STANDARDIZATION OF PROCESSING AND CONCENTRATION OF TURMERIC RHIZOMES *(Curcuma longa)* ESSENTIAL OIL AGAINST *Mycoplasma synoviae* in PHILIPPINE NATIVE CHICKEN

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A THESIS

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BACHELOR OF SCIENCE IN AGRICULTURE

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STANDARDIZATION OF PROCESSING AND CONCENTRATION OF TURMERIC *(Curcuma longa)* RHIZOMES ESSENTIAL OIL AGAINST *Mycoplasma synoviae* in PHILIPPINE NATIVE CHICKEN

Rubelyn T. Fuertes

ABSTRACT

Mycoplasma synoviae is a cell wall less pathogen that causes significant economic losses in broiler, layer, and breeder flocks by reducing weight gain, feed conversion efficiency, egg production, embryo mortality, and treatment costs. This study would (1) determine the standardized process of TREO using the various temperatures of the condenser of the rotary evaporator; (2) assess the minimum inhibitory concentration and zone of inhibition; and (3) evaluate the potency level of TREO. This study is a combination of qualitative design and experimental design. It involved 30 kilos of Turmeric rhizomes, 10.8 liters of ethanol, 18 vials, 30 Petri plates, 100µl *M. synoviae* bacteria, 500µl tylosin at a higher dose, 250µl tylosin at a lower dose, 500µl VCO, and 500µl distilled water. The concentrations were laid out using complete block design (CRD) and two-factor CRD. The results showed that at 36^{o}C water bath and -4^{o}C condenser temperature of the rotary evaporator, the best oil was produced. The MIC of the oil produced was 25% to 100%, while the ZOI showed significance at concentrations of 85% to 100%. The antibacterial potency of TREO against *M.synoviae* was 139.35%, which means that TREO is potent based on standard potency of 60 to 150 percent. Thus, in order to produce TREO that has antibacterial properties, the water bath should be at 36^{o}C and the condenser should be at -4^{o}C temperature, which requires about 80 revolutions per minute (RPM) for 15 minutes and 40 revolutions per minute for ten minutes of vacuum rotary evaporator.

CHAPTER I

INTRODUCTION

Background and Rationale

Today, the poultry industry is the most progressive and has made significant contributions to the agricultural sector of the country. The Philippine Statistics Authority (PSA) reports that it From October to December 2022, native chicken production accounted for 43.7 percent of total production. The poultry industry is important to the economy. As of December 31, 2022, Western Visayas had the highest native chicken inventory with 13.18 million birds (PSA, 2023). *Mycoplasma synoviae* is a cell wall-less pathogen that causes significant economic losses in broiler, layer, and breeder flocks by reducing weight gain, feed conversion efficiency, egg production, and hatchability; increasing embryo mortality, carcass condemnation, prophylaxis, and treatment costs (Yadav, 2022). According to Shoaib (2020), *Mycoplasma synoviae* causes infectious synovitis and respiratory disorders in chickens as well as joint abnormalities and eggshell and production loss.

The rise and spread of antibiotic resistance have become a major concern. For the last few decades, no significant new antibiotics have been developed, and practically all existing antibiotics are losing their efficacy against pathogenic microorganisms. The prevalence of multidrug resistance microorganisms has also grown (Agyare et al., 2019). One contributing aspect is the overuse of antimicrobials in food animal production (Nhung et al., 2017). In order to increase production rates, the poultry sector must discover an alternative technique for providing chickens with potential antibiotics.

Turmeric *(Curcuma longa)* is a medicinal herb that has recently been described as a natural antibiotic option for chicken farms. Turmeric contains curcumin, a polyphenolic phytochemical with anti-microbial, anti-inflammatory, anti-cancer, and antioxidant effects.

The scientific activity of Turmeric can improve growth performance and weight increase in poultry. This activity might be linked to general health, the metabolic system, immunomodulation, or antibacterial action (Mahesh et al., 2018). In line with this, the study would want to assess the antibacterial effectiveness of Turmeric essential oil generated in a vacuum rotary evaporator against *M. synoviae* of Philippine Native Chicken.

However, despite the fact that Turmeric rhizomes essential oil has significant antibacterial activity against infectious pathogens such as bacteria, there is insufficient evidence of its effects against diverse strains of microorganisms, and no study on *Mycoplasma synoviae* found in native chicken exists. Therefore, this study was conducted.

Objectives of the Study

The study was conducted to standardize the processing and concentration of Turmeric Rhizomes Essential Oil against the *Mycoplasma synoviae* of Philippine native chicken. Particularly, this would:

1. determine the standardized processing of Turmeric *(Curcuma longa)* rhizomes essential oil using various temperatures of the water bath and condenser of the rotary evaporator;
2. assess the Minimum Inhibitory Concentration and Zone of Inhibition; and
3. evaluate the Potency level of Turmeric *(Curcuma longa)* Rhizomes essential oil.

Time and Place of the Study

The study was conducted at CPU, Research Center for Product Development, Central Philippine University, Jaro, Iloilo City. The duration of the study was three (3) months, from December 18, 2022- February 04, 2023.

CHAPTER II

REVIEW OF RELATED LITERATURE

Mycoplasma synoviae

Mycoplasma species are considered significant pathogens for the poultry industry, *Mycoplasma synoviae* is considered the most relevant. These pathogens induce respiratory syndrome and articular disease and, as a consequence, severe economic losses especially in meat categories and in layers. Mycoplasmas are microorganisms that are found in both domestic and wild birds. Among the key species in laying hens, *Mycoplasma synoviae* has emerged as an emergent pathogen in recent years, generating significant economic losses due to declining eggs and decreased egg quality (Kaboudi & Jbenyeni, 2019). Transmission of *Mycoplasma synoviae* spreads horizontally, more quickly in multi-age sites, and vertically, reducing hatchability in breeding farms. Strains of *Mycoplasma synoviae* differ in the severity with which they cause disease in poultry from sub-clinical infection to upper respiratory disease and, occasionally, septicemia and synovitis (Hoerr and Lockaby, 2019).

Antibiotics are small, natural chemical substances that are produced and secreted by microorganisms into the environment to control other organisms (Hutchings et al., 2012). Since the early 1940s, antibiotics have been widely employed in the animal feeding sector. The use of antibiotics in veterinary medicine has transformed the industry. Antibiotics are mostly used to treat infections as a form of illness prevention, such as treating respiratory and enteric infections in the early stages of the animals' lives or treating bacterial infections, such as mastitis in the adult stage (Mungroo & Neethirajan, 2014).

Broiler meat is a necessary product for providing safe food. Protein that is both affordable and of high quality for humans' diet. Antibiotics are naturally occurring biological components that are produced naturally by a microorganism and are employed to hinder the growth of other microorganisms. These antimicrobial medicines are particularly effective at inhibiting bacterial development because they impair microorganism metabolism. An antibiotic can change the features of bacteria's cellular and metabolic activity, resulting in decreased growth or death. Antibiotics are used in chicken farms to improve growth, health, and immunity against several dangerous germs (Sahu, 2016).

