. It is also valuable as a seasoning for soups, salads,
stews and more. Another selection, the curly parsley, is less palatable than the flat-leaf. However, it is more
ornamental and it has its own use in culinary preparations (Biofarmer’s Digest, 2013).

Foreign Studies

In 2010, Devi et al. did a study using different concentrations of leaf extract of parsley using disc diffusion
method for antimicrobial property against various gram positive bacteria, (Staphylococcus aureus, Bacillus
subtilis and Micrococcus luteus) and gram-negative (Escherichia coli, Salmonella typhi and Pseudomonas
eaeruginosa) and as well as fungal species (Candida albicans and Aspergillus niger). The concentrations used
were one hundred (100) "g/disc, two hundred fifty (250) "g/disc and five hundred (500) "g/disc. All the
microorganisms that were tested exhibited susceptibility against the parsley leaf extracts. They also studied
diuretic effect and the result was a significant increase in sodium, potassium and chloride ions urine excretion
in a dose dependent manner. And in conclusion, the parsley leaves has statistically significant antimicrobial
and diuretic activity against the experimented microorganisms.

Wong and Kitts in 2003, experimented methanolic and water extracts of freeze-dried and irradiated parsley
and cilantro leaves and stems for their antibacterial and antioxidant properties. The reagent used to quantify
the total phenolic content was Folin-Ciocalteau. Different mechanisms of possible antioxidant activity of all
the extracts which includes ferrous ion-chelating activities, reducing power and relative free radical-
scavenging were tested. Iron induced linoleic acid oxidation model system was used to assess the total
antioxidant activity of the extracts. Cell damage to the various extracts was used to determine the sensitivity
of Escherichia coli and Bacillus subtilis. The results exhibited that the total phenolic content differed between
the plants used, the plant part used and the extracting medium used. Methanolic leaf extracts showed
significantly (p < 0.05 ) higher radical scavenging activity towards both lipid and water-soluble radicals, and
this represents the total phenolic content. Also, ferrous ion-chelating activity was significantly (p < 0.05)
higher in the methanolic stem extracts, and represented the antioxidant activity. In addition, prooxidant
activity was characteristic of all aqueous extracts and represented the reducing activity of both stem and leaf
parts of both cilantro and parsley. And the bacterial cell damage, which resulted in significant (p < 0.05) high
growth inhibition of E. coli and B. subtilis, showed the ferrous sequestering activity of methanolic stem
extracts.

In 2003, El-Astalet al tested three Palestinian medicinal plants, sage, parsley and thyme for antimicrobial
activity using aqueous, methanolic, ethanolic and phenolic solvents. The bacteria used were isolated from ten
UTI patients and the species were Proteus mirabilis, Escherichia coli, Enterobacter cloacae, Klebsiella
pneumonia, , Acinetobacter haemolyticus, Pseudomonas aeruginosa Enterococcus sp. and fungi Candida
albicans while Staphylococcus aureus and Salmonella typhi isolated from food poisoning patients; stools. The
concentrations used for each leaf extract of the three plants were 2.5, 5, 10, 20 and 40 mg/ml and were tested
using hole-plate technique. Results showed that aqueous extracts of sage and thyme inhibited most of the
experimented microorganisms. The phenolic extract of thyme and sage exhibited antibacterial property
against S. aureus and Enterococcus sp, respectively. Meanwhile, E. coli was more sensitive by the parsley
ethanolic extract which did not have significant effect against the used Gram positive bacteria. Results
revealed that Staphylococcus aureus was the most susceptible bacteria to most of the plant extracts.

Karimiet al., in 2014 evaluated the antimicrobial property of the essential oil of Parsley leaves and seeds sing
paper disc diffusion and microdilution technique against Staphylococcus aureus, Escherichia coli, Vibrio
cholera, Salmonella and Yersinia. Results showed four to thirty-two percent (4-32%) minimum inhibitory
concentrations (MICs) of essential oil from parsley seeds whereas zero point five to one percent (0.5 to 1%)
MICs of essential oil from parsley leaves. On the other hand, minimum bactericidal concentration (MBC) from
parsley seeds was found to be highest (4%) against Salmonella sp and E. coli, followed by S. aureus (16%) and
was found to be lowest (8%) against V. cholera. Meanwhile, MBC of essential oil from parsley leaves was
found to be highest (1%) again against E. coli and lowest (0.125%) against V. cholera and S. aureus.
Agar disk diffusion method and zone of inhibition diameters also assessed the effectiveness of the Parsley
essential oils in which S. aureus had the highest zone of inhibition diameter and lowest zone of inhibition was
obtained by Salmonella.

