COMPARISON OF THE ANTIMICROBIAL ACTIVITY OF RAW SOY BEANS, SOY FLOUR AND ROASTED SOY BEANS ON BACTERIAL GROWTH

BY

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A SPECIAL PROJECT SUBMITTED TO THE FACULTY OF BIOTECHNOLOGY, ASSUMPTION UNIVERSITY IN PART OF FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

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A Special project submitted to the faculty of Biotechnology Assumption University in part of fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology

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COMPARISON OF THE ANTIMICROBIAL ACTIVITY OF RAW SOY BEANS, SOY FLOUR AND ROASTED SOY BEANS ON BATERIAL GROWTH

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THE ADVISOR: A. SIRE

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CHAPTER I
INTRODUCTION

Soy beans are processed about 85 percent of the population in the world, therefore it has become one of the most widely grown and utilized type of legume by human beings. Soy beans are grown over three million only within Asian region itself. Usually soy beans grow in pods which contain edible seeds. (Ref: 1)Soy beans are mainly popular for its eight amino acids which play a very important role in human health. Production of edible oil using soy beans has become another popular trend. It's used as animal feed and used in the industries of soaps and biodiesel as well. Soy beans are processed in many ways such as soy milk, tofu, soy flour, roasted soy and many more. Amazing thing about soy beans is that it can be used to increase the nutrient quality of processed food.

Perfect example is the isolation of soy proteins which is used in the clear or acidic beverages. Key benefits of soy beans are its excellent protein content, its isoflavones, high levels of fatty acids and various vitamins and minerals. Isoflavones are very important natural chemical found in soy beans. It is structurally similar to that of estrogen hormone of women. It can cause estrogen like effect in the humans. (Ref: 2).

Antibacterial activity of soy beans is another important factor. Many research papers were published that isoflavone extracted from soy bean have the ability to inhibit many bacteria growth.
(Ref: 3)

In this study, the inhibition of bacterial growth using crude soy bean extracted by ethanol-hexane extraction was done. The three various materials of soy bean including raw soy beans, soy flour and roasted soy beans were studied. Soy beans extraction was done using 70% ethanol in order to isolate the isoflavone compound and hexane in order to remove lipid components of the soy beans which will migrate to the surface during the extraction. (Ref: 4)

The inhibition effect of crude soy extracts were tested on *Escherichia coli* and *Staphylococcus aureus*. *E. coli* is a gram negative bacteria which rod shaped and it is facultative anaerobic. *S. aureus* is a gram positive bacteria which are coccus shaped facultative anaerobic. Therefore it is very important to find a solution to inhibit these kinds of bacteria. (Ref: 5) The study of bacterial inhibition was done using disc paper method in which the inhibition zone was measured.

Chapter II

Literature review

Family: Fabaceae

Subfamily: Faboideae

Scientific name: *Glycin max*

Common names:

Asia

- It is native to central China.
- Called by names *Glycine soja/ G. ussuriensis*
- Introduced to many other countries such as India, Japan, Vietnam, Thailand etc:
- Many Asians use soy beans after fermentation process.

Africa

- Soy beans first cultivated in 1858.
- Soy based food were introduced in 1974 under the USDA food for program.

(Ref: 6)

Australia

- In 1770 two species of wild perennial soybeans were grown, Glycine tabacina and

  Glycine tomentosa.
- In 1804 first soy product soy sauce was produced.
- Discovered in northeastern part of Australia

(Ref: 7)

Soy beans:

Soy beans fall under the species and family of legumes. Soy beans were first found in East Asia. It is a very good sauce of protein and the its classified as an oil seed. Soy beans have the ability to grown on variety of soils and in varied ranges of climates. Protein content of the bean differs from land to land where the seeds are cultivated. The plant contains pods where the soy beans grow and mature. After these beans mature they become ripen and convert into dry beans. There are many varieties of colors such as yellow, black, brown and green color.

(Ref: 8)

(Ref: 9)

Roasted soy beans:

Roasted soy beans are produced by baking in the oven or by roasting using mono unsaturated oil which is considered to be very good for the health. Low sodium salt is used for seasoning. These kinds of salt contain 43% of salt less than normal day to day salt. Before soy beans are baked or roasted the beans are soaked in water so that process will be easier to carry. All the important proteins, dietary fibers and carbohydrates are found in sufficient amount for the consumers.

(Ref: 10)

Soy flour:

Soy flour is produced by heat processed soy beans. First the beans are dehulled or defatted. Then the soy beans ground until fine particles are produced. A hammer mill is used to ground the beans. There are two important mesh screen first 100- mesh and second 200-mesh. Soy beans will pass these two meshes. The finest mesh produces soy flour with the highest protein content. There is different heat processing methods used which depends with the end use of the soy flour. If the heat treatment creates a lot of moist then the protein content of the produced soy flour is the highest. Especially steam is used as the heat treatment method. If the protein content is higher the protein solubility reduces. Heating usually inactivates natural enzymes such as lipase, oxidase and peroxidase. (Ref: 11)

Properties of soy beans:

Soy beans are fallen under plant group Fabaceae and belong to the legume family. Usually the plant grow to height of 20cm to 2 m. These beans grow inside pods. Inside these pods it gets all the nutrition and the beans grow and eventually mature. Soy beans can be easily processed to different types of products and forms such as roasted for, granular form, powdered form or even liquid from like soy milk. Soy beans are considered as oil seeds in which roughly the amount of oil is 20% of one soy bean. Therefore production of soy vegetable oil has become possible as well. Soy beans can absorb water very easily; therefore crushing of beans can be done. in order to produce tofu, tempeh, miso soy beans are fermented.

Chemical composition:

There are about 60% of proteins in dry soybeans by weight. There is problem when the soy beans are consumed raw. There is chemical which called the Trypsin inhibitive factor, due to this reason if the soy beans are consumed raw typsin in humans cannot digest the proteins in the beans. Therefore necessary nutrients cannot be absorbed. Due to this reason the beans should be processed before consumption.
(Ref: 12)
(Ref: 13)
Soy proteins are heat stable therefore after the soy beans are processed the proteins inside the soy beans are not harmed. Heat stability unable to process the soy beans and get the sufficient nutrients. There are 35% carbohydrates and about 5% ash inside beans. Disaccharide sucrose is the main soluble carbohydrate found in mature soy beans. Apart of this type there are trisaccharide raffinose and tetrasaccharide stachyose which protect the viability of soy beans seed.

These are not digestible sugars therefore it is broken down in the intestine by the use of native microbes. After it is broken down products like hydrogen, methane and

carbon dioxide are produced. But when tofu, soy sauce or any other fermented product is made soluble carbohydrates are broken down due to the process of fermentation.

(Ref: 14)

(Ref: 15)

There is a large group of compounds derived in plats which are called phytoestrogens. Three types of major groups are isoflavones, lignans and coumestans. From these isoflavones are very rich in soy beans. Isoflavones in soy beans are produced by the enzyme chalcone isomerasewhich converts flavones precursor to isoflavones. These fall under polyphenol compounds therefore it shows antioxidant properties. Isoflavones are helpful to prevent diseases. There are 4 sub groups under this, which are aglycone, glucoside, acetyl glucoside, and malonylglucoside. Aglycone form and glucoside forms are the most prominent. Chemical structure of isoflavones are very similar to that of the hormone estrogen in women. Therefore it has the ability to balance the level of the hormones. Isoflavones has the ability to act as weak estrogen and bind to the estrogen receptors and block some detrimental effects of estrogen such as the growth of cancer cells. In the other hand women producing little estrogen can consume soy based products and produce enough estrogenic activity to relieve symptoms such as hot flushes. When isoflavones act as a antioxidant property it can remove the free radicals found in the body produced by oxidation.

(Ref: 16)

(Ref: 17)

(Ref: 18)

Medical applications:

There are many health benefits of soy beans discovered now worldwide. Majority of the Asians tend to consume soy food a lot in their diet. Soy beans contain a lot of phytoestrogen which are considered to be plant compound. This particular compound is structurally similar to estrogen and can act as the estrogen to a certain extent. Soy iso-flavones are fallen under the category of phytoestrogen which plays a main role when providing health benefits in humans.

Isoflavones are very important in the cases of sex hormone decline or imbalance. Most importantly it can be used against cancer and even osteoporosis. Some women have the problem of balancing the estrogen hormone therefore with the intake of soy beans or soy products it has the ability to balance the hormone level. Not only has this now have the scientists found that the soy beans had the ability of increasing the menstrual cycle where it positively affects the reproductive hormone level. Now women are able to use less of hormone replacement therapy by including soy food in their day today diet. Even by consuming 45 grams of soy flour per day women are able to reduce hot flashes after period of six weeks. Soy beans help to reduce cancer risks such as colon cancer, breast cancer and even prostate cancer. Women with high plasma estrogen level has a lower risk getting cancer and Asian men who are addicted for smoking and consuming alcohol even have a lower risk of cancer due to the high consumption of soy products.