Antibiotic resistance is a major public concern. Antibiotic-resistant bacteria associated with animals may be harmful to humans, posing a health risk that is transmitted to humans via food chains and widely dispersed in the environment by animal waste. The prevalence of antibiotic-resistant bacteria in Southeast Asia is of concern to global public health because this region is regarded as an antibiotic-resistant bacterium. Antibiotic resistance can only be reduced through appropriate regulation and policy (Manyi-Loh et al., 2018). However, there is a scarcity of reliable national data on antibiotic levels in this region due to a lack of effective surveillance methods. To combat the threat of antibiotic resistance, the World Health Organization (WHO) developed a global antimicrobial resistance action plan, which many Southeast Asian countries have adopted (Thapa, Shrestha, & Anal, 2020). Furthermore, alternatives to antibiotics in poultry and livestock production are being investigated. Individual strategies studied included direct-fed microbial and live microbial feed supplements. Organic acids with antimicrobial properties; herbs, spices, and other plant extracts and controlled organic production (Diarra & Malouin, 2014).

Essential oils contain a wide range of secondary metabolites capable of inhibiting or slowing the growth of bacteria, yeasts, and molds (Dosoky & Setzer, 2019). Furthermore, Turmeric essential oils have potent antioxidant and antibacterial properties, making them ideal for use in the pharmaceutical and cosmetic industries. Curcuma essential oils are extracted by hydro- or steam distillation of the fresh or dried rhizome.

The Turmeric powder was stored in the refrigerator to prevent moisture uptake. A solvent extraction method has been used to extract an active compound which was curcumin in *Curcuma Longa*. Ethanol has been used as a solvent to extract the curcumin. A Rotary evaporator was used to purify the sample. The samples were put in a rotary evaporator at temperatures 40°C, 50°C, 60°C and 70°C (Foozie et. al., 2016).

UV-Vis spectrometer is an analytical technique that measures the number of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. It relies on the use of light, which has a certain amount of energy that is inversely proportional to its wavelength. Electrons in different bonding environments in a substance require a different specific amount of energy to promote the electrons to a higher energy state, which is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380nm, which we see as violet, to 780nm, which we see as red. UV light has wavelengths shorter than that of visible light to approximately 100nm. UV light has wavelengths shorter than that of visible light to approximately 100nm, which can be used to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance (Tom, 2021).

UV-Vis spectrometer data can be processed for quantitative and qualitative analyses using an appropriate chemometrics algorithm (Fadhlurrahma et al., 2020). For the detection of curcumin, absorption detectors in the wavelength range of 350nm to 450nm or in the UV region using a common detection wavelength in the range of 250nm to 270nm are simple and extremely useful (Priyadarsini, 2014).

The minimal inhibitory concentration (MIC) defines the susceptibility or resistance of specific bacterial strains to an antibiotic in vitro. Reliable MIC assessment has a significant impact on the selection of a therapeutic strategy, which affects the efficacy of infection therapy (Kowalska Krochmal & Dudek-Wicher, 2021). The ethanolic extract of Turmeric *(Curcuma longa)* was subjected to a microbial susceptibility test using the agar well diffusion method. The extract was found to be active against the bacteria used as it inhibited all the organisms with the highest inhibition zone of 13.7mm recorded against *Shigella flexnerri* and the least inhibition of 2.3mm recorded against *Staphylococcus* epidermis. Minimum Inhibitory Concentration (MIC) values range from 6.25mg/ml to 200mg/ml. *Shigella flexnerri* gave MIC of 6.25mg/ml, S. aureus and, 25mg/ml, *E.coli*,50mg/ml, *S. epidermis*, *lactobacillus* and *P. aeruginosa*, 100mg/ml *K. pneumonia* and *V.cholera* and *S. typhi* 200mg/ml respectively (Oghenejobo, 2018).

Curcuma longa or known as Turmeric is a rhizomatous perennial herb that originated in southeast Asia. The plant's root system produces fleshy rhizomes that are brilliant yellow to orange in color and are the source of the commercially available spice Turmeric (Damlas, 2011). It contains curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin, as well as volatile oils (tumerone, atlantone, and zingiberone), carbohydrates, proteins, and resins (Akinyemi & Adeniyi, 2018). The major component of Turmeric is curcumin, which is extracted from Turmeric rhizomes using a solvent extraction process. Curcumin is stable at high temperatures and in acidic

environments, while it is unstable in alkaline conditions. It is soluble in oil and alkali but almost insoluble in water at acid and neutral pH. Water-soluble curcumin is produced by incorporating sodium dodecyl sulfate, cetyl pyridinium bromide, gelatine, polysaccharides, polyethylene, glycol, and cyclodextrins into different surfactant micellar systems (Iniaghe et al., 2019).

Curcuma species have anti-inflammatory, antibacterial, antiproliferative, anticancer, hypoglycemic, anti-hyperlipidemic, neuroprotective, hepatoprotective, hematologic and circulation abnormalities, infectious diseases, and cancer prevention properties, according to (Dosoky et al., 2019). They also supply flavoring and coloring compounds, cosmetics, fragrances, and decorative plants. Nonetheless, due to its limited absorbability, curcumin's usefulness in altering functioning and promoting gut health may be connected with a concentration in the intestine (Lopresti, 2018). Furthermore, Turmeric has a number of advantages for chickens. Improvement in immunological response can be achieved by a variety of methods, pathways, increased antioxidant activity, a decrease in the number of pathogenic microbe bacteria in the gut, and an improvement in blood markers associated with its nutrient content phytochemicals (Jurenka, 2009; Rahimi & Kazemi-Oskuee, 2014). Turmeric's immunomodulatory actions significantly strengthen the immune system, providing immediate natural antibacterial capability against pathogenic infection. Turmeric essential oil was shown to have higher antibacterial properties against *Escherichia coli, Proteus Vulgaris, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* Maximum antibacterial effects against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* have been found in ethanol extract (Gouda & Yashodhar Bhandary, 2018) . Despite its prominent organo-compounds and antibacterial activity, studies on its antibacterial action against *Mycoplasma synoviae*. The number of *M. synoviae* isolated from Philippine native chicken is limited. As a result, this study was conducted.

Minimum inhibitory concentrations (MICs)

MICs are the lowest antimicrobial concentrations (g/ml) that can inhibit visible bacterial growth after overnight incubation. They are used in diagnostic laboratories to confirm resistance, but they are also used in research to investigate the in vitro activity of new antimicrobials. Curcumin has considerably more antibacterial activity against Gram (+) bacteria than against Gram (-) bacteria. Curcumin was shown to be the most effective against S. aureus, regardless of antibiotic resistance. Because curcumin combined with antibiotics reduced *E. coli, E. faecalis*, in addition to *S. aureus*, which has a synergistic interaction in most instances, the potential use of such combinations to generate an alternative therapy in the treatment of particular human infections. Curcumin has a broad spectrum of antibacterial action, as demonstrated by the in vitro data reported in this study, including developing and multidrug-resistant pathogens. The antibacterial action, however, appears to be bacteria-dependent and can vary between bacterial species. However, the antibacterial effect appears to be bacterial-dependent and varies amongst bacterial species (Hussain et al., 2022).