Akrayi and Abdulrahman in 2013 assessed the antibacterial activity of black pepper, dry black lime, parsley,
chili pepper and thyme aqueous extracts against K. pneumoniae, E. coli, P. aeruginosa and P. mirabilis. Results showed that these bacteria were multi-drug resistant. Meanwhile the dry black lime aqueous extract showed the greatest susceptibility against the tested microorganisms whereas the black pepper aqueous extract did not exhibit any inhibitory effect. In 2014, Al-Janabi detected the antibacterial and the antioxidant activities of parsley oil using twenty-two (22) semen samples of infertile men. Bacterial culture of the semen samples was 68% positive and most isolated organisms 53% Staphylococcus aureus of fifteen (15) positive samples, followed by Escherichia coli and Proteus mirabilis 20% each, and 7% Klebsiella sp. Also revealed by the study was that parsley oil at different concentrations has inhibitory effect against the isolates and the most effective dose was 7x 10-4g/ml. The other concentrations (7×10-4 , 7x 10-6 , 7x 10-7, 7×10-8 g/ml) were also significant (P <0.5) for increasing the motile spermatozoa as compared to the control. On the other hand, 7×10-5 g/ml concentration was outperformed as compared with control (P < 0.01) as to the ability to increase the number of motile spermatozoa.

In 2014, Lenka et al. did a study to assess the antimicrobial properties of Petroselinum crispum, Thymus vulgaris, Melissa officinalis, Ocimum basilicum, Lavandula angustifolia, Allium schoenoprasum using aqueous solvent against Klebsiella oxytoca and pneumoniae, Escherichia coli, Raoultella terrigen and Hafnia alvei which were all isolated from dairy products except for the E. coli (CCM 929). Two concentrations 1:5 and 1:10 (herb : distilled water). Disk diffusion method was used and results showed lesser inhibitory effect from thyme and lavender extracts while basil, chive and parsley extracts exhibited higher inhibitory effect against the tested microorganisms.

Eskandari et al. in 2013 conducted a study to detect the antibacterial and antioxidant properties of Parsley extract on air packaged carp fillets shelf life at 4 °C storage. The fish fillets were divided into two groups one was dipped in distilled water as control and one was dipped in 1% extract of parsley. Air packing was done and were stored at 4 ° 1°C. Chemical (PV, TBA, TVB-N, FFA) and microbial (TVC, PTC) properties were analyzed over a 15-day period. Significant delay in lipid oxidation in the fish fillets was exhibited by parsley extract treatment. Results showed that the parsley extract treated samples remained fresh up to 12 days while control samples remained fresh up to 6 days. Thus, this study revealed that the parsley extract can be used as a natural preservative that extends the shelf life of silver carp fillets to 12 days compared to the control samples.

Wahba et al. in 2010 evaluated the usage of edible herbs, parsley, dill, green pepper and cayenne to Kareish cheese and detected their antimicrobial property against coliforms, normal flora, molds and gram positive bacteria, Staphylococcus aureus. Various concentrations of ethanol extracts were used to determine the minimum inhibitory concentration. Results showed that the highest inhibitory activity was obtained by cayenne and green pepper ethanolic extracts followed by parsley and dill against S. aureus when added to Kareish cheese. In addition, total bacterial count, coliform count, and yeast and molds count were tested to determine the effect on the microbial quality of ready-to-eat Kareish cheese when plant materials were added. And results showed that addition of the paint materials reduced the total coliform and bacterial counts. And all concentrations of the four herbs completely decreased the yeast count by 9% within 2 hours while within 2 days, green pepper and cayenne completely decreased the mold count whereas dill and parsley exhibited less effectivity. Therefore, the researchers prepared Kareish cheese with 1% cayenne pepper and 3% and 6% each of parsley, dill and green pepper and they were all preferred and accepted strongly by the consumers. Therefore in conclusion, this study showed that these herbs exhibited antibacterial property against the used microorganisms in Kareish cheese and that the addition of certain percentages of these herbs is preferable and acceptable to consumers and may greatly contribute to the new and safe development of Kareish cheese varieties.

According to Wright et al., in 2007 found out that the parsley leaves and stems are used in the cases of edema, cystitis, menstrual problems, kidney stones, indigestion, cramps, anorexia, prostatitis, arthritis, and rheumatism. Also, in 2001 according to Darías et al., parsley seeds because of its elevated essential oil content are also used like a diuretic.