Now hardening of and blocking of arteries have become a very big problem which is mainly caused by the oxidation of LDL cholesterol. Soy beans have the ability to reduce the levels of LDL compared to HDL cholesterol. Due to this reason the risk of cardiovascular diseases are inhibited to a certain extent. In women when estrogen level goes down it leads to increment of bone loss in the body. This leads to osteoporosis which is bone disorder. Now soy products have become good effective treatment for this disease.

Soy beans have the ability to act as antioxidants as well. Antioxidants have ability work against free radicals and neutralize it. Radicals are deadly because it causes oxidative damage to different systems in the human body and even increase the aging

process of the human body. Recent studies showed that by consuming 50-100 mg of soy beans per day can reduce oxidative damage to DNA.

There are many health benefits soy beans. Mainly iso flavones, geistein and daidzein play a very important role in providing these advantages. Soy beans are able to prevent cancer, osteoporosis, cardiovascular diseases and balance hormone levels in the body. Therefore now soy beans and soy based products are must in the day today diet of human beings. The recommended intake is 60 mg to no more than 120 mg per day.

(Ref: 19)

Food applications:

There are different soy based products found now. People focus a lot on healthy products and soy products are considered to be on top. Soy milk is one of the main products in the market. It's famous for many benefits and advantages. Soy milk is basically produced using whole soy beans. Due to this very reason it contains a nutty flavor. Nutrient content of soy milk is very high due to the usage of the whole soy bean itself. Production of soy milk is very simple procedure where the beans are soaked, ground and pressed to obtain milk from the soy beans. Flavors of soy milk found are chocolate, vanilla, almond and a lot more. There are many nutritional benefits of soy milk. It is a good source of proteins, vitamin B, vitamin D, Iron and even calcium. One of the best benefits of soy milk is that lactose intolerant people can easily consume soy milk and obtain the necessary nutrients and minerals. This is because soy milk, lack lactose which is found in normal fresh cow's milk.

(Ref: 20)

Next famous product is Tofu. Tofu is also called bean curd in many countries. Like mentioned earlier this is very alternative for lactose intolerant people. Tofu is best for the people who are allergic to eggs as well. It provides all the necessary nutrients. Tofu in considered a non fermented soy product.
http://www.inmamaskitchen.com/FOOD_IS_ART_II/health/tofu.html

Soy sauce is considered one of the main soy based products in the market. Main ingredients of soy sauce are soy beans and Aspergillus oryzae is used as the culture in order for fermentation process. Unlike tofu soy sauce is fallen under fermented products. Instead of the traditional method of making the soy sauce now made by using hydrolyzed soy proteins. It helps to enhance better favor, aroma, texture and it is able to increase the product's shelf life which is very important for the consumers.
(Ref: 21)

Soy ice cream is a very popular product as well. It is famous for its unique flavor, texture and aroma. Mainly it provides a nutty flavor to the ice cream. Soy ice cream is made using soy milk. Therefore people who are lactose intolerant can easily consume. Not only that ice cream is full of calcium therefore it is perfect for children consumption as well. Fat content of soy ice cream is less compared to normal dairy ice cream, therefore it unable to reduce cholesterol as well.

E. coli

Escherichia coli and also known as *E. coli* are type of bacteria found in intestinal tracts of human beings and other warm blooded animals. Scientists have found more than 700 serotypes of *E. coli* in the environment so far. The "O" and the "H" antigens on *E. coli* body and on flagella help to distinguish them into different groups. *E. coli* directly involves in food borne diseases such as gastrointestinal infections, urinary tract infections etc: But some kinds of *E. coli* strains are beneficial to human beings as well.

Escherichia coli fall to the category of gram negative bacteria in the environment. It appears red color under microscopic inspection when gram staining is used due to this reason. Being facultative anaerobic makes these microorganisms to survive in harsh environments. When the shape is concerned the most significant shape is rod shape. Usually 2 micrometers long succinate, ethanol, acetate etc due to the process of anaerobic fermentation. If the concentration of hydrogen gas increases it causes a problem to the microorganisms therefore it usually live around organisms such as Methanogens which consumes hydrogen. E. coli can be usually grown under the temperature 37°C to 49°C. Surprising ability of transferring genetic material from one generation to another by exchanging DNA using the process of bacterial conjugation is very important.

(Ref: 22)

E. coli 0157:H7 is famous for being a food borne pathogen. First outbreak was discovered in hamburgers in 1982. Shiga toxins produced by this type found to be the biggest cause of damage. It was found that the consumption of contaminated food types and beverages was biggest problem which will result in many diseases. These strains of *E.coli* were mostly found in cheese, milk unpasteurized apple juice, orange juice, ground beef etc;

(Ref: 23)

E. coli have this remarkable ability to survive outside the body for short period of time. Therefore mostly it is used as a indicator organism where it makes easier for indication of pathogenic bacteria and for detection of fecal contamination. Now the process of metagenics is used to grow the bacteria. *E. coli* can be easily grown and multiplied making it possible to study about it more.

S. aureus

Staphylococcus aureus falls under the category of gram positive where it is indicated as bluish purple under microscope when gram staining is used. These microorganisms are spherical in shape and also known as coccus. They usually appear in microscopic clusters making it similar to grapes. More than 20 species of staphylococcus species are found by scientists. From those indentified *Staphylococcus aureus* (yellow) and *Staphylococcus albus*(white) are very important. *S. aureus* usually shown interactions with humans especially in the areas of nasal passages, skin, oral cavity etc;

S. aureus was discovered in the 1880 by surgeon Sir Alexander Ogston. These microorganisms are found in large yellow colonies. Reason for this is the carotenoid pigment staphyloxanthin. This particular pigment helps it to survive death by reactive oxygen species. *S. aureus* microbes produce lactic acid using fermentation process. These are actually facultative anaerobes. There are many kids of diseases which *S. aureus* are responsible such as minor skin infections, wound infections and major diseases such as pneumonia, toxic shock syndrome etc;

This type of bacteria is catalase positive and oxidase negative therefore it has the ability to grow at a temperature range 15- 45°C and at the NaCl concentration as high as 15%. All the strains have the ability produce enzyme coagulase. *S. aureus* is considered as a potential pathogen.

(Ref: 24)

(Ref: 25)

(Ref: 26)

Chapter III

Objectives

- Study the extraction of crude extracts from Soy flour, Roasted soy beans, Raw soy beans
- To compare the inhibition effect of various soy bean samples on *E. coli* and *S. aureus*
- Study the ability of inhibition of various concentrations of soy crude extracts

Chapter IV

Equipments and reagents

I. Equipments

- Analytical balance (Ohause Anlytical plus AP 210S)
- Microscope (Nikon, SMZ-1)
- Autoclave (Hirayama, Model HA 300 M II)
- Incubator (Jouan, EB 280)
- Laminar flow (Dwyer Mark II, "Clean" Model H2)
- Spectrophotometer (Spectronic, GENESYS 5, Milton Roy)
- Shaker (KikaLabortechnik, KS 501 D)
- Separatery funnel (250ml)
- Single use filter disc (Minisart, 0.2μm)

II. Reagents

- Ethanol (70%)
- Hexane
- Nutrient agar (NA)
- Nutrient broth (NB)

III. Microorganisms

- *Escherichia coli*
- *Staphylococcus aureus*

IV. Samples

- Raw soy beans
- Roasted soy beans
- Soy flour

Procedure

I. Extraction from soy samples

200 grams of each sample raw soy beans, roasted soy beans and soy flour was weighed using analytical balance. Required volume of soy beans and roasted soy beans were crushed using the blender. For extraction 300ml of 70% ethanol was added to each sample respectively. Then all three samples were heated to 60°C for one hour while stirring at the same time. After this is done 20 ml of hexane was used to obtain the final supernatant using the seperatory funnel, this was repeated for 4 times for each sample respectively. In order to concentrate the supernatant the samples were kept in the water bath in 80°C until residues are obtained. Then 10 ml of autoclaved distilled water was added to each crude residue and filtered using filter disc (2µm). Final crude extract was obtained.

II. Preparing different concentrations of sample

After the residues were collected 750µl of crude extract was taken to a eppendorf tube and to it 250µl of autoclaved distilled water was added in order

to produce 75% concentration of each sample separately. Then 500µl of the crude residue sample was taken to an eppendorf tube and 500µl of autoclaved distilled water was added in order to dilute it up to 50%. The tube was shaken before the other dilution. From this particular tube another 500µl was taken to a new tube and to that another 500µl of distilled water was added, this was for the 25% of concentration. Same procedure was repeated for three times for the three concentrations 12.5 %, 6.25 %, and 3.125 % respectively.