Zone of Inhibition of (ZOI) of Turmeric Oil

The ethanol extract of Turmeric was used to determine the antibacterial sensitivity against *Staphylococcus aureus* and *E. coli*. The inhibitory zone in the media was measured using three different dosages. The findings revealed that the maximum inhibition zone in the case of broth bacteria was at 75µl level of ethanol extract of Turmeric, while at 100µl level the inhibition zone was 10.75mm and 9.5mm respectively for *E. coli* and *Staphylococcus aureus*, in that order. The bactericidal activity of Turmeric did not alter considerably ($p>.05$). Turmeric indicated a 9.00 mm inhibitory zone in *E.coli* at 50µl with *Staphylococcus aureus* measuring 8.5 mm. As a result, the data demonstrated

the antibacterial activity of C*. longa* ethanol extract against *E. coli* and *Staphylococcus aureus* (Islam, 2019).

Potency Test

Potency (strength) tests are performed to determine the amount of active ingredients in a sample. Antibiotic potency testing evaluates the degree of microbial growth inhibition produced by an antibiotic(s) in a large number of samples, providing real antibiotic potency verification. During antibiotic potency testing, many concentrations of the test antibiotic are tested against a live microbe to measure both antibiotic efficacies against microbiological growth and the concentration at which a specific antibiotic inhibits microbial growth. (Loyd, 2013).

Although studies have been conducted to produce Turmeric oil, there is no data on the standardized process of producing Turmeric essential oil using a vacuum rotary evaporator, and there is insufficient data on the use of Turmeric essential oil as an alternative antibiotic against the bacterium *Mycoplasma synoviae*. Furthermore, no studies have been undertaken using food-grade ethanol to extract the curcumin molecule. As a result, this research is critical for the safe and effective management of antibiotic resistance.

CHAPTER III

MATERIALS AND METHODS

This research is a combination of qualitative research design and experimental research design. This is composed of three sub-studies that include (1) determination of the processing of Turmeric rhizomes essential oil using the various temperatures of the water bath and condenser of a vacuum rotary evaporator; (2) assessment of the minimum inhibitory concentration and zone of inhibition; and (3) evaluation of the potency level of Turmeric rhizomes essential oil.

Laboratory Safety Measures

The researcher used an autoclavable hazmat suit, which is a whole-body garment worn to protect the user against harmful materials or substances from splashes, spills, or other exposures to chemical or biological agents in some cases. Safety goggles for eye protection, sterile gloves, a sterile mask, and a hair net were used as part of the researcher's personal protective equipment (Environmental Health and Safety, 2021).

DETERMINATION OF THE PROCESSING OF TURMERIC RHIZOMES ESSENTIAL OIL USING VARIOUS TEMPERATURES OF WATER BATH AND CONDENSERS OF THE VACUUM ROTARY EVAPORATOR

Sub-study 1

Turmeric (*Curcuma longa*) rhizomes were collected, washed, chopped into pieces, and dehydrated in the dehydrator at 50°C for three to five days. The dried Turmeric was ground using a grinder. The ground Turmeric rhizomes were soaked in 95% food-grade ethanol for 24 hours, and stored in the ultra-low freezer. The soaked Turmeric rhizomes powder was filtered using filter paper and a Buchner flask. The filtrate was subjected to the rotary evaporator at 36ºC, 38ºC, and 40ºC water bath and 0, -2, -4, -6, -8, and -10ºC condenser to separate ethanol from the essential oil. To purify the oil from ethanol, the sample was subjected to a magnetic stirrer at 70^{o}C temperature for 15 minutes to extract the remaining ethanol from the plant extract. A crude determination test was conducted by mixing the product with virgin coconut oil to determine if the product is an oil. The produced essential oil was tested under the Dual-beam UV Spectrophotometer from 190-1100nm.

Experimental Treatment and Design

The treatment was (36^{o}C), (38^{o}C), (40^{o}C) water bath at (0^{o}C), (-2^{o}C), (-4^{o}C), (-6^{o}C), (-8^{o}C), and (-10^{o}C) condenser. These treatments were replicated three times. A qualitative research design was used to determine the standardized process of Turmeric rhizomes essential oil in various temperatures of the water bath and condenser of the rotary evaporator.

Gathering & Preparation of Plant Material

The turmeric (*Curcuma longa*) rhizomes were already available at Central Philippine University, Research Center for Product Development, Jaro, Iloilo City. The plant that was utilized in this study was a randomly selected matured rhizome. Soil residues from the fresh Turmeric rhizomes were first removed using running water, after which, the rhizomes were chopped into pieces using a knife and set aside for essential oil preparation (Ching, 2014).

Dehydration and soaking of Turmeric Rhizomes. Fresh Turmeric rhizomes were dehydrated for three to five days using a dehydrator at 50°C temperature. After dehydration, the dried rhizomes were powdered using a grinder (Pardey et al., 2020). The Turmeric powder was weighed using a digital weighing scale to determine a certain ratio. Following a 1:4 ratio (600g of powder and 2,400 liters of 95% ethanol). Turmeric powder weighing 600g was transferred into a 4000ml beaker and 2,400 liters of food ethanol was added and placed at an ultralow-freezer temperature of -47^{o}C for 24hrs. Ethanol was used as a solvent to extract. Ethanol is widely used in oil extraction due to its ability to extract a large amount of oil, it is safely used in plant extraction to produce cooking oils and oils for personal care and cosmetics. Ethanol is particularly good at separating oils from the plant material and it's preferred by the FDA for food products (Ching, 2014; Mancuso, 2021).

Extract purification and vacuum filtration. After 24 hours of soaking, the soaked Turmeric rhizomes powder was squeezed using cheesecloth, and the extraction was filtered using a vacuum flask filter. The filter was done to separate the liquid from solid particles/residues using vacuum pressure suction. After the material has been filtered. The ethanolic Turmeric rhizomes extract was produced (Cole-Pamer,2020).

*Rotary evaporator***.** Since the product is still an ethanolic Turmeric rhizome extract. The use of a rotary evaporator was needed to produce essential oil. Therefore, the ethanolic Turmeric rhizomes extract was placed into a beaker for the process. Then, the water bath temperature was set at (36^{o}C), (36^{o}C), and (40^{o}C), and (0, -2, -4, -6,-8, and -10) degree celsius condenser and rotation was at 80rpm for 15 minutes and 40rpm for ten minutes (Ching 2014).

Test Purification of Essential Oil

Refinement of Turmeric oil. The Turmeric oil from the rotary evaporator was subjected to a magnetic stirrer at 70^{o}C temperature for 15 minutes to extract the remaining ethanol from the plant extract. A magnetic stirrer was used in this study to reduce the consumption of organic solvent ethanol and improve efficient extraction (Hingmire et al., 2017).

