Minimum Inhibitory Concentration of Crude Leaf Extracts of *Garcinia mangostana* Against *Staphylococcus aureus*

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By

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APPROVAL SHEET

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"Minimum Inhibitory Concentration of Crude Leaf Extracts of *Garcinia mangostana* Against *Staphylococcus aureus*"

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ABSTRACT

Alpha-mangostin found in hulls and leaves of *Garcinia mangostana* are known to possess antibacterial activity against *Staphylococcus aureus.* The study determined the minimum inhibitory concentration of crude leaf extracts of *Garcinia mangostana* against *Staphylococcus aureus.* Crude extracts were made from using solid-liquid phase extraction method and minimum inhibitory concentration was determined using broth method by serially diluting extracts using a two-fold method. All three replicates showed the same minimum inhibitory concentration. Minimum inhibitory concentration of crude leaf extracts of G. mangostana against S. aureus was 6.25-3.125 µg/ml.

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CHAPTER I

INTRODUCTION

A. Background of the Study

The curative potentials of plants have long been recognized, and to date, plants remain a stable source of drugs and medicines. More and more people are looking at plants as an alternative way to treat bacterial infections, viral infections, and cancer. And the mangosteen, *Garcinia mangostana*, is considered to be a very important plant, due to its many reported benefits.

The mangosteen is a tropical fruit of the family Guttiferae. Its tree is a slow growing evergreen with a pyramidal crown, and the fruit, which is round and dark-purple to reddish-purple in color, with four to eight triangular segments of white, soft and juicy flesh within (Morton 1987 and Templeman 2005). The leaves are leathery and thick; oblong or elliptic in shape, and have a conspicuous, pale midrib (Morton 1987).

The fruit hull is used in Southeast Asia as a traditional medicine, possessing anti-inflammatory, anti-diarrhea, anti-ulcer, and antiseptic properties. Mangosteen has been the subject of many scientific studies, wherein many of its properties have indeed proven true (Nakatani and others 2002).

In the field of scientific research, compounds from the fruit hulls and leaves of *G. mangostana* have shown a variety of biological activities, but notable is a constituent in the fruit hulls and leaves called α-mangostin, which has been found to have antibacterial activity against

s. aureus. The results of the said study show that α-mangostin inhibited the activity of vancomycin-resistant Enterococci (VRE) as well as methicillin-resistant S. aureus (MRSA) (Iinuma and others 1996).

Minimum inhibitory concentrations (MICs) are considered the "gold standard" for determining the susceptibility of organisms to antimicrobials. It is defined as the lowest concentration of an antimicrobial that will inhibit the growth of a microorganism after overnight incubation (Andrews, 2001).

B. Statement of the Problem

The study determined the minimum inhibitory concentration of crude leaf extracts of *G. mangostana* against *S. aureus.*

C. Objectives of the Study

1. Produced crude extracts from the leaves of *G. mangostana.*
2. Determined the minimum inhibitory concentration of crude *G. mangostana* leaf extracts against *s. aureus*.

D. Significance of the Study

Staphylococcus aureus, the most virulent of all staphylococcal species, has demonstrated its adaptability by remaining a major cause of morbidity and mortality despite the availability of numerous effective anti-staphylococcal antibiotics (Fauci and others 2008).

Successive acquisition of resistance to most classes of antimicrobial agents, including penicillin, chloramphenicol, and tetracycline, has made the treatment and control of staphylococcal infections increasingly difficult (Smith and others 1998).

With the appearance of these multidrug-resistant strains, it becomes imperative that newer medications be produced for the treatment of staphylococcal infections, especially for the highly adaptive *S. aureus* (Gill and others 2004).

E. Scope and Delimitation of the Study

The research was designed to determine the minimum inhibitory concentration of crude leaf extracts of *G. mangsotana*. Only the bacteria *S. aureus* were used as a subject for testing. Three trials were performed to ensure validity of the study. A positive and negative control is used which are commercial antibiotics and water respectively. These were done in the Research Laboratory of Philippine Science High School.

F. Definition of Terms

α-mangostin – a xanthone found in the crude extract of mangosteen fruit hulls and leaves that is considered responsible for mangosteen's antibacterial properties (Sakagami and others 2005 and Nakatani and others 2002).

Antibacterial property – the ability of a substance to kill or inhibit the growth of bacteria.

Culture – (noun) refers to a group of microorganisms allowed to multiply in a predetermined culture media under controlled laboratory conditions

– (verb) the act of allowing microorganisms to multiply in a predetermined culture media under controlled laboratory conditions.

Cutaneous infection – an infection of the skin.

Extract – a substance made by extracting a part of a raw material, often by using a solvent such as ethanol or water.

– to take out the substance needed.

Inoculation – the placement of an organism (bacteria) to where it will grow or reproduce. An inoculum refers to the microorganism used in the inoculation.

Methicillin-Resistant S. aureus (MRSA) – a strain of *Staphylococcus aureus* that has developed resistance against a large group of antibiotics, including methicillin, dicloxacillin, nafcillin, and oxacillin.

MIC- is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

Nosocomial infection – an infection that results from treatment in a hospital or healthcare service unit, but secondary to the patient's original condition.