III. Preparation of culture

Two strains of microorganism *E. coli* and *S. aureus* were used in this experiment. One loopful of active culture from nutrient agar (NA) was transferred to nutrient broth (NB), The concentration of the culture used was controlled by using spectrophotometer with the control absorbance as 0.59 and 0.55 for *E. coli* and *S. aureus*, respectively.

IV. Preparation of media

a. Nutrient broth (NB)

Peptone	5 grams
Beef extract	3 grams
Agar	15 grams
Distilled water	1000 ml

b. Nutrient agar (NA)

Peptone	5 grams
Beef extract	3 grams
Agar	15 grams

V. Test for microbial inhibition

First one loop of desired microorganism (*E. coli and S. aureus*) was inoculated using 50 ml of nutrient broth. It was kept overnight until microbial growth reached the stationary phase. The inhibition test was done using the disc diffusion method. Disc papers were cut to circles increasing its width using three disc papers attached together and autoclaved. After the desired number of plates of nutrient agar was prepared 200µl of inoculated nutrient broth was added to each plate and spread using inoculated glass rod by spread plate technique. Plates were left to dry for 15min. Meanwhile 8µl of each concentration of the sample was added to each disc paper. After the plates are completely dry the disc papers are placed in the specific areas of the plates with desired concentrations of soy crude extracts of all three samples respectively. 70% ethanol (Obtained from the laboratory and from the drug store), ampicillin and hexane were used as the positive controls and autoclaved distilled water was used as negative control. Even for the controls disc paper was used in a separate plates. Each concentration of soy sample and the controls was triplicate. The plates were incubated finally in 37°C and the results were checked after keeping the plates overnight. The length of inhibition area was measured for each plate while comparing with the negative and positive control. After taking down the results all the plates were kept overnight once again and diameter of inhibition zones of each plate was checked to observe a difference in results. All the results were analyzed using excel and statistical methods in order to compare.

VI. Chemical test and the recovery yield

Percentage recovery was found after obtaining the soy crude extract. In order to calculate it initial sample weight which was 200 grams was used. Equation shown below was used.

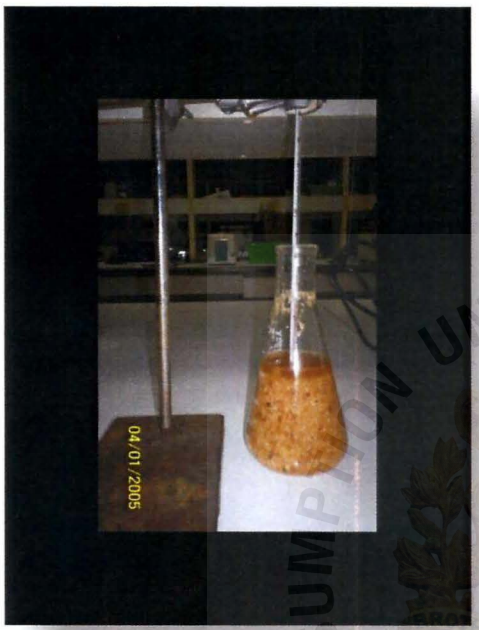
$$\% \text{ yield (w/w)} = \frac{\text{weight of dried crude extract (g)}}{\text{Weight of initial soy sample (g)}} * 100$$

pH was tested for all three types of crude extracts soy flour, raw soy beans and roasted soy beans using the pH paper.

VII. Statistical Analysis

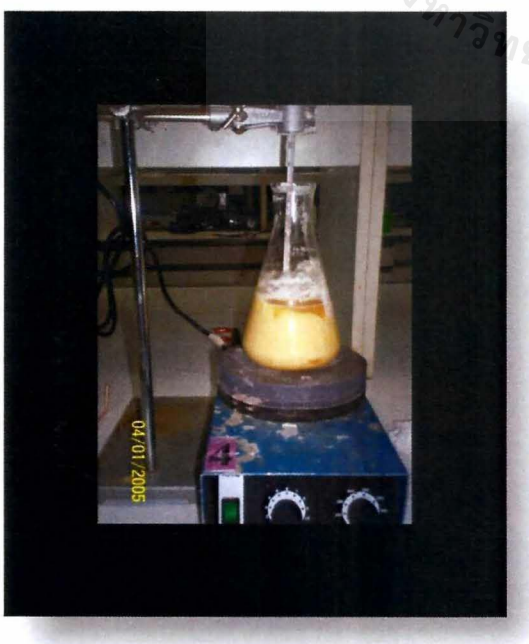
Results were determined by SPSS using RCBD, Duncan's test. Differences at $p < 0.05$ were considered to be significant level.

Soy crude extraction



Add 70% ethanol, 300ml

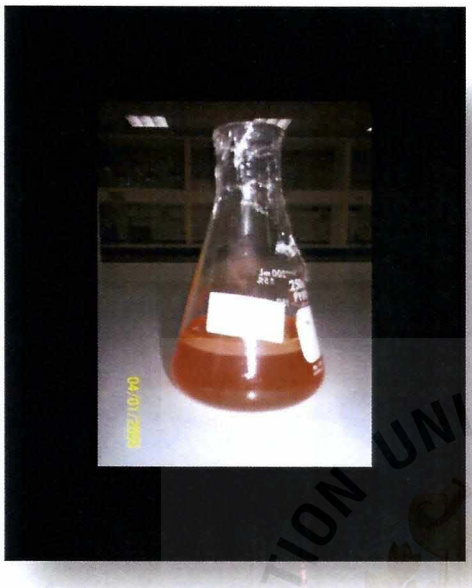
Figure 1: Represents after adding 300ml of 70% ethanol to the soy sample



Heat @60°C + stir, 1 hour

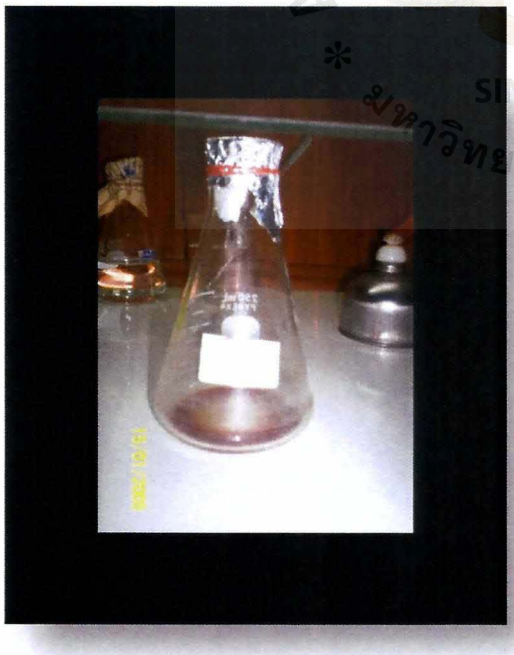
Figure 2: Represents when the soy sample was

heated at 60°C for one hour



Extraction with hexane 20ml * 4

Figure 3: Represents when the soy sample was extracted using 20 ml of hexane for 4 times.



Collect residues

Figure 4: Represents when the soy crude extract

Chapter VI

Results

1. Percent yield of crude soy samples after extraction

Table 1: Percent yield of dried crude soy samples

Soy samples	% Yield of soy sample crude extract (w/w)
Soy flour	4.4
Soy beans	4.5
Roasted soy beans	4.3

Table 2: Measured pH of dried crude soy samples

Soy samples	pH readings
Soy flour	7
Soy beans	6
Roasted soy beans	6

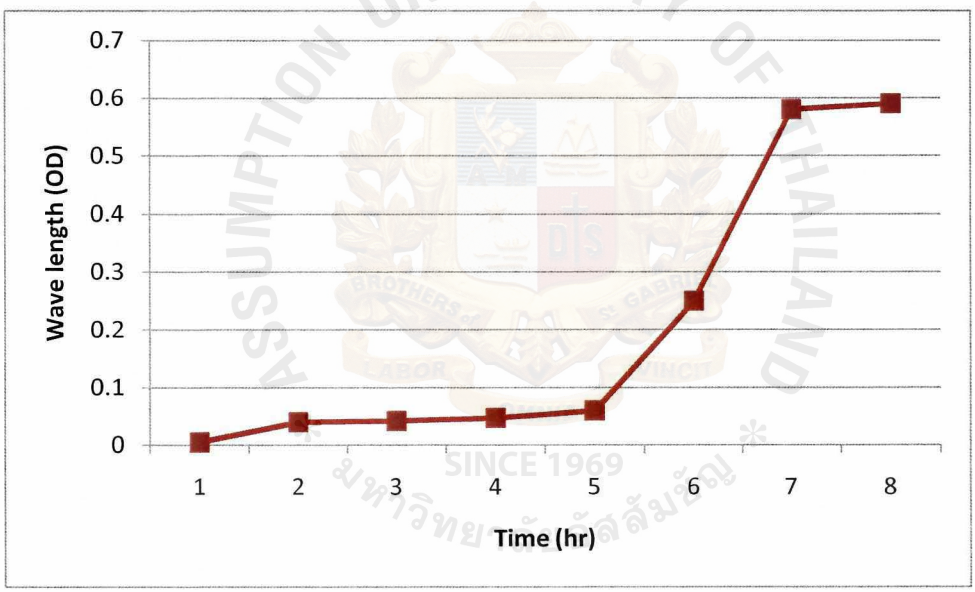
Percentage of yields of the dried crude samples of soy beans, roasted soy beans and soy flour are shown in Table 1 above. Highest percentage yield was shown by soy beans and lowest percentage yield was shown by roasted soy beans. Table represents the pH of each crude sample and all showed a neutral pH.