Crude Test Determination Oil. The Turmeric rhizomes essential oil produced in the rotary vacuum evaporator was mixed with virgin coconut oil (VCO) to determine whether the product is oil or not. Due to the volatility of the product, it was able to mix with the carrier oil. These volatile oils were lipophilic, meaning they are fat soluble or fat lovers (Miller, 2015).

Dual-beam UV Spectrophotometer. The visible spectroscopy principle is that chemical substances absorb ultra-violet or visible light to create distinctive spectra. The produce Turmeric rhizomes essential oil in 36^{o}C, 38^{o}C, and 40^{o}C temperatures of water bath and 0^{o}C, -2^{o}C, -4^{o}C, -6 ^{o}C, -8^{o}C, and -10^{o}C condensers were further analyzed using Dual-beam UV Spectrophotometer to measure its light energy absorbance and used VCO as the baseline of every sample (Bosch & Sanchez, 2013).

ASSESSMENT OF THE MINIMUM INHIBITORY CONCENTRATION AND ZONE OF INHIBITION

Sub-study 2

Two-fold dilution was used to determine the Minimum Inhibitory Concentration. Using a multi-channel micropipette, 300 microliters of treatments were placed from A1 to A7 of the microplate, and 150 microliters of Brain Heart Infusion (BHI) from columns two to twelve. In column A, 150 microliters of the treatments were withdrawn and transferred to each subsequent column until the tenth column, where 150 microliters of the treatments were discarded. Using a multi-channel micropipette, ten microliters of *Mycoplasma synoviae* were diluted in ten milliliters of water and were shaken for 60 seconds. The dilution of 150 microliters was placed from columns two to eleven, sealed, and incubated for 24 hours. The 96-plate reader was used to determine the MIC.

The resulting concentration determined by the MIC was used to test the zone of inhibition of Turmeric rhizomes essential oil produced in 36 ^{o}C, 38 ^{o}C, and 40^{o}C water baths and 0^{o}C,- 2^{o}C, -4^{o}C, -6 ^{o}C, -8^{o}C, and -10^{o}C temperatures of the condenser of rotary evaporator against *Mycoplasma synoviae*. Brain Heart Infusion (BHI) was prepared and placed into each Petri dish. A hole was punched in the center of the solidifier agar using a 9.33mm sterile micropipette tip then 100 microliters of diluted *Mycoplasma synoviae* in pure water (ten microliters: ten milliliters) was spread onto the agar. The Petri dishes were incubated for 72 hours. The data were analyzed using ANOVA for a two-factor, completely randomized design and Duncan's multiple range test at the 5% level of significance.

Experimental Treatment and Design

The experimental treatments for the minimum inhibition concentration include various concentrations of Turmeric rhizomes essential oil to VCO. It consists of 100%, 75%, 50%, and 25% concentrations of Turmeric rhizomes essential oil and virgin coconut oil, pure VCO, tylosin (+), and water (-) were used in the study. These treatments were replicated three times and laid out in a completely randomized design (CRD).

The experimental treatments for the potency test include 100%, 85%, 70%, 55%, 40%, and 25% concentrations of TREO to VCO, and pure VCO, tylosin and distilled water were used as the positive and negative control respectively. These treatments were replicated three times and laid out in a two-factor completely randomized design (CRD).

Collection of Bacterial Samples

The bacterial source of *Mycoplasma synoviae* were already available at Central Philippine University, Research Center for Product Development**.**

MIC Determination by Microtiter Broth Dilution Method

The determination efficacy of Turmeric rhizomes essential oil against the bacterium *Mycoplasma synoviae* was assessed using a 96-plate reader. In a microtiter plate reader, 300µl of Turmeric rhizomes essential oil consisting of the level of treatment (100%, 75%, 50%, 25%), tylosin (+), water (-), and virgin coconut oil was placed in A1 to G1 respectively. Using a multi-channel micropipette, 150µl of Brain Heart Infusion (BHI) was placed from column two to column twelve. Using a multi-channel micropipette, 150µl were withdrawn from column one and added to column two and were mixed by sucking up and down four to five times. As a result, column one

gets diluted twice in column two. A 150µl was withdrawn from column two and added to column three up until column ten, the operation was repeated. The full dilution series was completed with the same set of tips. A 150µl was withdrawn from column ten and was discarded rather than putting it in column eleven. Ten microliters of the bacteria were diluted in ten milliliters of pure water. Using the multi-channel micropipette, 150µl of the dilution were put from column two to eleven. The four sides of the microplate were sealed using tape and placed in a CO_2 incubator set at 37°C prior to detection by a 96-plate reader (Hossain et al., 2017).

Zone of Inhibition (ZOI)

*Preparation of Experimental Treatment***.** The result of (MIC) was used to determine the (ZOI) of the Turmeric rhizomes essential oil produced in 36ºC water bath and -4ºC condenser temperatures of rotary evaporator against *Mycoplasma synoviae* of Philippine Native Chicken. The concentrations of essential oil were 100% (500µl TREO), 85% (425µl TREO + 75µl VCO), 70% (350µl TREO +150µl VCO), 55% (275µl TREO + 225µl VCO), 40% (200µl TREO + 300µl VCO) 25% (125µl TREO + 375µl VCO), positive control tylosin higher and lower dose (500µl), (250µl) and distilled water (500µl).

Preparation of Agar Media. The Brain Heart Infusion (BHI)powder was obtained at the Central Philippine University- Research Center for Product & Development. The BHI was prepared according to the label indicated in the container. Mettler digital weighing scale was used to measure nineteen grams of BHI. A pan was prepared with 500ml distilled water at 60ºC to 80ºC and waited until it boiled. The BHI powder was put and stirred upon boiling to dissolve the powder uniformly. The cooked BHI was transferred to a beaker and ten milliliter was placed in each Petri dish. The Petri dishes

containing the culture media were autoclaved for fifteen (15) minutes at a temperature of 121^{o}C. After 15 minutes, the Petri dishes were removed from the autoclave and placed inside the biosafety cabinet for the culture media to cool and solidify. The use of a biosafety cabinet lessens contamination among treatments.

Inoculation of Mycoplasma synoviae. Using aseptic techniques, 100µl of *Mycoplasma synoviae* was inoculated into the culture media with the use of a pipette and evenly spread with the use of a glass spreader. The glass spreader was then sterilized for a few seconds by flaming it in the alcohol lamp.

Impregnation of Treatments. The study utilized an Agar Well Diffusion Method. A hole was punched in the center of the solidified agar using a 9.33mm sterile micropipette tip. The well was filled with 40µl of each experimental treatment. The Petri dishes were placed in an incubator at the temperature of 37°C and cultured for twenty-four (24) hours (Blazekovic et al., 2011).

Data Collection. This study measured the zone of inhibition (ZOI) a halo/clear form that can be seen in Petri dishes. The measurements were taken in the horizontal and vertical planes of the petri dish using the digital caliper. The measurements were computed to serve as the means of determining the zone of inhibition.

$$\text{Zone of inhibition} = \frac{(Horizontal\ measure - 9.3) + (Vertical\ measure - 9.3)}{2}$$

Statistical Analysis

Data that was obtained from MIC were analyzed statistically using analysis of variance (ANOVA) for a completely randomized design and for ZOI were analyzed statistically using analysis of variance (ANOVA) for a two-factorial completely randomized design. Significant differences between or among treatment means were further analyzed using Duncan's multiple range test at the 5% level of significance.