Xanthone – is an organic compound with the molecular formula $C_{13}H_8O_2$.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. *Garcinia Mangostana*

Garcinia mangostana is a tropical evergreen tree, believed to have originated in the Sunda Islands and the Moluccas of Indonesia. The tree grows from 7 to 25 m (20–80 ft) tall. The rind (exocarp) of the edible fruit is deep reddish purple when ripe (Dweck 1999).

A.1 Alpha-mangostin

Extracts of *Garcinia mangostana* have shown inhibitory effects against the growth of *S. aureus*. In a test conducted by one active isolate, alpha-mangostin, had a minimum inhibitory concentration of 1.57-12.5 micrograms. Other related isolates were also examined to determine their anti-MRSA activity (Linuma 1996).

Garcinia mangostana is used with lime water to treat blisters and necrotic wounds. The mangosteen peel extract contains a major component called alpha-mangostin, which is responsible for its antibacterial activity against *Staphylococcus aureus* (Chatchai 2005).

The fruit rind is ground and used in the treatment of diarrhea and dysentery, and for skin diseases. A tea made from the leaves and bark is used to lower fever and for urinary disorders. Made into an ointment, it is used to treat eczema and other kinds of skin disorder and infections (Morton 1987).

B. *Staphylococcus aureus*

Staphylococcus aureus is a type of bacteria commonly carried on the skin or in the nose of people. Staphylococcus bacteria are one of the most common causes of skin infections. *S. aureus* is transmitted most frequently by direct skin-to-skin contact or contact with shared items or surfaces that have come into contact with someone else's infection (Fleming 2008).

S. aureus produces two kinds of toxins namely Pyrogenie toxin superantigens and Exfoliate toxin. Pyrogenie toxin superantigens which usually cause food poisoning and is the main source of TSS (toxic shock syndrome). Targets of the toxin are virtually every organ and tissue in the body. Exfoliate toxin is a toxin that cause Staphylococci Scaled-skin syndrome. This usually affects newborns as their skin is very vulnerable (Centers for Disease Control 2004).

C. Extraction

Extraction of *G. mangostana* leaves is done by solid to liquid phase extraction method. Solid-phase extraction is a separation process that is used to remove compounds from a mixture. Solid phase extraction can be used to isolate analytes of interest from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissue. Solid-phase extraction uses the affinity of solutes dissolved or suspended in a liquid or mobile phase and for a solid through which the sample is passed to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase.

The principle of extraction applies that the solvent must have the same polarity. The closer the polarity of the solvent to the analyte, the more efficient the extraction may be (Nollet 2004).

D. Determining Minimum Inhibitory Concentration

Minimum inhibitory concentration are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. The lower the concentration the higher is its potency (Andrews, 2001).

D.1 Broth Method

Inoculates are suspended in test tubes with broth and with decreasing concentration of antibiotic agents. MIC is determined by the lowest concentration that will inhibit growth of organism which is determined by the test tubes that do not exhibit the growth of organisms by difference of the turbidity of the solution (University of Maryland 2000).

D.2 Agar Method

Nutrient agars with wells containing diluted concentration of extracts are made to diffuse into the plate. Inoculates are then streaked onto the plates and are incubated overnight and checked for microbial growth. The lowest concentration of antibiotic agents is the MIC (Andrews, 2001).

CHAPTER III

METHODOLOGY

A. Collection

Leaves of *g. mangostana* were collected from a tree in Sapian, Capiz. The leaves were hand-picked and were placed in a Ziploc bag and will be processed within 24 hours of picking.

B. Extraction of *G. mangostana* leaves

B.1 Materials

1-liter beaker	500ml beaker
Oven mitts	Aluminum foil

The leaves were washed thoroughly with distilled water to remove any dirt. They were left to air-dry. Ten grams of the *G. mangostana* leaves were weighed. A 500ml beaker was filled with 150 ml of methanol and was placed on the hot plate. The leaves were put in and left to boil for 3 minutes. The extracts obtained were placed in a 1-L beaker. The same leaves were then extracted again using the same procedure three times. The solution was then concentrated with the rotary evaporator until the extracts began sticking to the wall of the flask. The flask was then rinsed with a small amount of methanol. The contents were then transferred into a small beaker and placed on a hot plate to remove the excess methanol.

C. Test of antibacterial activity

C.1 Sterilization

13 Volumetric Pipette Forceps

10 test tube

Materials to be used are 10 test tubes and a volumetric pipette which were acquired from the SRA of the school. Glass wares were washed thoroughly with soap and water. Then they were air-dried. They were then wrapped in paper and placed in the autoclave at 120°C for 30 minutes under 15psi. After the autoclave was turned off, apparatus are left to cool before they were taken out.

C.2 Media Preparation

Suspension of 5.4 gram of TSB into 180 ml of distilled water was made. The broth was then heated to dissolve the media completely. The solution was then autoclaved for 15 minutes at 121°C and at 15 psi.

C.3 Bacteria culture and testing of extracts

Test tubes were filled with solutions of antibacterial extracts with decreasing concentration by two-fold. A volume of two milliliter of antibacterial extracts with a concentration of 100µg/ml was introduced into the test tube by the use of a pipette. Then the addition of one milliliter of tryptic soy broth to all other tubes was made. Then transfer one milliliter of antibacterial extracts from the first test tube to the second tube. Using another pipette, mix the content of the second test tube and transferred one

milliliter of the second tube to the third test tube. This process was continued up to the tenth test tube. One milliliter was then removed from the tenth tube and was discarded.