In 2013, Al-Hadi et al., performed the phytochemical screening wherein results showed the presence of primary and secondary metabolites in alcohol, chloroform, petroleum ether and in water soluble parts in the drug. The detected metabolites were saponins, alkaloid, amino acids and proteins, carbohydrate, tannins,
phenolic compound and flavonoids. Parsley extract concentrations of 125 ug/mL, 250 ug/mL, 500 ug/mL and 1000 ug/mL were prepared for the antibacterial activity testing. Results revealed that good inhibitory activity against bacteria S. aureus, E. coli, K. pneumoniae and P. aeruginosa was obtained by the alcohol and petroleum ether parsley extracts.

Also, in 2015, Mulugeta found out that the essential oil parsley leaves was 0.6 " 0.29 per 300 gram of dry sample. A phytochemical test was done and results showed the presence of various constituents including terpenoids, tannins, phenols, flavonoids and saponins in methanolic crude extract while flavonol, alkaloids, terpenoids, steroids and tannins were present in ethanolic parsley extract.

Yanardag et al. in 2003 investigated the chemical constituents of parsley and results revealed the presence of flavonoids, furanocoumarins, ascorbic acid, coumarins, phenylpropanoids, carotenoids, apiole, myristicin, various terpenoid compounds, phthalides, and tocopherol. Among which, furanocoumarins and furanocoumarins were found to exhibit antibacterial properties wherein furanocoumarins inhibit bacterial growth by reacting with DNA and disrupting DNA replication as studied by Manderfield et al., in 1997. In 2006, Wong and Kits studied the phenolic compounds present in parsley leaf also showed bactericidal effect by impairing cellular function and membrane integrity as found out by Raccach in 1984. Also, in 1996 Jay found out that these phenolic compounds are capable of chelating transition metals such as iron and copper, which will result to lower the metal ion reactivity by the formation of inert metal-ligand complex and thus reduces bioavailability needed for bacterial growth.

According to Tajkarimi et al., in 2010, essential oil compounds such as sabinene, "-pinene, "-thujene and myristicin were reported to contribute antifungal and antibacterial properties to herbs and spices. These foreign studies mentioned are important in this research paper because they will serve as support materials to strengthen the objectives of this research.

Local Studies

In a study conducted by Batol et al. (2007), the antibacterial property of Petroselinum crispum was tested against selected clinical isolates. These clinical isolates were Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa. The method used to determine the antibacterial property of Parsley against the said microorganisms was the Kirby-Bauer technique. Results showed that Parsley exhibited no effect on the mentioned organisms.

In contrast with the study conducted by Batol et al., this research paper made use of the following clinical isolates: Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). The same method, Kirby-Bauer disk diffusion test, was performed to assess the susceptibility of the selected pathogens against the ethanol and chloroform extract of the parsley stem.

CHAPTER III
RESEARCH DESIGN AND METHODOLOGY

This chapter includes all the methods and procedures that are necessary in comparing the antibacterial activities of ethanolic parsley stem extract and chloroform parsley stem extract against the test bacteria.

Research Design

The researchers of this study used an in vitro experimental research design, wherein the dependent variable antibacterial activity of ethanolic parsley stem extract was tested against selected bacterial strains namely Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). The study sought to determine the difference in antibacterial activity of the plant’s stem using two different solvents for extraction namely ethanol and chloroform. The independent variable was further manipulated thru the use of different concentrations, which includes 125 "g/mL, 250 "g/mL, 500 "g/mL and 1,000 "g/mL. Amoxicillin disc, with a concentration of 25 "g was used as positive control.

Research Locale

The researchers gathered the selected pathogens, Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922), from the Medical Technology laboratory of Angeles University Foundation, which were available.

The researchers purchased the parsley stem from a supermarket within Angeles City and its authenticity as Petroselinum crispum was verified by the Philippine National Herbarium. The plant extraction was carried out in the Research Laboratory of Angeles University Foundation. The first
susceptibility testing was carried out by the researchers of the study at the Medical Technology Laboratory of Angeles University Foundation, where the equipment and reagents needed for attaining the desired result were accessible and available for use. As for the repeated susceptibility testing, it was carried out in the Microbiology Section of the Bulacan Medical Center. The phytochemical screening for flavonoids and tannins of the Petroselinum crispum stem was carried out in the Medical Technology Laboratory of Angeles University Foundation as well.