EVALUATION OF THE POTENCY LEVEL OF TURMERIC RHIZOMES ESSENTIAL OIL

Sub-study 3

The antimicrobial potency of the Turmeric rhizomes essential oil was evaluated using the formula by Sovasia and Hala (2016). It was used to calculate the percent (%) potency, after 72 hours of post-exposure TREO against *Mycoplasma synoviae* of the Philippine native chicken.

Experimental Treatment and Design

The experimental treatments for the potency test include 100%, 85%, 70%, 55%, 40%, and 25% concentrations of TREO to VCO, and pure VCO, tylosin, and distilled water were used as the positive and negative control respectively. These treatments were replicated three times and laid out in a two-factor completely randomized design (CRD).

Potency Test

After 72 hours of post-exposure to Turmeric rhizomes essential oil, the antimicrobial potency of the TREO against *Mycoplasma synoviae* was evaluated using the formula Sovasia and Hala, (2016).

The formula of Sovasia and Hala (2016), was used to calculate the % potency.

$$A = \frac{(TS1+TS2)-(S1+S2)}{(TS1+TS2)+(S1-S2)}$$

$$\text{Potency of sample} = \frac{Potency\ x\ assumed\ potency\ sample}{100}$$

Waste Disposal

Waste was properly sorted and segregated. Before washing, waste that may include bacteria or other biological material was autoclaved at 121°C for 15 minutes. Certain materials generated were disposed of in accordance with regulations, including the classifying, packing, and labeling of biohazardous and infectious waste.

CHAPTER IV

RESULTS AND DISCUSSION

DETERMINATION OF THE PROCESSING OF TURMERIC RHIZOMES ESSENTIAL OIL USING VARIOUS WATER BATHS AND CONDENSERS OF ROTARY EVAPORATOR

Sub-study 1

Turmeric Rhizomes Essential Oil Production

In this sub-study, the collected rhizomes of Turmeric were washed, sliced, and dried set at 50°C for 3-5 days in a dehydrator. The dried Turmeric was ground using a grinder. The ground Turmeric rhizomes were soaked in 95% food-grade ethanol, then filtered using a vacuum Buchner filter flask. The filtered extract was processed using a vacuum rotary evaporator with a temperature of water bath set at 36^{o}C, 38^{o}C, and 40^{o}C and a condenser set at 0^{o}C, -2^{o}C, -4^{o}C,-6^{o}C,-8^{o}C, and -10^{o}C. While the revolutions per minute (RPM) was set to 80 for 15 minutes and 40 for 10 minutes. The produced essential of Turmeric rhizomes in 36^{o}C, 38^{o}C, and 40^{o}C temperature water bath and 0^{o}C, -2^{o}C, -4^{o}C, -6^{o}C,-8^{o}C, and -10^{o}C temperature condensers were tested for light energy absorbance using Dual-beam UV spectrophotometer. The results of the studies were presented below.

As shown in Table 1, the qualitative assessment of Turmeric Rhizomes Essential Oil produced in a vacuum rotary evaporator set at various temperatures of water bath and condensers. The table results showed that at 36^{0}C temperature of the water bath, (-2^{0}C) and (-4^{0}C) condensers were homogenously mixed with the virgin coconut oil, which indicates that it needs agitation to be mixed with virgin coconut oil.

The temperature in the condenser set at (0^0C), (-6) and (-8^0C) showed a homogenously mixed which indicates that it needs agitation to be mixed with virgin coconut oil. At the 38^0C temperature of the water bath, (-6^0C), (-8^0C), and (-10^0C) of condenser produced oil and it's considered homogeneously mixed, requiring agitation to mixed with the carrier oil. Some temperatures of the condenser were paste indicating that there is no oil produced at that temperature. At the 40^0C temperature of the water bath, only (-6^0C), (-8^0C), of the condenser produced oil and it's considered homogeneously mixed, requiring agitation to mix with the carrier oil and only (-10^0C) of the condenser shows no separation with the carrier oil indicating that it is already an oil without agitation needed.

Table 1

Determining the Appropriate Temperature of The Condenser of the Vacuum Rotary Rotary Evaporator for Production of Turmeric Rhizomes Essential Oil

Temperature of Water Bath	Temperature of Condenser	WS	HM	Remarks
36	-0,-6,-8		/	Requires agitation to mix, but no separation was detected after.
	-2,-4		/	Requires agitation to mix, but no separation was detected after.
	-10			There is no essential oil produced, considered as a paste.
38	-0,-2,-4			There is no essential oil produced, considered as a paste.
	-6,-8,-10		/	Requires agitation to mix, but no separation was detected after.
40	-0,-2,-4			There is no essential oil produced, considered as a paste.
	-6,-8		/	Requires agitation to mix, but no separation was detected after.
	-10	/		A separation of turmeric oil and virgin coconut oil was detected in the sample.

Note: WS – with separation; HM – homogeneously mixed; * It was done by mixing the produced essential oil with Virgin Coconut Oil. The oil that was homogeneously mixed was considered the Turmeric Rhizomes Essential Oil.

The finding of the study reveals that in the -4^{o}C condenser temperature and 36^{o}C water bath temperature of the vacuum rotary evaporator, the best oil was produced. This is due to the relationship between temperature and volatile compounds present in an extracted plant material. Wherein, as the temperature of the condenser rises the more volatile compound losses (Valdivieso-Ugarte et al., 2019). The loss of volatile compounds present in the produced essential oil greatly affects its efficiency**.**

There are no known studies on the use of condensers 0^{0}C, -2^{0}C, -4^{0}C, -6^{0}C, -8^{0}C, and -10^{0}C also 36^{0}C, 38^{0}C, and 40^{0}C water bath temperatures of a vacuum rotary evaporator, in the process of producing Turmeric rhizomes essential oil. But, in a study by Nong et al. (2016), reveals that Turmeric rhizomes essential oil is produced at a maximum temperature of 45^{0}C. But, the study of Goncalves et al. (2019) reveals that *Curcuma longa* rhizomes essential oil were produced in a water bath temperature at 50 ^{o}C and 40rpm for 30 minutes. The major components present in essential oil remain active such as zingiberene (11%), sesquiphellandrene (10%), β-turmerone (10%), and α-curcumene (5%). The essential oil was efficient at inhibiting the growth of *Staphylococcus aureus* (MIC of 38.8µl/ml), *Staphylococcus epidermidis* (MIC of 50.0µl/ml), *Escherichia coli* (MIC of 44.4µl/ml), and *Pseudomonas aeruginosa* (MIC of 27.7µl/ml). While in the study of Ching et al. (2014), reveals that *Curcuma longa* rhizomes essential oil produced in a rotary evaporator at 40 ^{o}C, 50 ^{o}C,60 ^{o}C,70 ^{o}C water bath temperatures with constant speeds of rotation at 90rpm show the different percentage of curcumin the major component. Factors such as the extraction method, the solvents used for extraction, the extraction time and temperature, the solvent ratio, and extraction pressure were among the significant factors that were shown to be able to influence the efficiency of curcumin extraction.