A pure culture was obtained and 3 to 5 identical sized colonies were selected and picked up with an inoculating loop and was then transferred into a test tube with 5ml of tryptic soy broth. Turbidity of the solution was then adjusted by comparison of the turbidity of the 0.5 McFarland standard. The solution was then diluted by pipetting 0.2ml of the solution into 40ml of TSB. Then the addition of one milliliter of diluted culture suspension to each test tube. The tubes are then incubated for 18 hours at 37°C.

D. Determination of Minimum Inhibitory Concentration

The tubes are examined for bacterial growth by the turbidity of the solution. The TSB control is used as comparison for the growth of the bacteria. The lowest concentration that inhibits bacterial growth is taken as MIC.

Chapter IV

Results and Discussion

The study aimed to produce crude extracts from the leaves of *Garcinia mangostana*.

The study aimed to determine the minimum inhibitory concentration of crude leaf extracts of *G. mangostana* against *S. aureus*.

Crude leaf components of *G. mangostana* were extracted by a methanol solvent using solid-liquid phase extraction. The extracts were then tested for their minimum inhibitory concentration by culturing bacteria in series of test tubes with different concentrations of extracts ranging from 25-0.098 µg/ml by using a two-fold dilution method. Minimum inhibitory breakpoint was determined by observation of tubes with and without bacterial growth. Further validation of results was made by sub culturing the bacteria from the tubes to agar plates.

A. Results

The dry weight of leaves was 10g and the weight of the crude leaf extracts of *G. mangostana* were at 0.31g, 0.47g and at 0.44g after extraction process was finished.

Crude leaf extracts of *G. mangostana* showed a minimum inhibitory concentration of 6.25-3.125 µg/ml in culture of *S. aureus*. All three replicates showed the same inhibitory concentration value. The study also used water and Ampicillin as controls. Growth of bacteria was observed in the water control and no bacterial growth was observed in the Ampicillin control.

Test tubes with extracts changed its color from dark green to red after sterilization.

B. Discussion

Crude leaf extracts of *G. mangostana* has a minimum inhibitory concentration of 6.25-3.125 µg/ml in culture of *S. aureus*. This is the minimum concentration of extracts needed to inhibit the growth of *S. aureus*. A study conducted in 1996 by Linuma and others showed that methanolic extracts from fruit hulls and leaves contain an antibacterial property known as

alpha-mangostin and showed that alpha-mangostin has a MIC of 1.57-12.5 µg/ml against *S. aureus*. The inhibitory strength is different as compared to the MIC of the crude leaf extracts due to the concentration of alpha-mangostin in the leaves and the extraction of alpha-mangostin was crude.

The solution of extracts with broth turned from dark green to red after sterilization in the autoclave. This was observed on all three replicates but the content of the crude leaf extracts was not compromised as xanthones is a heat stable molecule and are able to withstand temperatures up to 180-182°C (Morton 2005).

Chapter V

Summary of Findings, Conclusion, Recommendations

The study determined the minimum inhibitory concentration of crude leaf extracts of *G. mangostana* against *s. aureus*.

This study specifically aimed to:

1. Produce crude extracts from the leaves of *G. mangostana*.
2. Determine the minimum inhibitory concentration of crude *G. mangostana* leaf extracts against *s. aureus*.

A. Summary of Findings:

1. The weight of the crude leaf extracts of *G. mangostana* were at 0.31g, 0.47g and at 0.44g after extraction process was finished.
2. Crude leaf extracts of *G. mangostana* showed a minimum inhibitory concentration of 6.25-3.125 µg/ml in culture of *S. aureus*.

B. Conclusions:

Minimum inhibitory concentration of crude leaf extracts of *G. mangostana* against *S. aureus* was 6.25-3.125 µg/ml.

C. Recommendations:

It is recommended that further studies be made on the following:

1. A study on the cytotoxicity of crude leaf extract of *G. mangostana.*
2. A study on the concentration of alpha-mangostin in the leaves of *G. mangostana*.
3. A study on the minimum inhibitory concentration of leaf extract of *G. mangostana* using agar dilution method.