Research Methods

To achieve the objectives of this study on the comparison of the antibacterial properties of parsley stem in different solvents, the researchers used the Disk Diffusion Method for the antibacterial susceptibility testing, the Broth Microdilution Method for the determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration using the isolates Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). All these procedures were discussed below and were followed accordingly. The flow chart of the general procedures shown in Figure 3.1 (Parija, 2009)

Petroselinum crispum Collection
Preparation of Organic Extracts
Antibacterial Screening

Figure 3.1 The Flow Chart of the General Procedures

A. Plant Collection and Preparation
Petroselinum crispum was purchased from the Hypermarket of SM City Clark, Angeles City, Pampanga. The authenticity of the plant used as an extract was verified by the Philippine National Herbarium. The parsley stems were taken and were washed with distilled water then, these stems were left out in shade until completely dried. The dried stems were then powdered using an osterizer. The flowchart of the collection and preparation procedure is shown in Figure 3.2. (Chan et. al. 2012)

Petroselinum crispum stems
Washed with Distilled Water
Shade dried
Powderized using an osterizer

Figure 3.2: Flow Chart of the Plant Collection and Preparation

B. Preparation of Organic Extracts
Petroselinum crispum stem powder (10g) were soaked in 100 mL ethanol and 100 mL chloroform. The solvents were decanted out and filtered under vacuum using Buchner apparatus to give a clear solution. They were evaporated at low pressure using rotary evaporator to obtain the crude extracts. These crude extracts were dissolved using 10% DMSO to obtain the concentrations: 125 “g/mL, 250 “g/mL, 500 “g/mL and 1000 “g/mL. The flowchart of the organic extract preparation is shown in Figure 3.3.

Ground Dried Parsley Stem (10 grams) soaked in 100 mL Ethanol and Chloroform
Decant and Filter using Buchner Apparatus
Evaporate at Low Pressure using Rotary Evaporator
Dissolve with 10% DMSO to obtain concentrations (125 “g/mL, 250 “g/mL, 500 “g/mL and 1000 “g/mL)

Figure 3.3: Flow Chart of the Organic Extract Preparation

C. Antibacterial Screening
The antibacterial activities of different stem extracts were tested by the disc diffusion method. The assay employed strains of gram positive bacteria, Staphylococcus aureus ATCC 25923, and gram negative bacteria, Escherichia coli ATCC 25922. Inoculums (100 “L) were spread evenly onto MHA set in 90 mm Petri dishes using a sterile cotton swab. Sterilized paper discs were impregnated with parsley stem extract using a micropipette and were firmly placed onto the inoculated agar, ensuring even distribution to avoid overlapping of zones. Amoxicillin susceptibility discs (25 “g) were used as positive controls. After incubation overnight at 37°C, the mean diameter of inhibitory zone (DIZ) in millimeters was measured using a vernier caliper. The flowchart of the Disk Diffusion Method is shown in Figure 3.4 (Chan et. al. 2012)
Phytochemical Screening for Flavonoids and Tannins

I. Flavonoids
A. Extraction of Flavonoids (Leucoanthocyanins, \( \beta \)-benzopyrone nucleus)
1. Saturate the ethanolic parsley extract with 1 drop of 1% hydrochloric acid for about 15-30 minutes.

B. Chemical tests
Preparation of test solution
1. Defat the residue collected from the extraction of flavonoids with 9mL hexane and 1mL distilled water with the use of separatory funnel. Discard the hexane extract and collect the aqueous layer.
2. Dilute the aqueous layer with 10mL 80% ethanol.
3. Filter and divide the filtrate into three portions.
A. Bate-Smith and Metcalf method (Test for Leucoanthocyanins)
1. Treat the first part of the sample solution with 0.5mL concentrated hydrochloric acid.
2. Observe for any color change.
3. Immerse in a water bath for 15 minutes and observe for any change in color.
Positive result: A strong violet or red color indicates presence of leucoanthocyanins.
B. Wilstatter 'cyanidin' test (test for \( \beta \)-benzopyrone nucleus)
1. Using another portion of the sample solution, treat it with concentrated hydrochloric acid.
2. Add 3-4 pieces of magnesium turnings.
3. Observe for any change in color within 10 minutes.
Positive result: Color ranging from orange to red, to crimson and magenta and occasionally green or blue color may be observed.