As shown in Table 2, the absorbance coefficient of several samples of Turmeric rhizomes essential oil. Figures 1,2 and 3 with a 36^{o}C, 38^{o}C, and 40^{o}C water baths and 0^{o}C, -2^{o}C,-4^{o}C,-6^{o}C,-8^{o}C, and -10^{o}C condenser show the absorbance coefficient of Turmeric rhizomes essential oil at a range of 190nm-1100nm.

The findings of this study reveal that as the temperature of the condenser increases, the oil becomes less concentrated. This results in a gradual change in the absorbance coefficient of TREO.

Table 2

Absorbance Coefficient of Turmeric Rhizomes Essential Oil at 190nm – 1100nm

Condenser Temperatures	Water Bath Temperatures		
	36^{o}C	38^{o}C	40^{o}C
0^{o}C	871nm-939nm	No sample tested	No sample tested
-2^{o}C	788nm-1054nm	No sample tested	No sample tested
-4^{o}C	779nm-1088nm	No sample tested	No sample tested
-6^{o}C	No sample tested	867nm-940nm	892nm-1034nm
-8^{o}C	840nm-1057nm	901nm -949nm	788nm-1059nm
-10^{o}C	No sample tested	No sample tested	773nm-1090nm

The variations in the absorbance coefficient of Turmeric rhizomes essential oil vary from the applied temperature of the water bath and condenser of the rotary evaporator. The condenser in a vacuum rotary evaporator is a coolant that speeds up the phase and changes its process from the gas to the liquid phase (Santos et al., 2021).

Therefore, the higher the set temperature of the condenser used the more the volatile compounds were lost.

There are no existing studies related to the absorbance coefficient of Turmeric rhizomes essential oil using various temperatures of the condenser of the rotary evaporator. But the study of Kamble et al. (2011) uses Turmeric *(Curcuma longa)* rhizomes as plant material to produce essential oil, using various temperatures of water bath consists 40^{o}C, 50^{o}C, 60^{o}C and 70^{o}C in a rotary evaporator. It reveals that the active compounds of Turmeric rhizomes which is curcumin were found at 425nm. It supports the study Priyadarsini (2014), that curcumin can be detected at the wavelength range from 350nm to 450nm range or in the UV region using a common detection wavelength in the range of 250nm to 270nm.

Figure 1

The absorbance coefficient of Turmeric Rhizomes Essential Oil. In a 36-degree C. water bath and 0,-2,-4,-6,-8 and -10-degree C. condensers

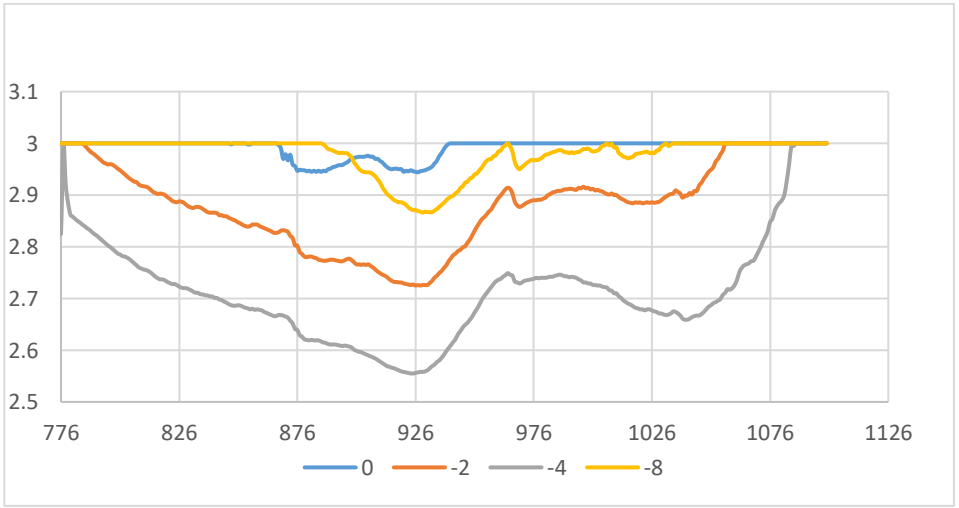


Figure 2

The absorbance coefficient of Turmeric Rhizomes Essential Oil. In a 38-degree C. water bath and 0, -2,-4,-6,-8 and -10-degree C. condensers

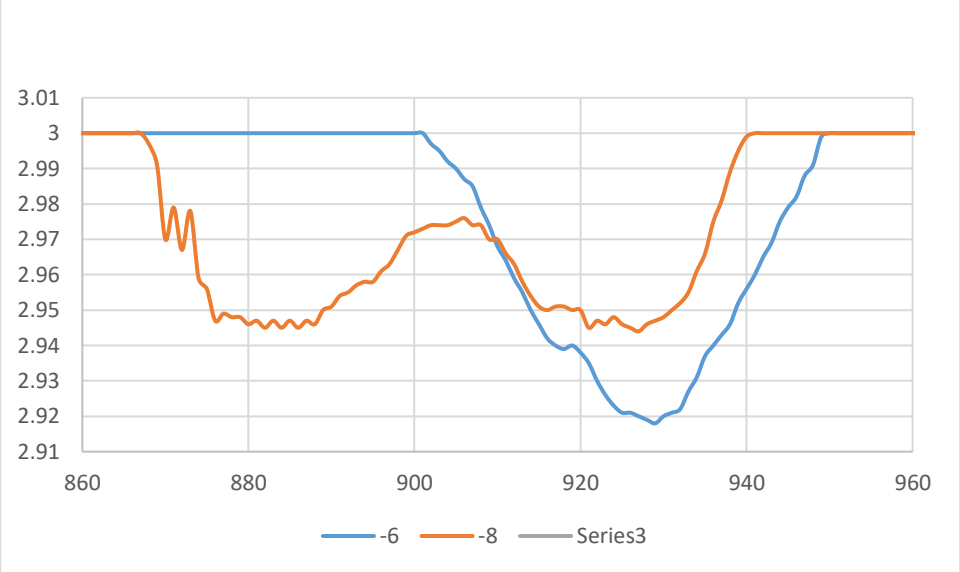
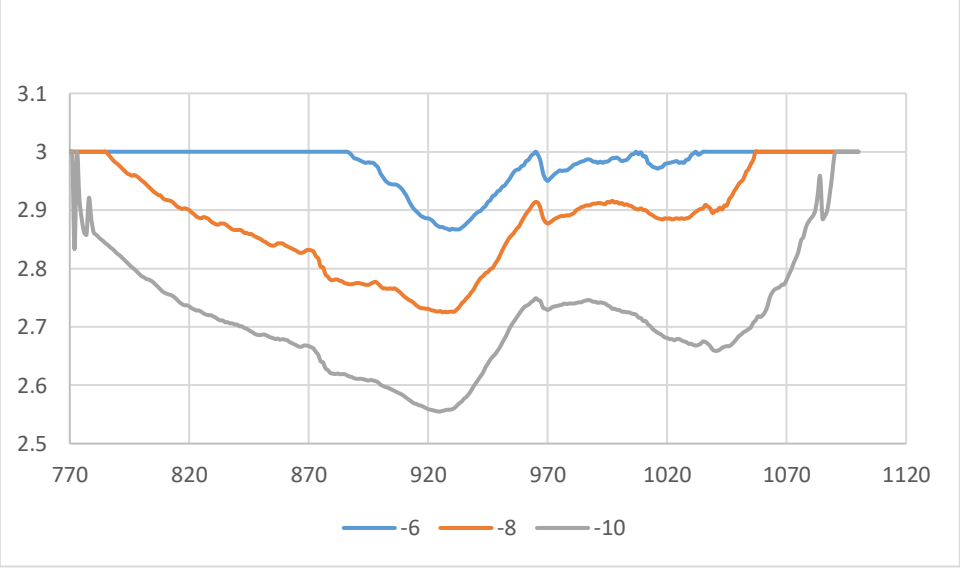