2. Plotting the growth curves of *E. coli* and *S. aureus*

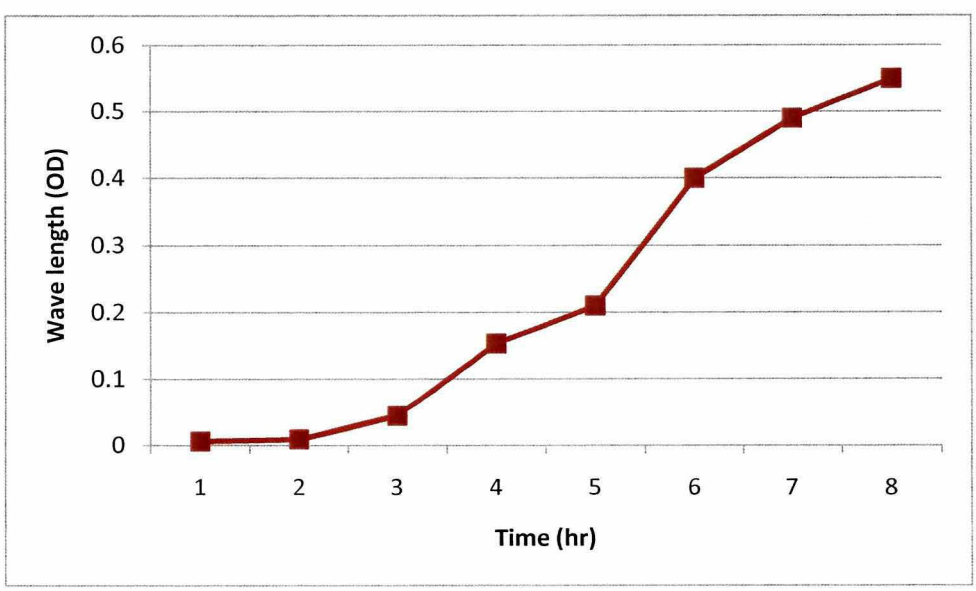
Table 3: Represents the wavelength (OD) measured for each hour to plot growth curve

Microbe type	Wavelength (OD)							
	9.00 am	10.00am	11.00am	12.00am	1.00am	2.00pm	3.00pm	4.00pm
E. coli	0.005	0.04	0.042	0.047	0.06	0.25	0.58	0.59
S. aureus	0.006	0.009	0.044	0.153	0.21	0.40	0.49	0.55

Graph 01: Growth curve of *E. coli*



Graph 02: Growth curve *of S. aureus*



3. Colony forming unit count for bacterial starter cultures

Table 4: Average number of *Escherichia coli* in all three replicates

Dilution	N= Average Number of bacteria
10^{0}	TNTC
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	TNTC
10^{-4}	98 ± 8
10^{-5}	21 ± 4
10^{-6}	TLTC
10^{-7}	TLTC

Table above shows the average amount of bacteria counted per each plate of *E. coli* which was triplicate.

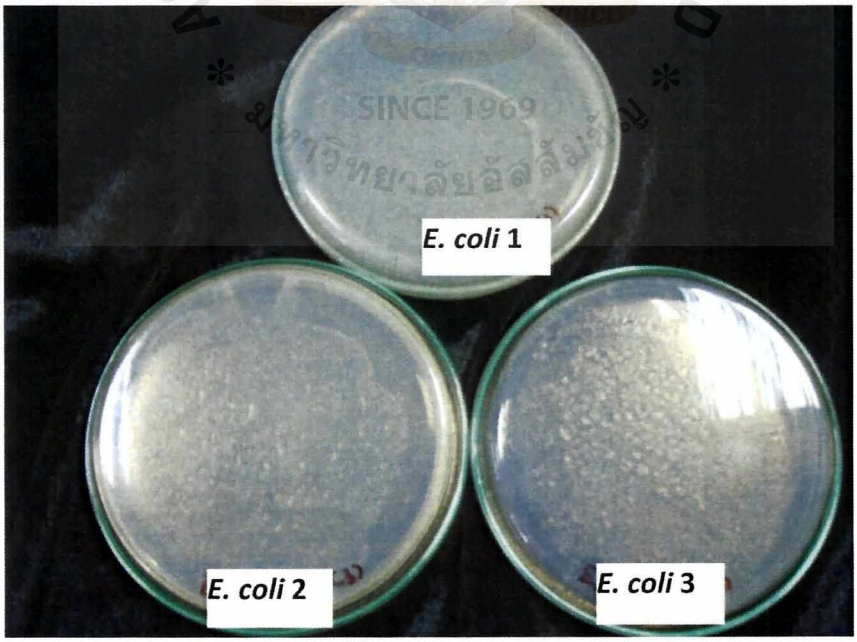


Figure 5: Shows the plates of *E. coli* used to count cfu value.

Table 5: CFU value (cfu/mL) of the starter culture of *E. coli*

Dilution	CFU value (cfu/mL)
10^{0}	TNTC
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	TNTC
10^{-4}	$10.6*10^{5} \pm 9*10^{6}$
10^{-5}	$25*10^{6} \pm 17*10^{6}$
10^{-6}	TLTC
10^{-7}	TLTC

Table above represents the cfu/mL calculated for each dilution of *E. coli*

Table 6: Average number of *Staphylococus aureus* in all three replicates

Dilution	N= Number of bacteria
10^{0}	TNTC
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	TNTC
10^{-4}	111 ± 10
10^{-5}	16 ± 4
10^{-6}	TLTC
10^{-7}	TLTC

Table above shows the average amount of bacteria counted per each plate of *S. aureus* which was triplicate.

Table 7: CFU value (cfu/mL) of the starter culture of *S. aureus*

Dilution	CFU value (cfu/mL)
10^{0}	TNTC
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	TNTC
10^{-4}	$12.1*10^{5} \pm 10.1*10^{5}$
10^{-5}	$2*10^{7} \pm 12*10^{6}$
10^{-6}	TLTC
10^{-7}	TLTC

Table above represents the cfu/mL calculated for each dilution of *S. aureus*

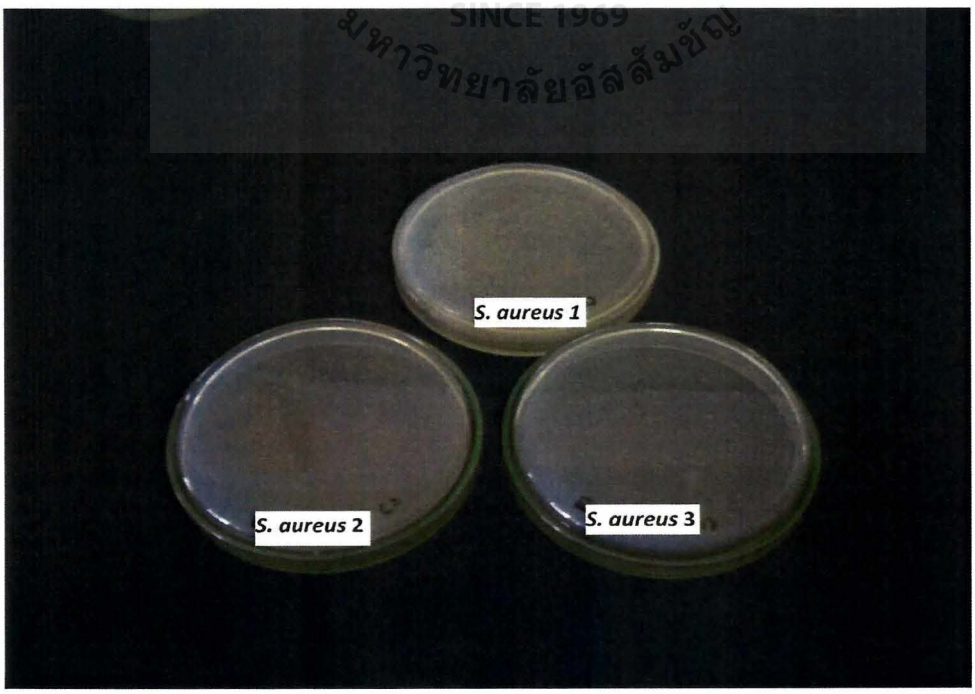


Figure 6: Shows the plates of *S. aureus* used to count cfu value.