II. Tannins
A. Chemical test
Preparation of test solution
1. Prepare test solution by extracting it with 20mL of hot distilled water. Afterwards, add 5 drops of 10% NaCl solution.
2. Filter the mixture and divide the filtrate into three portions and put it in test tubes. Use one test tube as control.
A. Gelatin test
1. Treat one test tube containing the tannin extract with three drops of gelatin-salt reagent.
Positive result: Formation of jelly like precipitate.
B. Ferric Chloride test
1. Treat the other test tube with the tannin extract with drops of Ferric chloride solution.
Positive result: Blue-black color indicates the presence of hydrolysable tannins, a brownish-green color indicates presence of condensed tannins.

Statistical Analysis
For descriptive purpose, mean \( \pm \) standard deviation of zones of inhibition was presented.
For the statistical testing, the tool that was used is one-way analysis of variance with Duncan’s Multiple Range Test as post-hoc test.
The method used today for comparisons of three or more groups is called one-way analysis of variance (ANOVA). This method has the advantage of testing whether there are any differences between the groups with a single probability associated with the test.
On the other hand, Duncan’s multiple range tests provide significance levels for the difference between any pair of means, regardless of whether a significant F resulted from an initial analysis of variance. It is used as a post hoc test to identify which pairs of means resulting from a group comparison study with more than two groups are significantly different from each other.

CHAPTER IV
PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA
The data gathered based on the obtained results from the experiment are presented, analyzed and interpreted in this chapter.
Phytochemical Screening for Flavonoids and Tannins
Petroselinum crispum ethanolic and chloroform stem extracts were subjected to phytochemical screening to establish the presence of chemical constituents, specifically Flavonoids and Tannins that might be responsible for the possible antibacterial activity of the extracts. The chemical tests that were performed revealed the absence of both tannins and flavonoids in the sample. The actual results of the phytochemical screening test are shown in Appendix C.

### Table 4.1.1: Phytochemical screening test for Flavonoids and Tannins results of Petroselinum crispum ethanolic extract

<table>
<thead>
<tr>
<th>Constituent Tested and Chemical Test</th>
<th>Positive Result</th>
<th>Experimental Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids (Bate-Smith and Metcalf Test)</td>
<td>Red/violet color</td>
<td>Colorless</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavonoids (Wilstatter test)</td>
<td>Orange/Red Colorless</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Tannins (Gelatin test)</td>
<td>Jelly-like precipitate</td>
<td>No jelly-like precipitate</td>
<td>Negative</td>
</tr>
<tr>
<td>Tannins (Ferric chloride test)</td>
<td>Blue-black color</td>
<td>Brown</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 4.1.1 presents the visible results produced by the phytochemical screening test of Petroselinum crispum ethanolic extract. Flavonoids were absent in Petroselinum crispum ethanolic extract due to failure to achieve strong red or violet color in Bate-Smith and Metcalf Test and lack of color ranging from orange to red, to crimson and magenta and occasionally green or blue color in Wilstatter test. Tannins were also absent in Petroselinum crispum ethanolic extract due to the nonexistence of jelly like precipitate in the Gelatin Test and a blue-black color in the Ferric chloride test.

### Table 4.1.2: Phytochemical screening test for Flavonoids and Tannins results of Petroselinum crispum chloroform extract

<table>
<thead>
<tr>
<th>Constituent Tested and Chemical Test</th>
<th>Positive Result</th>
<th>Experimental Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids (Bate-Smith and Metcalf Test)</td>
<td>Red/violet color</td>
<td>Colorless</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavonoids (Wilstatter test)</td>
<td>Orange/Red Colorless</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Tannins (Gelatin test)</td>
<td>Jelly-like precipitate</td>
<td>No jelly-like precipitate</td>
<td>Negative</td>
</tr>
<tr>
<td>Tannins (Ferric chloride test)</td>
<td>Blue-black color</td>
<td>Brown</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 4.1.2 presents the evident results gathered from the screening tests done for the phytochemical constituents of Petroselinum crispum chloroform extract. Absence of flavonoids in Petroselinum crispum chloroform extract was strongly indicated by the lack of strong red or violet color in Bate-Smith and Metcalf Test and lack of color ranging from orange to red, to crimson and magenta and occasionally green or blue color in Wilstatter test. Tannins were also absent in Petroselinum crispum chloroform extract due to the absence of jelly like precipitate in the Gelatin Test and failure to achieve blue-black color in the Ferric chloride test.