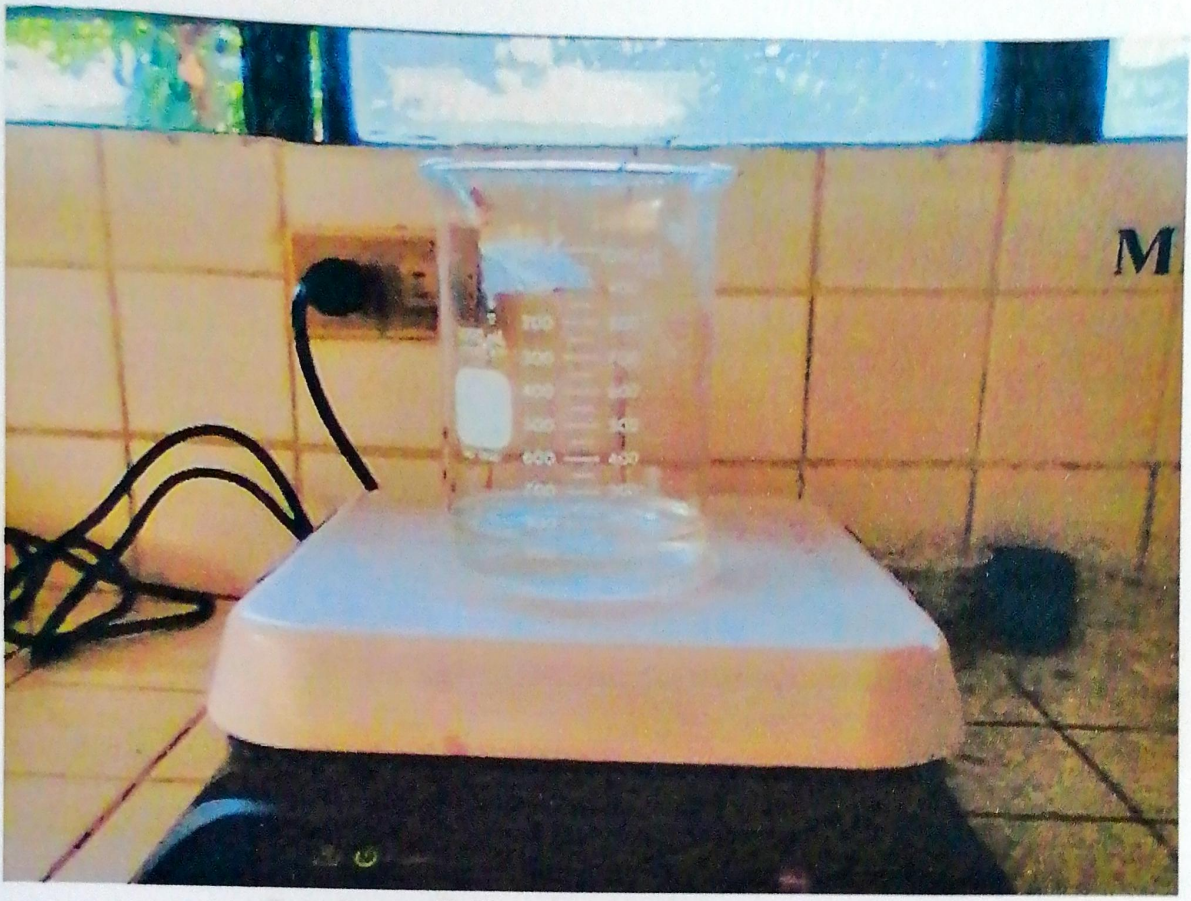
Appendix

A. Table

Replicates No.	Weight of Leaves	Extracted Weight	MIC
1	10 grams	0.31	6.25-3.125 µg/ml
2	10 grams	0.47	6.25-3.125 µg/ml
3	10 grams	0.44	6.25-3.125 µg/ml