4. Inhibition of the growth of microorganisms using the 100% soy crude extract

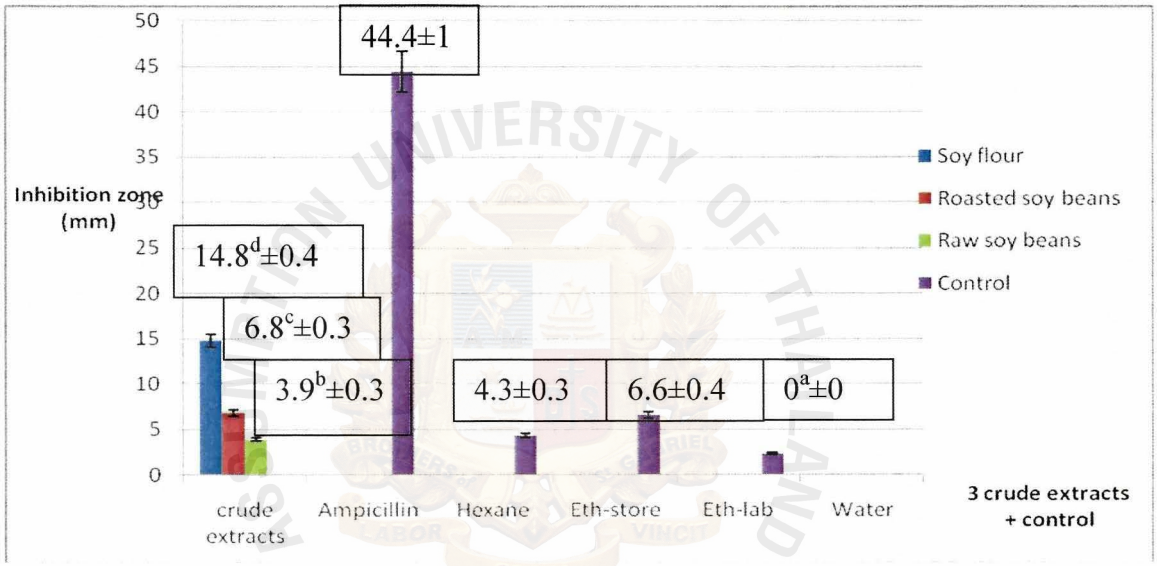
Table 8: Measurement of the inhibition zones (mm) using the average number of

	E. coli	*S. aureus*
Soy flour	$14.8^{d} \pm 0.4$	$6.6^{d} \pm 0.3$
Roasted soy beans	$6.8^{c} \pm 0.3$	$4.9^{c} \pm 0.2$
Raw soy beans	$3.9^{b} \pm 0.3$	$2.6^{b} \pm 0.1$
Ampicillin (+)	44.4 ± 0.6	39.7 ± 1
Ethanol- Store (+)	6.8± 0.3	6.2± 0. 4
Hexane(+)	3.9± 0.2	4.1± 0.3

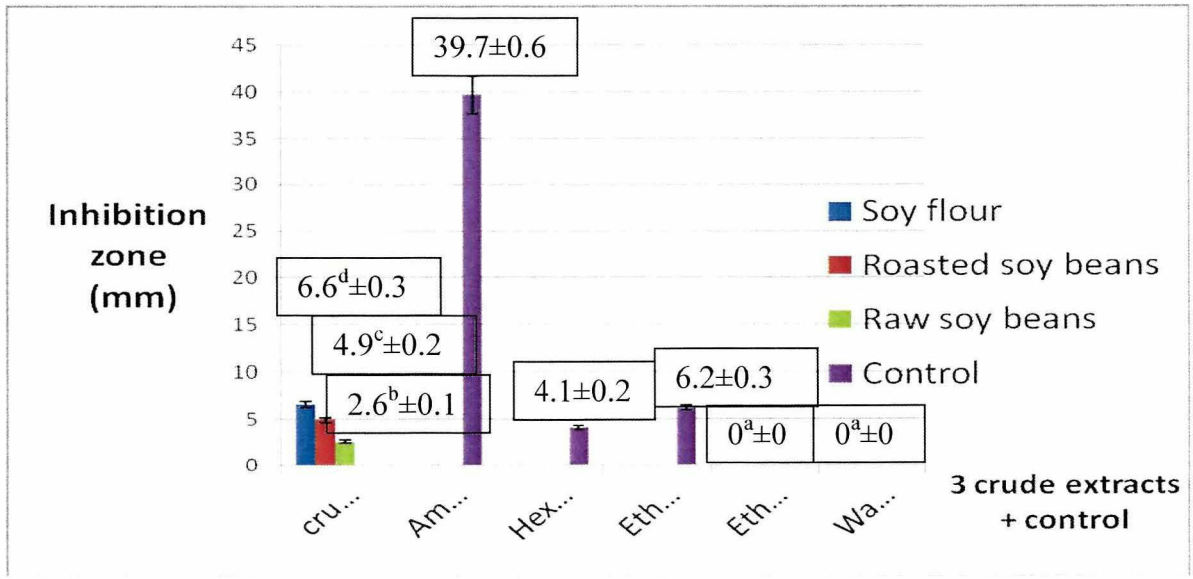
The table above represents the inhibition zones obtained after using the three crude extracts. The statistical values were obtained after comparing the inhibition zones obtained for all three samples with the negative control distilled water. Comparing was done for *E. coli* and *S. aureus* separately.

5. Inhibition of the growth of microorganisms using the three soy crude extractions.

Graph 3: Represents the inhibition zones (mm) of all three soy crude extracts on the growth of *E. coli*



Graph 4: Represents the inhibition zones (mm) of all three soy crude extracts on the growth of *S. aureus*



Statistical analysis

1. Inhibition of the growth of microorganisms using three soy crude extractions.

Table 9: Shows the diameter (mm) of the inhibition zones of growth of *E. coli* after using the three types of soy crude extracts.

Type of Soy crude extract	100% concentration of crude extract
Soy flour	14.8^{d}
Roasted soy beans	6.8^{c}
Raw soy beans	3.9^{b}
Negative control- water	0.0^{a}

The different subscript letters (a, b, c, d) means there is a significant difference between the inhibition zones of *E. coli* obtained by soy crude extract sample of all 3 types and the negative control at ($p<0.05$).

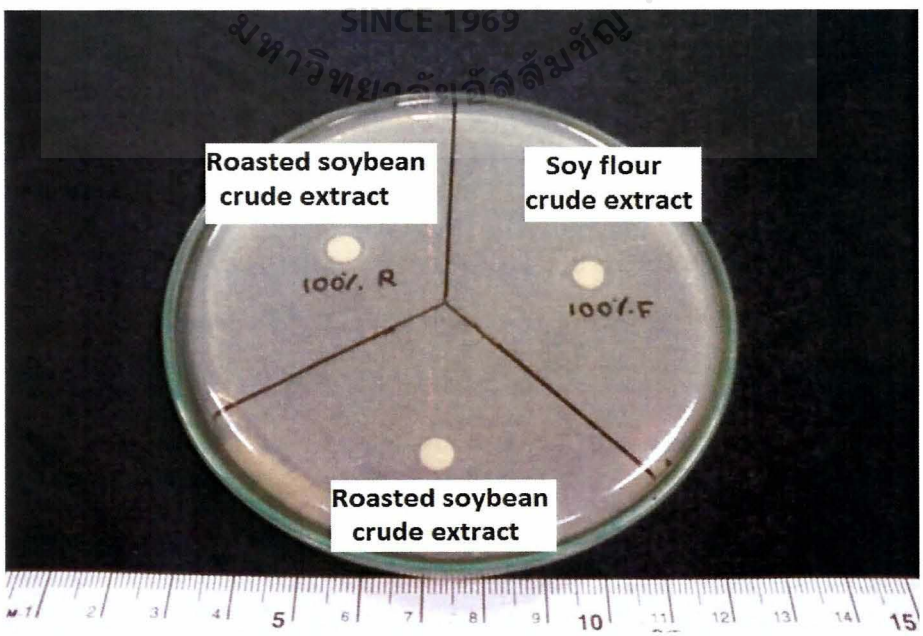


Figure 7: Represents the inhibition zones of *E. coli* after using three crude extracts.