### Zone of inhibition (in mm)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Staphylococcus aureus ATCC 25923</th>
<th>Escherichia coli ATCC 25922</th>
<th>Amoxcillin (Control: S. aureus)</th>
<th>Amoxcillin (Control: E.coli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>250 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>500 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>1000 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>36</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 4.2.1: Antibiotic Susceptibility Testing results of Petroselinum crispum ethanolic stem extract

Table 4.2.1 presented the zones of inhibition yielded by the Petroselinum crispum ethanolic stem extract.
against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) inoculated on Mueller-Hinton agar and incubated at 37°C for 24 hours. All concentrations, 125 ug/mL, 250 ug/mL, 500 ug/mL and 1000 ug/mL were negative to both bacterial isolates which means no zone of inhibition was produced. Results showed that the Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) were resistant to P. crispum ethanolic stem extract.

Table 4.2.2: Antibiotic Susceptibility Testing results of Petroselinum crispum chloroform stem extract

Zone of inhibition (in mm)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Staphylococcus aureus ATCC 25923</th>
<th>Escherichia coli ATCC 25922</th>
<th>Amoxcillin (Control: S. aureus)</th>
<th>Amoxcillin (Control: E.coli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>250 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>500 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>1000 ug/mL</td>
<td>Negative</td>
<td>Negative</td>
<td>36</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 4.2.2 showed the zones of inhibition produced by the Petroselinum crispum chloroform stem extract against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) inoculated on Mueller-Hinton agar and incubated at 37°C for 24 hours. All concentrations, 125 “g/mL, 250 “g/mL, 500 “g/mL and 1,000 “g/mL were negative to both bacterial isolates which means no zone of inhibition was produced. Results showed that the S. aureus ATCC 25923 was resistant to P. crispum chloroform stem extract.

Both the ethanolic and chloroform parsley stem extract did not produce any zone of inhibition in all of the concentrations against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).

The results of the present study were similar with the study of Petrolini et al., (2013) wherein both studies utilized Petroselinum crispum as the sample and had similar objectives in investigating the antibacterial potential of the plant towards bacterial isolates. The differences were the plant part, the extract and the method used. Petrolini’s group used crude hydroalcoholic extract of the aerial parts of Parsley against several gram-negative bacteria including E. coli ATCC 25922 and utilized MIC and MBC to evaluate the antibacterial activity of the plant. P. crispum extract did not lead to satisfactory results for the bacteria tested including E. coli ATCC 25922.

Another local study by Batol et al., (2007) also showed the same results. Both studies used Petroselinum crispum as the plant sample and utilized Kirby-bauer technique to determine the antibacterial activity of the said plant. The difference was the solvent used for the extract. Batol’s group made use of the aqueous extract of P. crispum and tested it against Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa. The study revealed that plant had no activity against the tested organisms.

CHAPTER V
SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

Nowadays, the use of plant extract has been frequently utilized by researchers, most commonly in the field of medicine. With this, the researchers aimed to investigate and determine the antibacterial activity of their chosen plant, Parsley. The purpose of this study was to determine the antibacterial activity of parsley (Petroselinum crispum) stem extract against selected pathogens. The researchers would like to lift the knowledge and importance regarding herbal plant extracts as another option of treatment. The study is well concerned with the antibacterial property of Petroselinum crispum extract on Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) as test organisms. Ethanol and Chloroform were used in the extraction process. Antibacterial activity of the said extracts were determined using disk diffusion assay and the zones of inhibition were measured.

The crude extracts were acquired by soaking air-dried and macerated stem of Petroselinum crispum in ethanol and chloroform, and were concentrated using a rotary evaporator. The said organisms were inoculated in the MHA agar. Whatman (no. 1) filter paper disks were impregnated with the extract and the antibacterial property was determined using disk diffusion assay. The results from the disk diffusion assay showed 0 mm zone of inhibition for both Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) in all concentrations (125 “g/mL, 250 “g/mL, 500 “g/mL and 1,000 “g/mL) of ethanol and chloroform.
extract indicating that the test organisms were resistant to the ethanol and chloroform extract of Parsley stem. The positive control, which is Amoxicillin, showed 34 mm average zone of inhibition indicating that it is an effective antibacterial drug.

A phytochemical screening test using Bate-Smith and Metcalf Test and Wilstatter test for Flavonoids as well as Gelatin Test and Ferric chloride test for Tannins were performed in order to determine whether the aforementioned antibacterial constituents were present or not in the Parsley ethanol and chloroform extract. Results showed negative in all chemical tests, thus confirming the absence of both Flavonoids and Tannins. Flavonoids and tannins were picked out of the many constituents because various studies which used essential oil of parsley (Mulugeta et al, 2015; Al-Hadi et al, 2013; Farzaei et al, 2013) found out that these bioactive compounds were the ones responsible for antibacterial property of parsley.