B. Plates

Weighing of leaves

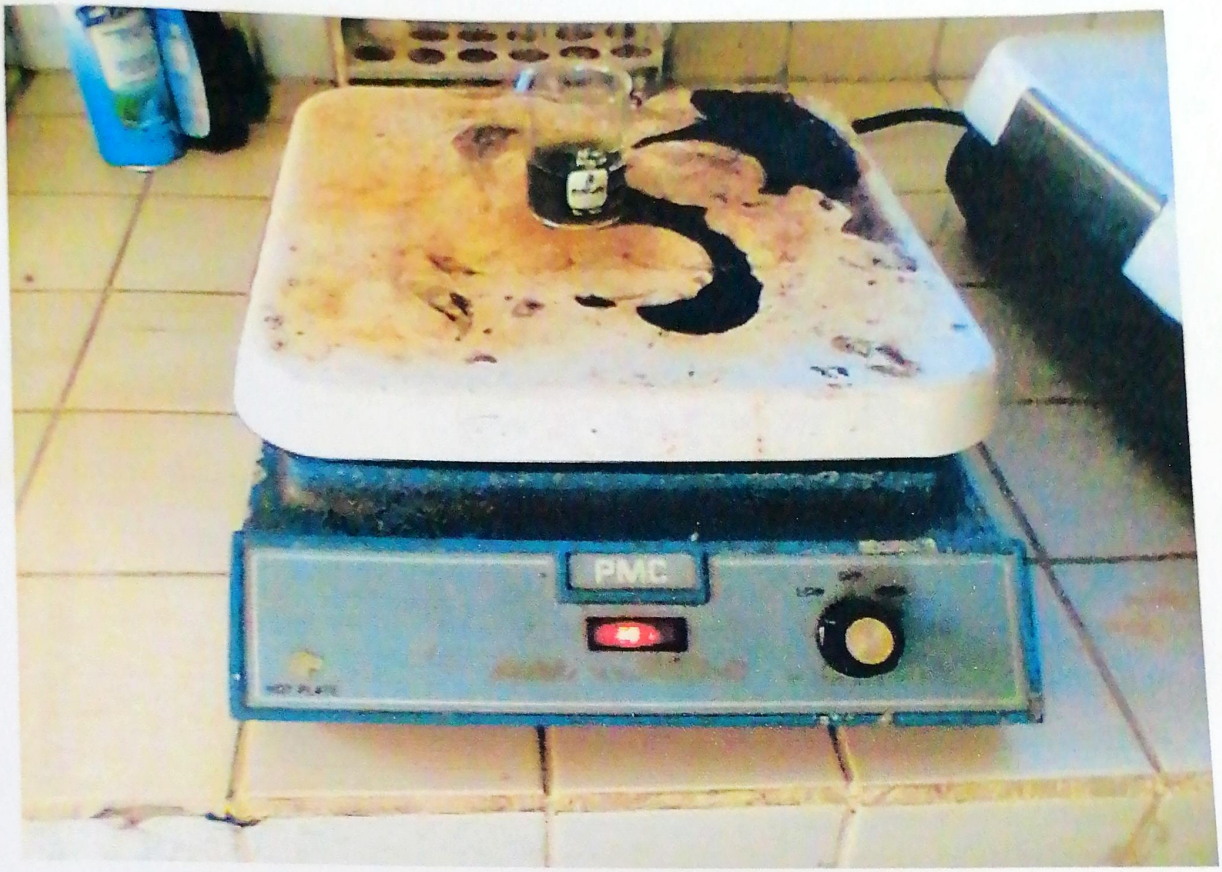


Boiling of methanol

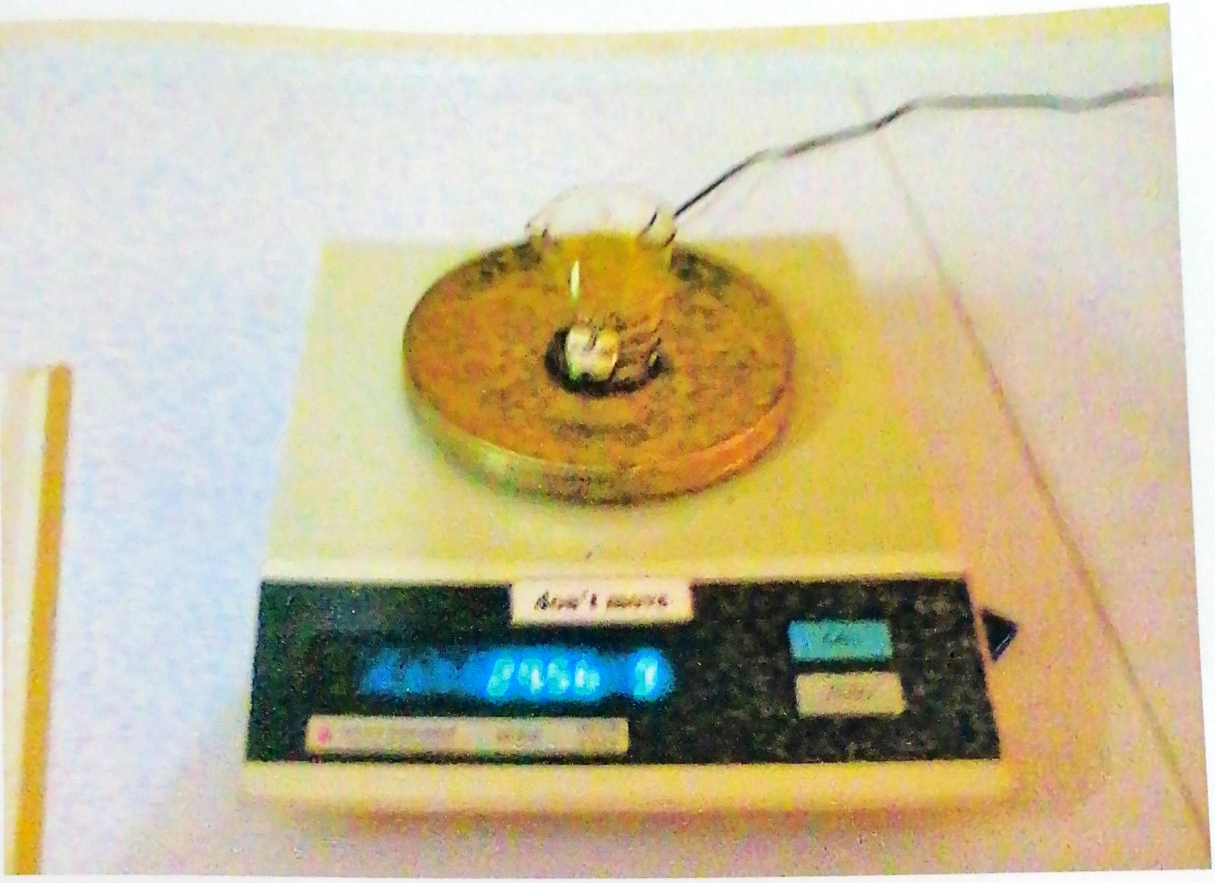
Transferring of extracts from the first extraction