Table 10: Shows the diameter (mm) of the inhibition zones of growth of *S. aureus* after using the three types of soy crude extracts.

Type of Soy crude extract	Soy crude extract
Soy flour	6.6^{d}
Roasted soy beans	4.9^{c}
Raw soy beans	2.6^{b}
Negative control- Distilled water	0.0^{a}

The different subscript letters (a, b, c, d) means there is a significant difference between the inhibition zones of *S. aureus* obtained by crude extract sample of all 3 types and the negative control distilled water at ($p<0.05$).

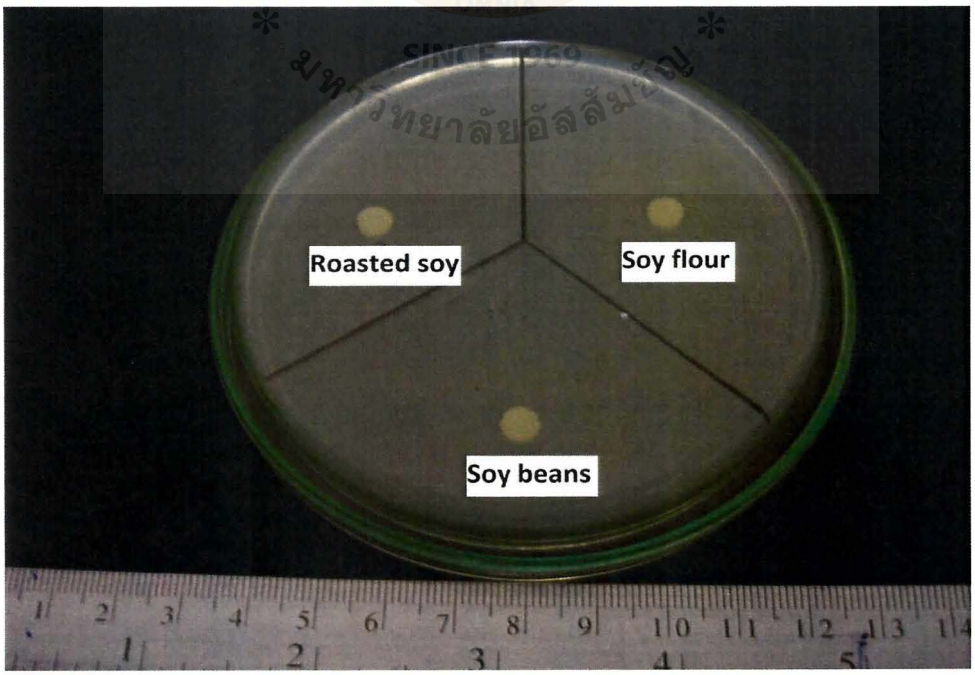


Figure 8: Represents the inhibition zones of *S. aureus* after using three crude extracts.

2. Inhibition of the growth of microorganisms using varying concentrations of soy crude extracts

Table 11: Shows the diameter (mm) of the inhibition zones of growth of *E. coli* after using the three types of soy crude extracts.

Type of microbe- *E. coli*	**% Concentration of soy flour extracted by distilled water with negative control, water**						
	75	**50**	**25**	**12.5**	**6.25**	**3.125**	**Control (Water)**
Soy flour	$7.2^{d}\pm0.3$	$4.9^{c}\pm0.5$	$3.1^{b}\pm0.1$	$2.8^{b}\pm0.2$	$2.6^{b}\pm0.3$	0.0^{a}	0.0^{a}
Roasted soy beans	$4.9^{d}\pm0.07$	$3.9^{c}\pm0.1$	$2.2^{b}\pm0.1$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Raw soy beans	$4.6^{c}\pm0.1$	$2.7^{b}\pm0.3$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

The different subscript letters (a, b, c, d) means there is a significant difference between the inhibition zones of E. coli and the varying concentrations of soy crude extract and negative control distilled water at ($p<0.05$).

Figure 9: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of soy flour extracted using ethanol-hexane extraction method.

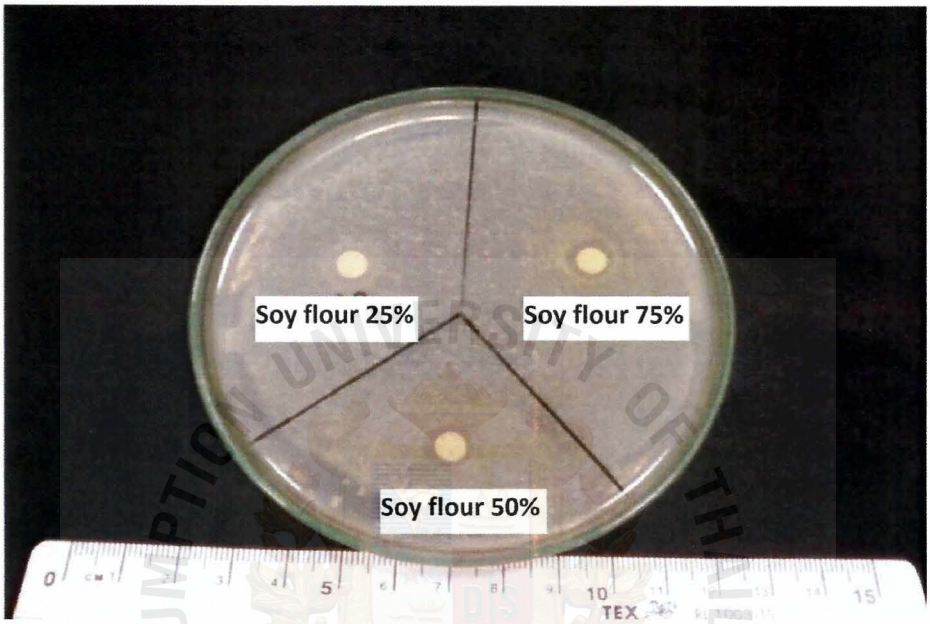


Figure 10: Represents the inhibition zones obtained using the 12.5%, 6.25%, 3.125% concentrations of soy flour extracted using ethanol-hexane extraction method.

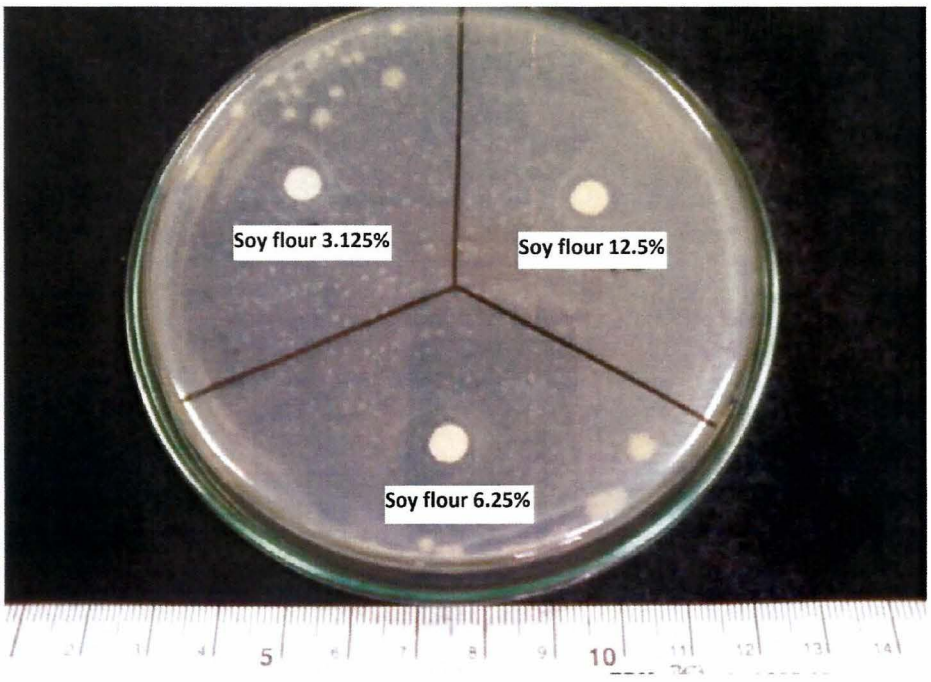


Figure 11: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of Roasted soy beans extracted using ethanol-hexane extraction method.