**Major Findings**

The study ought to investigate the efficacy of parsley (Petroselinum crispum) stem extract as an antibacterial agent against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). Based on the results of the experiments, the researchers were able to arrive at the following answers:

1. Phytochemical screening test of the ethanol and chloroform extract of parsley stem revealed the absence of flavonoids and tannins. Chemical tests namely, Bate-Smith and Metcalf Test and Wilstatter test for Flavonoids and Gelatin Test and Ferric chloride test for Tannins were employed in determining the presence of these metabolites.

2. No zones of inhibition were produced using the ethanol extract of parsley stem with the concentrations of 125 "g/mL, 250 "g/mL, 500 "g/mL and 1,000 "g/mL.

3. No zones of inhibition were observed using chloroform extract of parsley stem with the concentrations of 125 "g/mL, 250 "g/mL, 500 "g/mL and 1000 "g/mL.

4. There is no significant difference between the antibacterial property of ethanol and chloroform parsley stem extract in different concentrations (i.e. 125 "g/mL, 250 "g/mL, 500 "g/mL and 1000 "g/mL).

5. There is a significant difference between the antibacterial property of ethanol and chloroform parsley stem extract and the positive control, Amoxicillin.

**Conclusions**

Based on the foregoing findings, the researchers therefore conclude that Petroselinum crispum ethanol and chloroform stem extracts, are not comparable to Amoxicillin in terms of antibacterial activity in various concentrations, hence, cannot be used as source of potential alternative drug for treating infections caused by Staphylococcus aureus and Escherichia coli. Further, indicated results can be explained by the absence of flavonoids and tannins during phytochemical screening.

**Recommendations**

Based on the data gathered and conclusions made in this study, recommendations were established for the utilization of the future researchers. The use of parsley leaves instead of stems would probably yield better antibacterial effects. The researchers also recommend the use of increased concentrations to the test the efficacy of the extract. The antibacterial property of parsley may also be tested in vivo in the form of a juice or tea. This study made use of ethanol and chloroform as solvents, but for future researchers, other solvents may also be used considering the fact that these solvents did not yield the researchers’ expected results.

**BIBLIOGRAPHY**

**Books**


**Journals**


APPENDICES

APPENDIX A

AUTHENTICATION OF PLANT SAMPLE

APPENDIX B

DOCUMENTATION OF THE EXPERIMENT

Grinding of Parsley stem

Soaking of ground Parsley stem (10 grams) in 100 mL ethanol and chloroform

Filtration of ethanol and chloroform extract

Preparation of culture media

Preparation of assay disk

Inoculation of bacteria

APPENDIX C

Phytochemical Screening Test for Flavonoids and Tannins Results

(Left to Right)

Chemical Tests: Wilstatter and Bate-Smith and Metcalf method, respectively
Extract used: Ethanol extract
Positive result: Wilstatter (Orange/Red color); Bate-Smith and Metcalf method (Red/Violet color) (Left to Right)
Chemical Tests: Gelatin test and Ferric chloride test, respectively
Extract used: Ethanol extract
Positive result: Gelatin test (jelly-like precipitate); Ferric chloride test (Blue-black color) (Left to Right)
Chemical Tests: Wilstatter and Bate-Smith and Metcalf method, respectively; Third tube is control
Extract used: Chloroform extract
Positive result: Wilstatter (Orange/Red color); Bate-Smith and Metcalf method (Red/Violet color) (Left to Right)
Chemical Tests: Gelatin test and Ferric chloride test, respectively
Extract used: Chloroform extract
Positive result: Gelatin test (jelly-like precipitate); Ferric chloride test (Blue-black color)

APPENDIX D
Sample Table of Data Presentation
Table 1. Antibacterial activities of ethanolic parsley stem extract and chloroform parsley stem extract against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Ethanolic Parsley Stem Extract</th>
<th>Chloroform Parsley Stem Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>125 ug/mL</td>
<td>250 ug/mL</td>
</tr>
<tr>
<td></td>
<td>500 ug/mL</td>
<td>1000 ug/mL</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Amoxcillin (Control : S. aureus)</td>
<td>35 35 35 35 35 35 35 35</td>
<td></td>
</tr>
<tr>
<td>Amoxcillin (Control: E.coli)</td>
<td>35 35 35 35 35 35 35 35</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Ethanolic Parsley Root Extract</th>
<th>Chloroform Parsley Root Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MIC MBC MIC MBC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