Combination and Storing of extracts

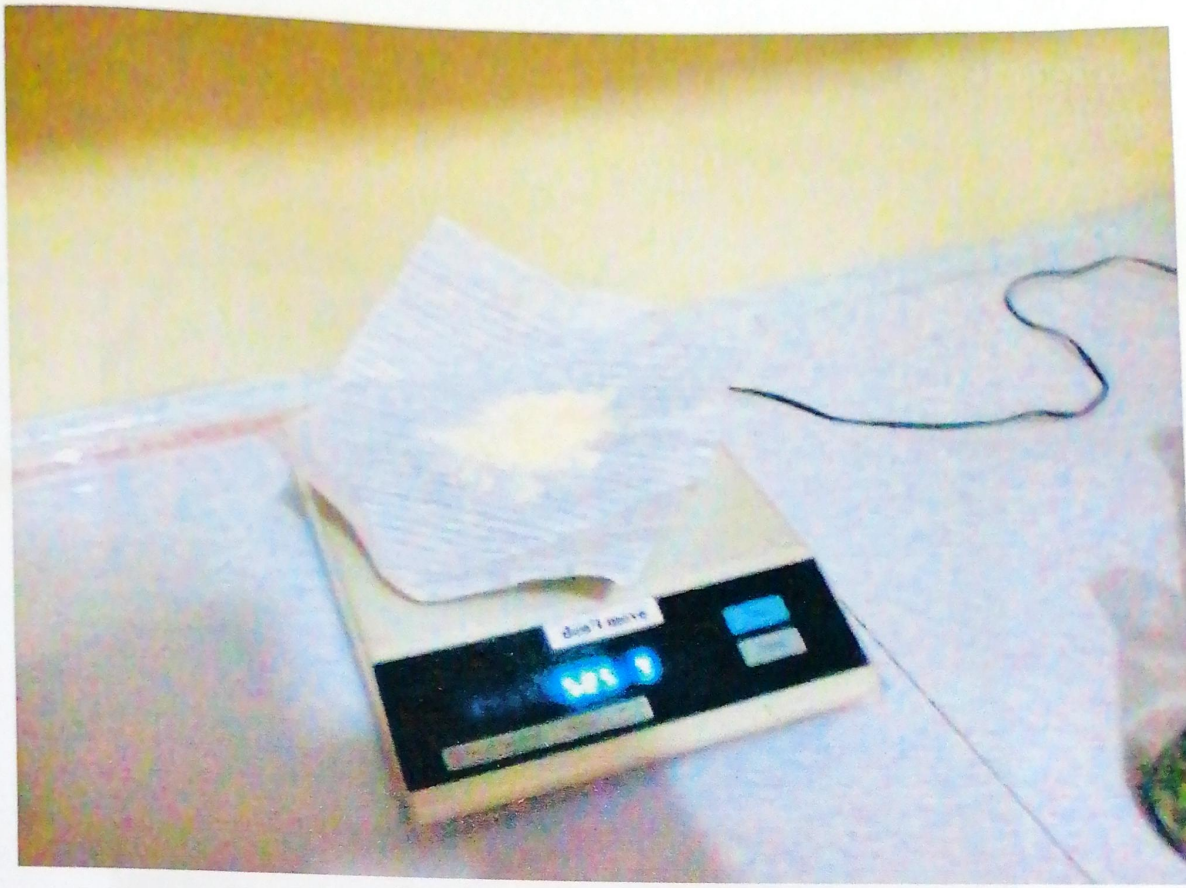
Concentrating of extracts



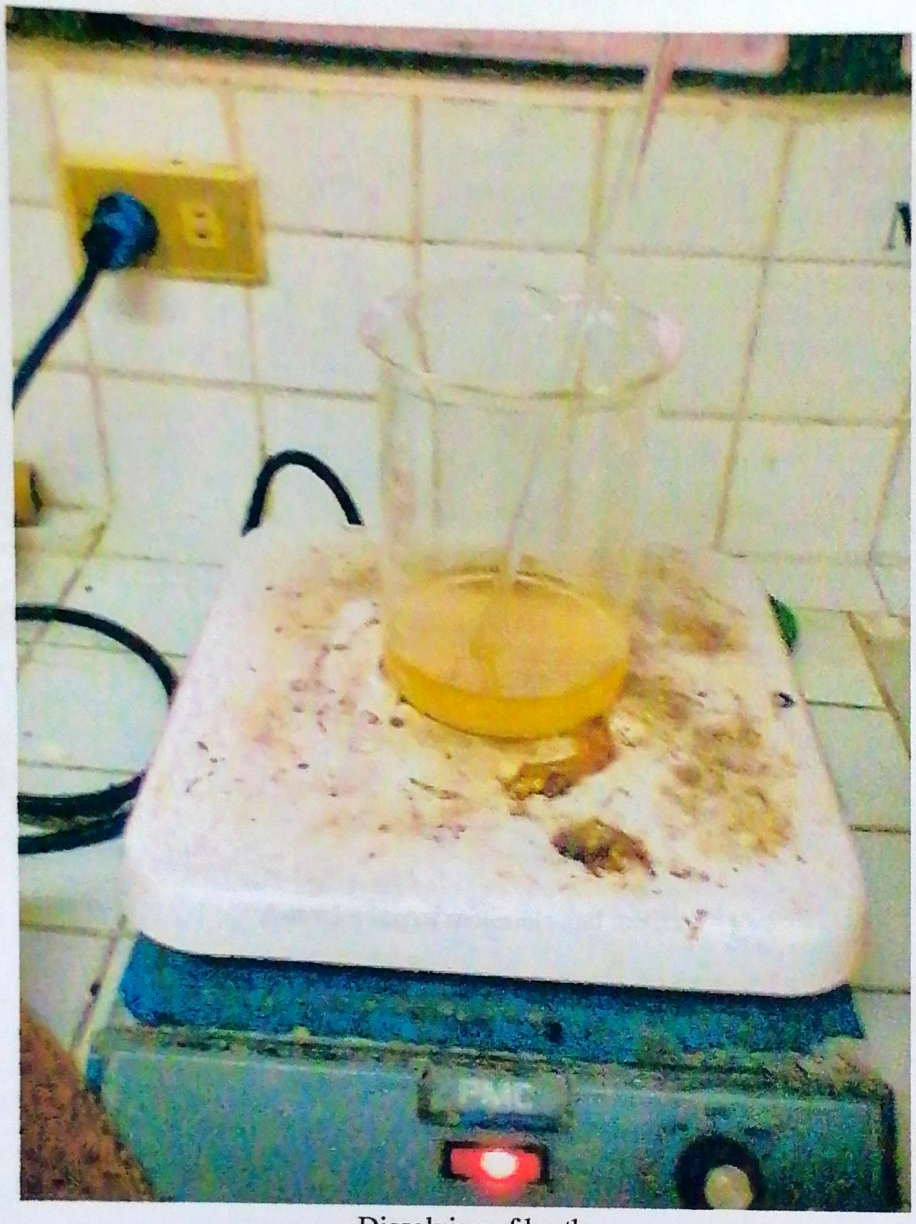
Further concentrating and removal of methanol