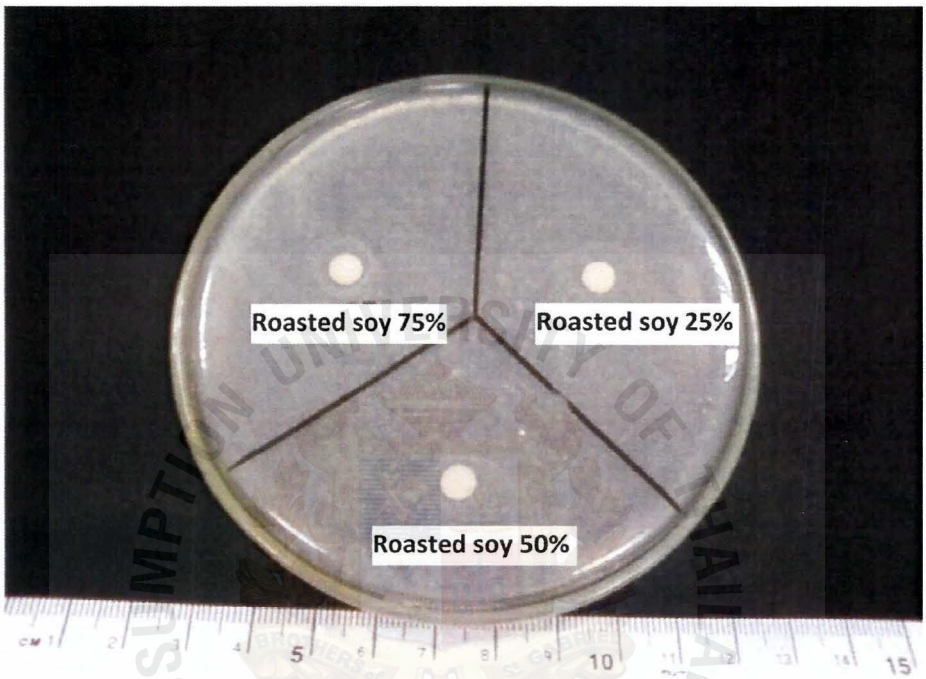


Figure 12: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of raw soy beans extracted using ethanol-hexane extraction method.

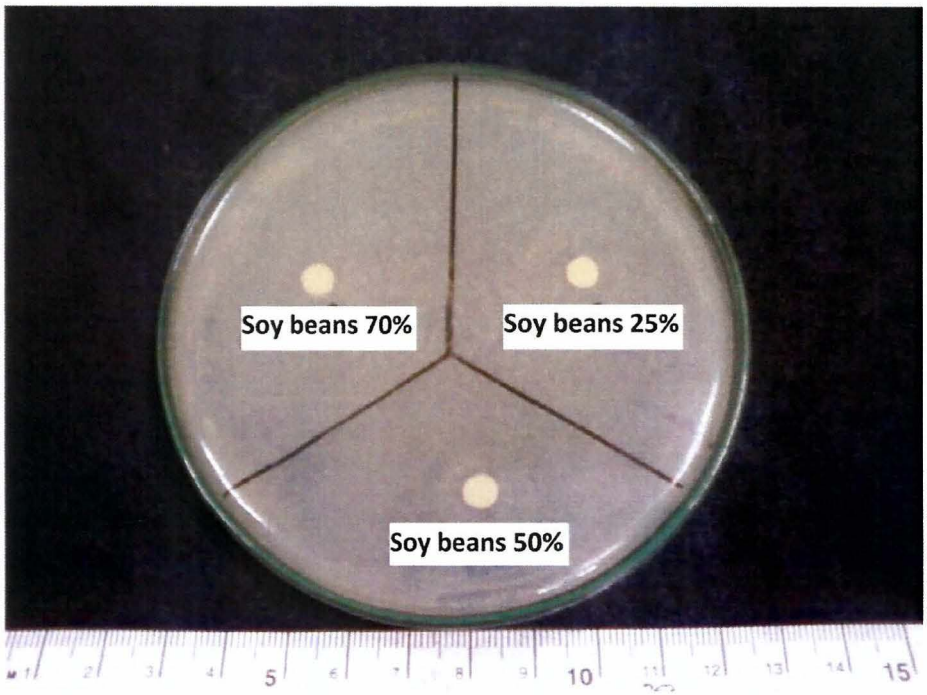


Table 12: Shows the diameter (mm) of the inhibition zones of growth of *S. aureus* after using the three types of soy crude extracts.

Type of microbe – *S. aureus*	**% Concentration of soy flour extracted by distilled water with negative control, water**						
	75	**50**	**25**	**12.5**	**6.25**	**3.125**	**Control (Water)**
Soy flour	$6.7^{d}\pm0.1$	$5.5^{c}\pm0.25$	$4.8^{b}\pm0.1$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Roasted soy beans	$4.9^{d}\pm0.1$	$2.9^{c}\pm0.1$	$1.7^{b}\pm0.1$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Raw soy beans	$0.9^{c}\pm0.1$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

The different subscript letters (a, b, c, d) means there is a significant difference between the inhibition zones of *S. aureus* and the varying concentrations of soy crude extract at ($p<0.05$).

Figure 13: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of soy flour extracted using ethanol-hexane extraction method

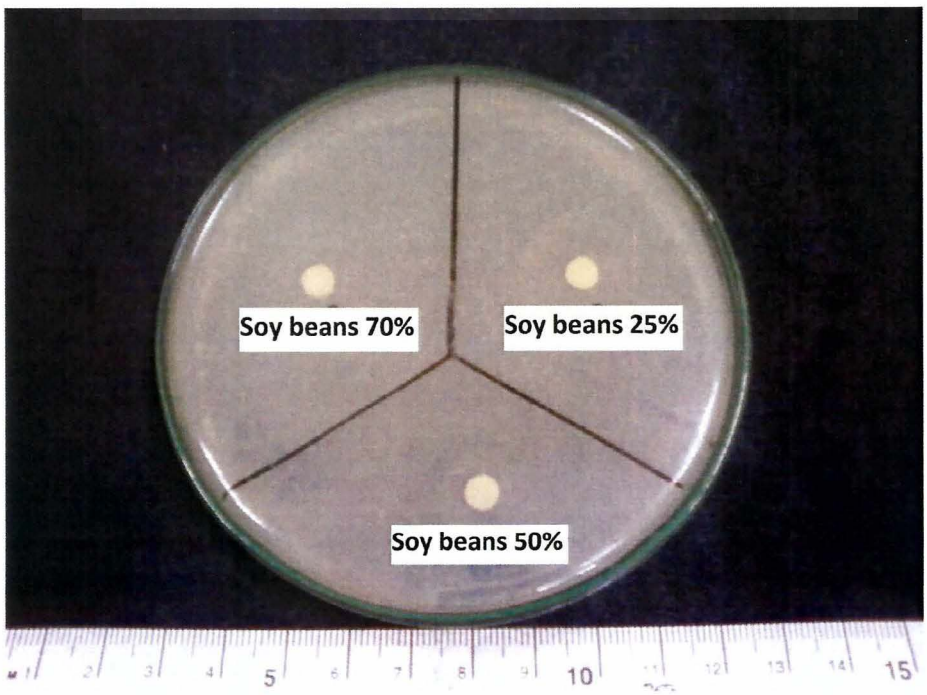


Figure 14: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of Roasted soy beans extracted using ethanol-hexane extraction method