APPENDIX E
Curriculum Vitae
Name: Jacel Krisha Y. Bonifacio
Birthday: September 28, 1996 Age: 19
Address: Villa Barcelona Sindalan, City of San Fernando, Pampanga
Contact no.: 09361461728
Email address: jacel_krisha28@yahoo.com
Educational Attainment:
Elementary: Masantol Elementary School
High School: Masantol High School
College: Angeles University Foundation
Affiliation Center: 1st – Bulacan Medical Center; 2nd- Armed Forces of the Philippines Medical Center
Name: Jessica Marie Escoto
Birthday: April 17, 1996 Age:20
Address: De Leon St. San Rafael Proper, Tarlac City
Contact no.: 09253041796
Email address: jem_1710@yahoo.com
Educational Attainment:
Elementary: College of the Holy Spirit of Tarlac
High School: College of the Holy Spirit of Tarlac
College: Angeles University Foundation
Affiliation Center: 1st- Bulacan Medical Center; 2nd- Dr. Paulino J. Garcia Memorial Research and Medical Center
Name: Amica Coleen A. Lawingco
Birthday: March 12, 1996 Age: 20
Address: #124 Taurus st., Dona Adela Subd. Phase 1, Sta. Cruz, Porac, Pampanga
Contact no.: 09366388082
Email address: amicalawingco@gmail.com
Educational Attainment:
Elementary: Montessori School of St. Nicholas
High School: Angeles University Foundation Integrated School
College: Angeles University Foundation
Affiliation Center: 1st- Philippine Orthopedic Center; 2nd- Angeles University Foundation Medical Center
Name: FrancedGwendale P. Pinazo
Birthday: August 15, 1995 Age: 20
Address: Pascual Village Bancal, Guagua, Pampanga
Contact no.: 09369396723
Email address: francedpinazo@gmail.com
Educational Attainment:
Elementary: St. Mary’s Academy of Guagua
High School: St. Mary’s Academy of Guagua
College: Angeles University Foundation
Affiliation Center: 1st- Philippine Orthopedic Center
here...

About Essay Sauce

EssaySauce.com is a completely free resource to help students research their academic work and learn from great essays!

View all posts by Essay Sauce

...(download the rest of the essay above)

About this essay:

This essay was submitted to us by a student in order to help you with your studies.

If you use part of this page in your own work, you need to provide a citation, as follows:

Review this essay:

Please note that the above text is only a preview of this essay. The full essay has 4240 words and can be downloaded free in PDF format, using the link above.

Latest reviews:

Science essays
- Drug administration and Drug Delivery Systems
- Identify the correlation of economic growth (GDP), poverty reduction and reduction in income inequality in Tanzania

Search for student essays:

Search ...

About EssaySauce, the student essay site:

EssaySauce.com is a free resource for students, providing thousands of example essays to help them complete their college and university coursework. Students can use our free essays as examples to write their own.
Latest student essays:

Ocular disease
HUMAN action recognition
Analysing data production
Desorption study
Surfactants (surface active agents)
Islamic Finance and Its Impact on Customer Satisfaction
Persian gulf
Feminist approach (Bhumika) (notes)
What does it mean to be a Muslim woman in 21st century? (Shari’ah)
Appellate Body’s analysis under section XIV(c)

Student essay categories:

Accounting essays
Architecture essays
Business essays
Computer science essays
Criminology essays
Economics essays
Education essays
Engineering essays
English language essays
English literature essays
Environmental studies essays
Finance essays
Geography essays
Health essays
History essays
Hospitality and tourism essays
Human rights essays
Information technology essays
International Relations
Law essays
Leadership essays
Linguistics essays
Literature essays
Management essays
Marketing essays
Media essays
Medicine essays
Miscellaneous essays
Music Essays
Philosophy essays
Photography and arts essays
Politics essays
Project management essays
Psychology essays
Religious studies and Theology essays
Science essays
Social work essays
Sociology essays
Uncategorized
Zoology essays

Average review:

Overall rating: 0 out of 5 based on 0 reviews.
Q: Is EssaySauce.com free?

Yes! EssaySauce.com is a completely free resource for students. You can view our terms of use here.

Why use Essay Sauce?

The brightest students know that the best way to learn is by example! EssaySauce.com has thousands of great essay examples for students to use as inspiration when writing their own essays.

Is Essay Sauce completely free?

Yes! EssaySauce.com is a completely free resource for students. You can view our terms of use here.

Info:

About
Content policy
Essay removal request
Privacy
Terms of use