Figure 3

The absorbance coefficient of Turmeric Rhizomes Essential Oil. In a 40-degree C. water bath and 0,-2,-4,-6,-8 and -10-degree C. condenser



ASSESSMENT OF THE MINIMUM INHIBITORY CONCENTRATION AND ZONE OF INHIBITION

Sub-study 2

Minimum Inhibitory Concentration and Zone of Inhibition

This sub-study presents the results of minimum inhibitory concentration and zone of inhibition. The MIC of the organism was determined following the method described by Hossain et al. (2017). The Turmeric rhizomes essential oil used varying concentrations ranging from 25% to 100%, tylosin as a higher dose and lower dose of positive control, whereas VCO and distilled water as negative control were prepared by microtiter broth dilution using Brain Heart Infusion (BHI). Each concentration was inoculated by the bacteria *Mycoplasma synoviae* overnight and incubated at 37ºC for 24 hours.

The result of the MIC was used to test the zone of inhibition of Turmeric rhizomes essential oil against *Mycoplasma synoviae*. The antimicrobial activities of Turmeric rhizome essential oil were evaluated using the agar well diffusion method (Blazekovic et al., 2011). A hole was punched in the center of the solidified agar using a 9.33mm micropipette tip, then the *Mycoplasma synoviae* inoculum was streaked onto a sterile Brain Heart Infusion agar plate and incubated at 37°C. for 72 hours.

As shown in Table 3, the concentrations of Turmeric rhizome essential oil from 25%,50%,75%, and 100% together with antibacterial tylosin as the positive control and virgin coconut oil as the negative control. These were used against *Mycoplasma synoviae* for its minimum inhibitory concentration. The study reveals that after an overnight incubation visible growth of bacteria was inhibited at 25 % to 100% concentration of TREO.

Table 3

Percentages of Minimum Inhibition Concentration of Turmeric Rhizomes Essential Oil Against Mycoplasma synoviae of Philippine Native Chicken

	Replication			
Treatment	I	II	III	Mean
100	90.65	90.43	92.55	91.21[a]
75	79.05	80.94	81.59	80.67[c]
50	73.52	73.19	73.03	73.24[d]
25	73.00	72.48	72.98	72.82[e]
PC	89.98	89.94	91.23	90.38[b]
VCO	71.00	71.16	71.28	71.15[f]
cv	15.76			

Note: * Means with the same are not significantly different.

The study reveals that 100% TREO has minimum inhibitory concentration against *Mycoplasma synoviae* of Philippine native chicken. This means that 25% to 100% concentrations of TREO had bacterial inhibition against *Mycoplasma synoviae*. Therefore, concentrations of TREO were subjected to the zone of inhibition (ZOI) against *Mycoplasma synoviae* of Philippine native chicken.

The minimum inhibition concentration of Turmeric rhizomes essential oil agrees with the study of Persaud et al. (2020), that Turmeric rhizomes extract in 75% concentration had a maximum antibacterial activity and inhibits the three tested bacteria namely *E. coli, and S. aureus*. This is parallel to the study of Maulidi et al. (2020), which revealed that Turmeric rhizomes extract at 25% concentration has a weak inhibitory zone in the growth of *Bacillus cereus* bacteria.

The fractional inhibitory concentration index of Turmeric rhizomes essential oil mixed with virgin coconut oil as a carrier against *Mycoplasma synoviae* in Philippine native chicken is presented in Table 4. Based on the stated means (2.38, 2.36, and 2.25) of concentrations 75% to 25% TREO mixed with VCO showed fractional inhibitory concentration against *Mycoplasma synoviae* and indifferent interactions with the carrier oil VCO. Based on the given indication more than 0.5 but less than or equal to 4.0 indicates indifferent interactions. Therefore, Turmeric rhizomes essential oil possesses antibacterial action against *Mycoplasma synoviae* in Philippine native chicken.

Table 4

Fractional Inhibitory Concentration Index of Turmeric Rhizomes Essential Oil mixed with Virgin Coconut Oil as Carrier Against Mycoplasma synoviae in Philippine Native Chicken.

Treatment	Replication			Mean
	I	II	III	
75	2.36	2.36	2.42	2.38
50	2.29	2.43	2.38	2.36
25	2.24	2.21	2.29	2.25

Note: less than or equal to 0.5 means synergistic interactions between VCO and Turmeric rhizomes essential oil; more than 0.5 but less than or equal to 4.0 means indifferent interactions; more than 4.0 antagonistic interactions

The use of VCO as a carrier oil enhances product penetration in essential oils. It has antibacterial characteristics capable of inhibiting pathogenic bacteria and a high lauric acid content (Barrel, 2021). VCO is used as a carrier oil for dilution in the production of essential oil from Turmeric rhizomes using a rotary evaporator in order for the oil to be completely penetrated. The carrier oil used was autoclaved to destroy its property since oil loses its property when treated to high temperatures.

This agrees with the report by Willet (2018), that exposure to 350^{o}F warm can destroy fat lauric acid which serves as an antibacterial property of VCO. A ponder by Listones (2023) also concurs that virgin coconut oil (VCO) and coconut oil lana exhibit no antibacterial activity against *B. subtilis, S. aureus, E. coli.* But it was in contrast to the report of Boyers (2023), hat both unrefined and refined coconut oils have a high concentration of fat lauric acid, which has antibacterial properties against pathogenic microbes.

As shown in Table 5, the means of concentrations of Turmeric rhizomes essential oil exposed to *Mycoplasma synoviae* from 24 to 72 hours. The concentrations employed were 25% TREO to 100% TREO, tylosin as a positive control, and distilled water and VCO as a negative control. The table shows that the time of exposure of Turmeric rhizomes essential oil with concentrations of 85% to 100% does not affect its antibacterial efficacy against *Mycoplasma synoviae* of Philippine native chicken.