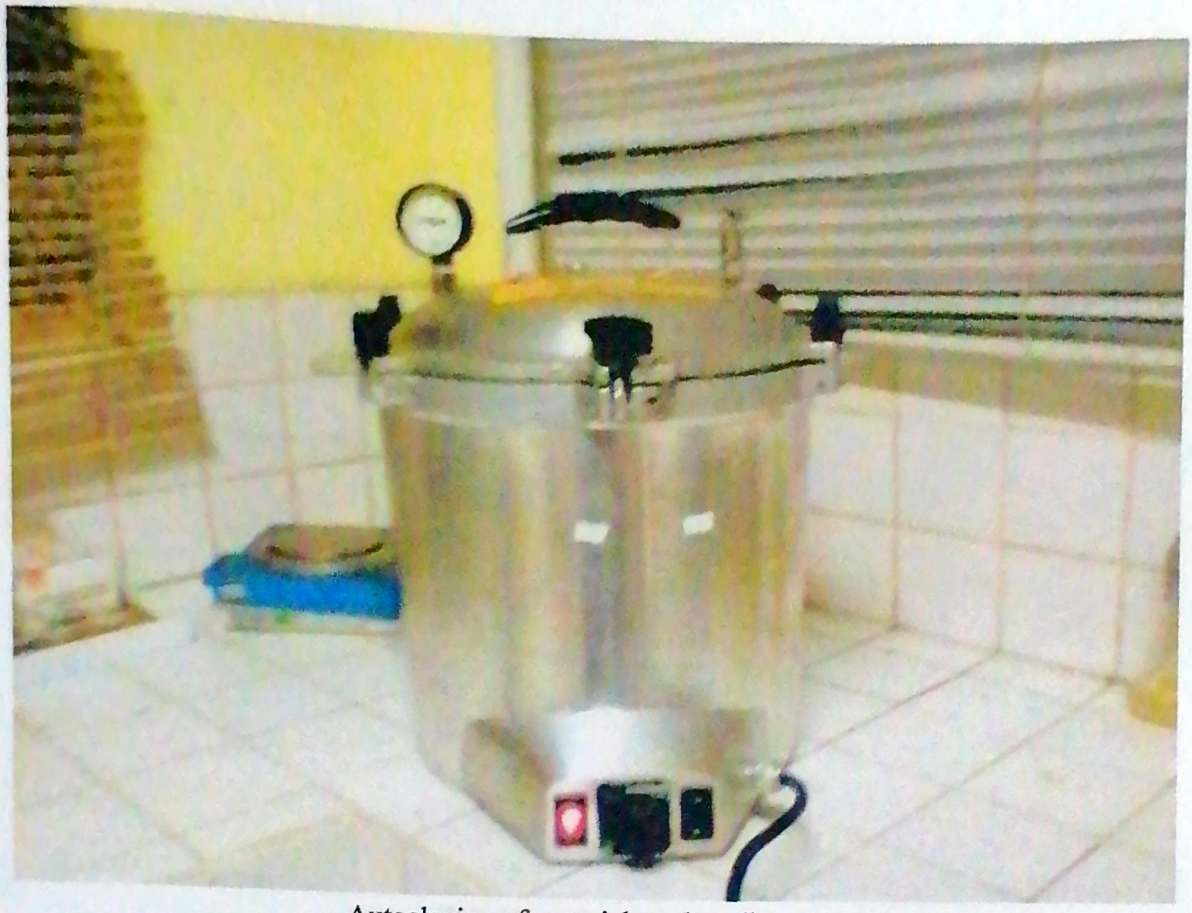
Weighing of extracts



Weighing of Tryptose Broth



Dissolving of broth

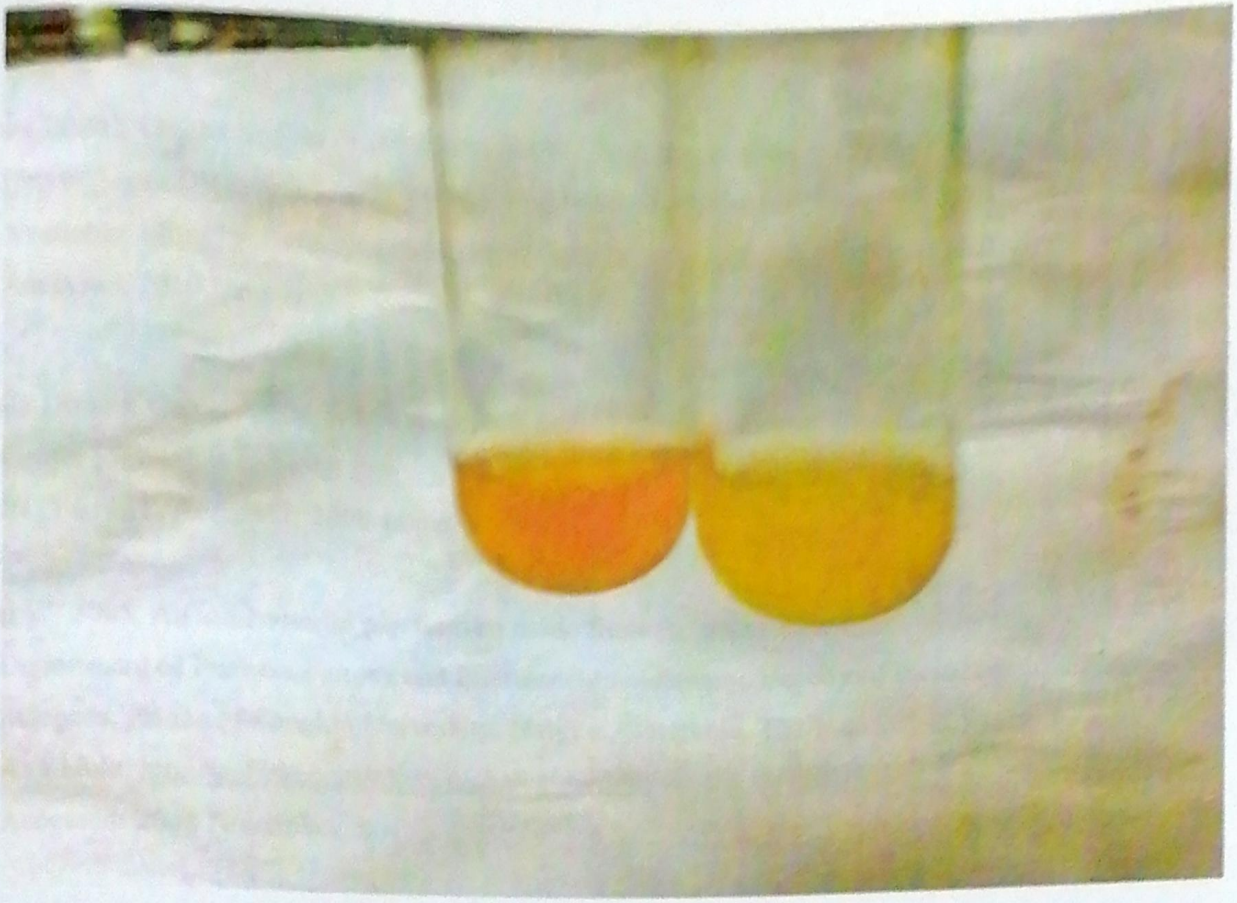


Autoclaving of materials and media

Weighing of extracts

Labeling of tubes

Addition of inoculums



Determination of bacterial growth

Linuma et al. 1996. Antibacterial Activity of Xanthone from Guttifaraceous Plants Against Methicillin-Resistant *Staphylococcus aureus*. Department of Pharmacognosy, Gifu Pharmaceutical University.
Available: www.nutritionhealthinfo.com/.../mangosteen_0179_001.pdf via the INTERNET. Accessed: 2008 November 5.

Montoso Gardens. Garcinia mangostana (Clusiaceae). 2007. Montoso Gardens [online].
Available: http:// www.montosogardens.com via the INTERNET.
Accessed: 2008 November 5.

Morton D. 2005. The Xanthone Effect, Orem, Utah, Sound Concepts [online].
Available: http://www.mangosteen-natural-remedies.com/xanthones.html via the INTERNET. Accessed: 2010 February.

Morton, J. 1987. Mangosteen. Fruits of warm climates [online].
Available: http://www.hort.purdue.edu/newcrop/morton/mangosteen.html via the INTERNET. Accessed: 2008 November 5.

Nollet L.2004. Handbook of Food Analysis. CRC Press [online].
Available: http://books.google.com.ph/books?id=Z1YlZrKqg3EC&dq=polarity+extraction&hl=en&source=gbs_navlinks_s via the INTERNET.
Accessed: 2009 July 1.

Smith et al. 1998. Emergence of vancomycin resistance in Staphylococcus aureus. The New England Journal of Medicine [online].
Available:http://content.nejm.org/cgi/content/abstract/340/7/493?ijkey=1f83527900f9f1b663a260f2c2250379d541079f&keytype2=tf_ipsecsha via the INTERNET.
Accessed: 2008 November 5.

Templeman F. 2005. Mangosteen The X Factor. Sound Concept [online]. Available: http://www.mangosteen-natural-remedies.com/xanthones.html via the INTERNET. Accessed: 2010 February.

University of Maryland. 2000. Minimal Inhibitory Concentration: Broth Tube Dilution Method. University of Maryland [online]. Available: http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/BrothTubeMIC.htm via the INTERNET. Accessed: 2010 January 27.