Table 5

Zone of Inhibitions (mm) of Mycoplasma synoviae after 72 hours of post-exposure time

Concentrations	Hours			Mean
	24	48	72	
100%	5.72	5.46	5.45	5.72[a]
85%	5.15	4.91	5.15	5.18[ab]
70%	3.56	4.92	4.99	4.97c
55%	2.95	4.40	4.42	4.42d
40%	2.41	4.13	4.41	4.16[d]
25%	1.65	3.28	3.0	3.22e
H2O	0.71	0.71	0.71	0.71[f]
PC	5.63	5.48	5.51	5.54[a]
VCO	0.71	0.71	0.71	0.71[f]
cv	5.09			

*Means with the same letter are not significantly different at a 5% level of probability.

The concentrations of 85% to 100% of Turmeric rhizomes essential oil showed significant differences with the positive control. This indicates that the concentrations of 85% to 100% were comparable with the positive control, tylosin. Therefore, it states that any of the 85% to 100% concentrations could be used as an effective antibacterial against *Mycoplasma synoviae* in Philippine native chicken.

The study of Maulidi (2020), found that the ethanol extract of turmeric *(C. longa)* rhizomes had antimicrobial properties. At greater concentrations, longa possesses antimicrobial action. According to Gupta et al. (2015), turmeric rhizome extract includes phytochemical substances such as tannins and phenolic compounds, protein, flavonoids, steroids, triterpenoids, glycosides, carbohydrates, and so on that can suppress pathogenic microorganisms. In contrast to the study of González et al. (2019), which discovered that the extract of turmeric rhizomes had substantial antibacterial activities at all doses against pathogenic bacteria such as *E. coli* and *S. aureus.*

EVALUATION OF THE POTENCY LEVEL OF TURMERIC RHIZOMES ESSENTIAL OIL

Sub-study 3

In this sub-study, the antimicrobial potency of the Turmeric rhizomes essential oil was evaluated using the formula Sovasia &Hala (2016). This was used to calculate the percent (%) potency of Turmeric rhizomes essential oil against *Mycoplasma synoviae* of Philippine native chicken, after 72 hours of post-exposure.

Results revealed that Turmeric (*C. longa)* rhizomes essential oil against *Mycoplasma synoviae* showed antibacterial potency. Based on Table 6, it has 139.35% percent potency which was accepted to the standard level of potency. The result was based on the percent potency test formula of Sovasia & Hala (2016).

Table 6

Potency Percentage of Turmeric Essential Oil Against Mycoplasma synoviae

Treatment	Potency Percentage Tylosin	The standard level of Potency 60% to 150%
Turmeric Rhizomes Essential Oil	139.35%	p

Note: p – the treatment was considered potent; np – the treatment was considered not potent.

Turmeric rhizomes have been found rich in phenolic compounds such as alkaloid, flavonoid, saponin, tannin, curcuminoids, and terpenoid compounds that contributed

to antioxidant activities (Ching, 2014). *Curcuma longa* is a source of various phytochemicals that justify the use of plants as bactericidal agents for the treatment of so many diseases such as cancer, inflammations, microbial infections, diabetes, arthritic, muscular disorders, biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, and sinusitis(Pizzorno et al., 2016). Therefore, it was proven that the standardized process of Turmeric rhizomes essential oil exhibit antibacterial activity against *Mycoplasma synoviae.*

The antibacterial potency of Turmeric *(Curcuma longa)* rhizomes essential oil supports the study of Hussain et al. (2022), which revealed the efficacy of the *C. longa* leaf against *Mycoplasma synoviae.* This also affirms the study of Singh et al. (2017), which revealed that *C. longa* rhizomes have been shown to have inhibitory effects on bacterial strains such as *Mycoplasma synoviae.*

CHAPTER V

SUMMARY, CONCLUSION, AND RECOMMENDATION

Mycoplasma synoviae is a cell wall-less pathogen that causes significant economic losses in broiler, layer, and breeder flocks by reducing weight gain, feed conversion efficiency, egg production, and hatchability; increasing embryo mortality, carcass condemnation, prophylaxis, and treatment costs. To inhibit the growth of this bacterium, Turmeric rhizomes essential oil (TREO) was employed. Turmeric rhizomes are rich in substances such as curcumin, tannins, flavonoids, and triterpenoids that are responsible for inhibiting the growth of pathogenic bacteria. This study would (1) determine the standardized process of Turmeric rhizomes essential oil using the various temperatures of the condenser of a vacuum rotary evaporator; (2) assess the minimum inhibitory concentration and zone of inhibition; and (3) evaluate the potency level of Turmeric rhizomes essential oil. This study is a combination of qualitative design and experimental design. This research involved a total of 30 kilos of Turmeric rhizomes a total of 10.8 liters of ethanol, 18 vials, and a total of 30 Petri plates 100µl *Mycoplasma synoviae* bacteria, 500µl tylosin at a higher dose, 250µl tylosin at a lower dose, 500µl VCO, and 500µl of distilled water. The concentrations were laid out using a complete block design (CRD) and a two-factor complete block design.

The results showed that at a 36^{o}C water bath and -4^{o}C condenser temperature of the rotary evaporator, the best oil was produced. The minimum inhibitory concentration of the oil produced shows inhibition at concentrations of 25 %TREO to 100% TREO. However, the zone of inhibition shows significance at concentrations of 85% TREO to 100% TREO toward the bacterium *Mycoplasma synoviae.* On the other hand, the antibacterial potency of Turmeric *(Curcuma longa)* rhizomes essential oil against

Mycoplasma synoviae shows 139.35% potency. This only means that the antibacterial property of TREO is potent based on the standard potency of 60 to 150 percent. Thus, in order to produce TREO that had an antibacterial property against *Mycoplasma synoviae* of Philippine native chicken, the water bath should be 36^{o}C and the condenser should be -4^{o}C temperature. It requires about 80 revolutions per minute (RPM) for 15 minutes and 40 revolutions per minute (RPM) for ten minutes of vacuum rotary evaporator. Further studies are recommended, including in vivo studies, the use of the lower temperatures of the water bath of a rotary evaporator, and the use of other plant parts of the turmeric *(Curcuma longa)* plant.

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APPENDICES

May 19, 2023

CERTIFICATION

This is to certify that the paper entitled STANDARDIZATION OF PROCESSING AND CONCENTRATION OF TURMERIC *(Curcuma longa)* RHIZOMES ESSENTIAL OIL AGAINST *Mycoplasma synoviae* in PHILIPPINE NATIVE CHICKEN by Rubelyn T. Fuertes has undergone grammar evaluation and has passed.

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This is to certify that the research paper entitled **"STANDARDIZATION OF PROCESSING AND CONCENTRATION OF TURMERIC *(Curcuma longa)* RHIZOMES ESSENTIAL OIL AGAINST *Mycoplasma synoviae* IN PHILIPPINE NATIVE CHICKEN"** by **Rubelyn T. Fuertes** has undergone Turnitin Similarity Checking and with a passing percentage of 15% and have passed the requirements (Chapters 1-5).

Prepared by:

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