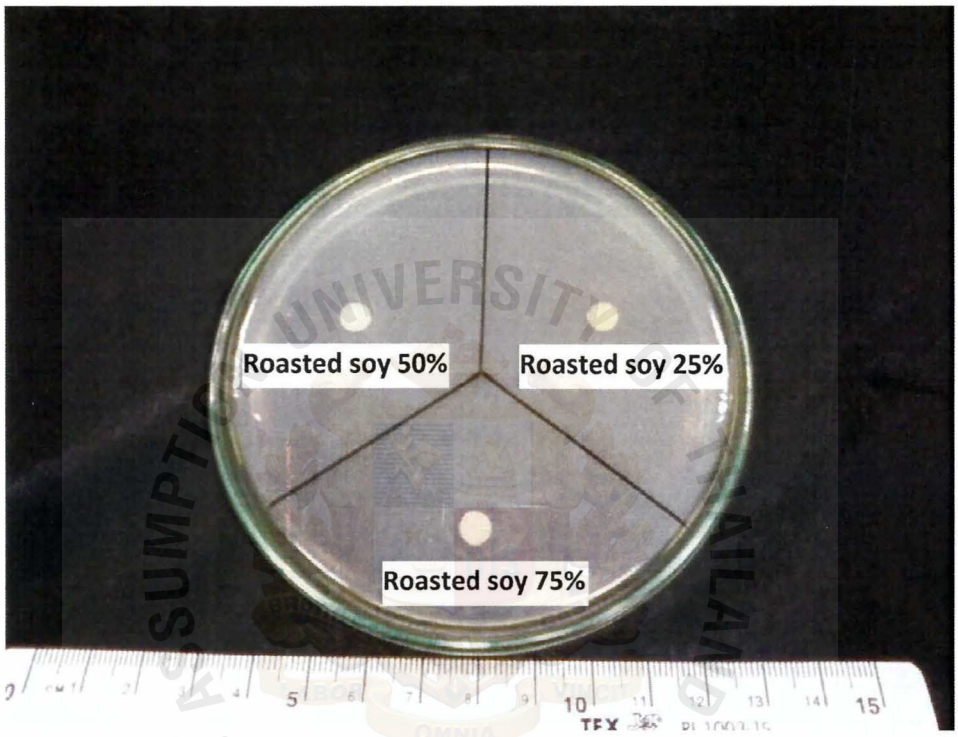
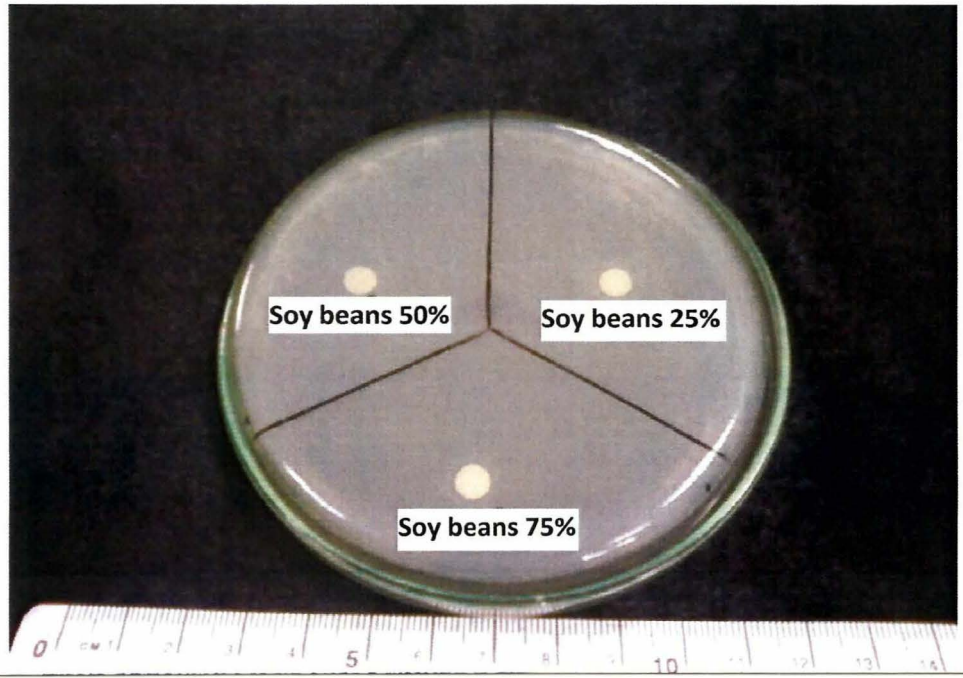
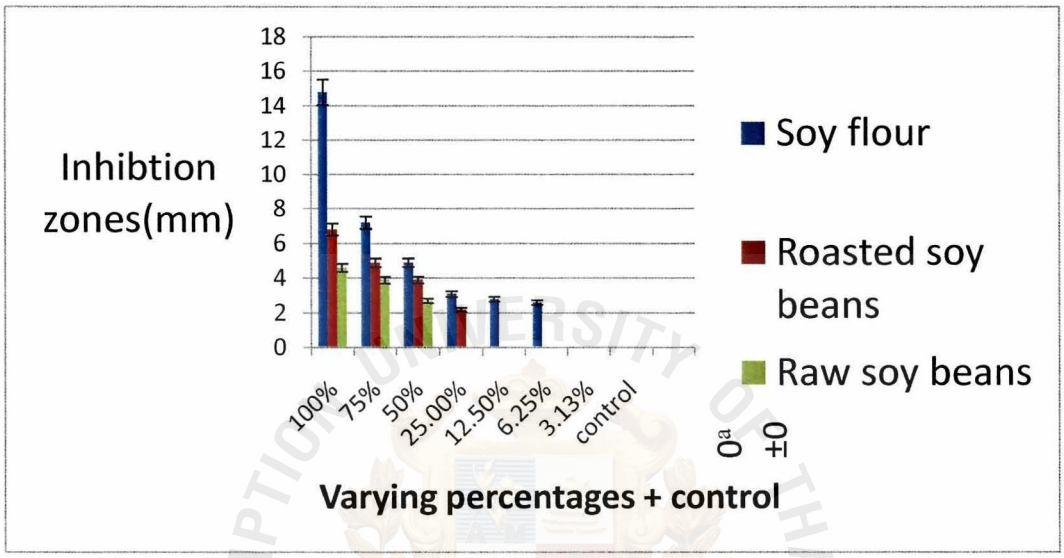


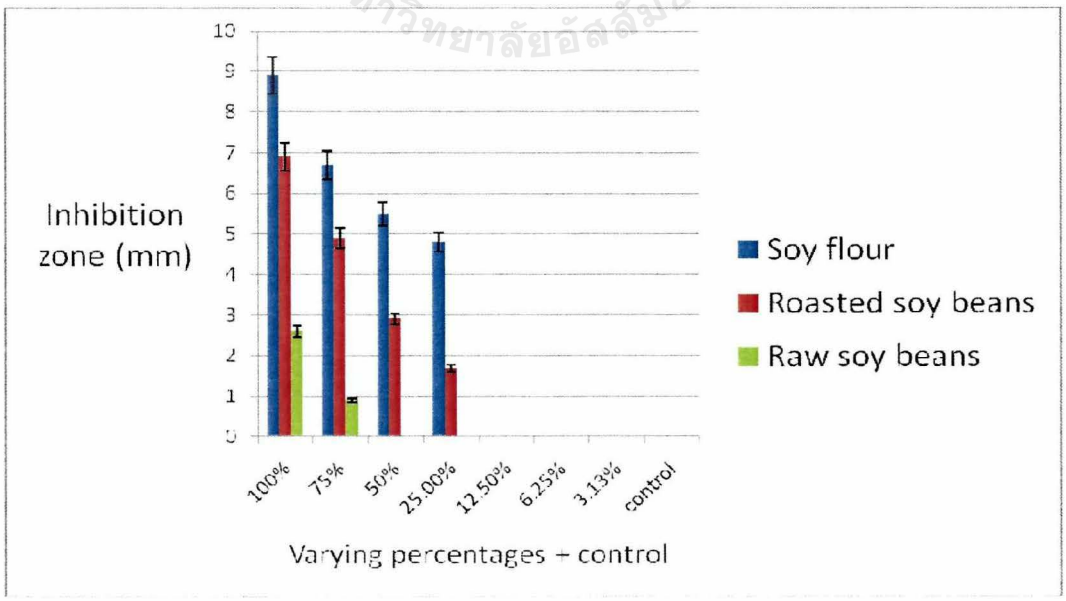
Figure 15: Represents the inhibition zones obtained using the 75%, 50%, 25% concentrations of Soy beans extracted using ethanol-hexane extraction method



Graph 5: ***E. coli*** - Comparison of the inhibition zones (mm) obtained varied concentrations of soy crude extracts with negative control



Graph 6: ***S. aureus*** - Comparison of the inhibition zones (mm) obtained varied concentrations of soy crude extracts with negative control



3. Inhibition of the growth of microorganisms using the soy flour extracted by distilled water

Table 13: Shows the diameter (mm) of the inhibition zones of growth of *E. coli* and *S. aureus* after using varying concentrations of soy flour extracted by distilled water.

Type of microbe	**% Concentration of soy flour extracted by distilled water with negative control, water**						
	20	**15**	**10**	**7.5**	**5.0**	**2.5**	**Control (Water)**
E. coli	$5.1^{d} \pm 0.1$	$3.8^{c} \pm 0.3$	$2.9^{b} \pm 0.2$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
S. aureus	$3.1^{c} \pm 0.1$	$1.8^{b} \pm 0.3$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

The different subscript letters (a, b, c, d) means there is a significant difference between the inhibition zones of *E. coli* and *S. aureus* at varying concentrations levels of soy flour extracted by distilled water at ($p<0.05$).

Figure 16: Represents the inhibition zones of *E. coli* obtained using the 20%, 15%, 10% concentrations of Soy flour extracted using distilled water.

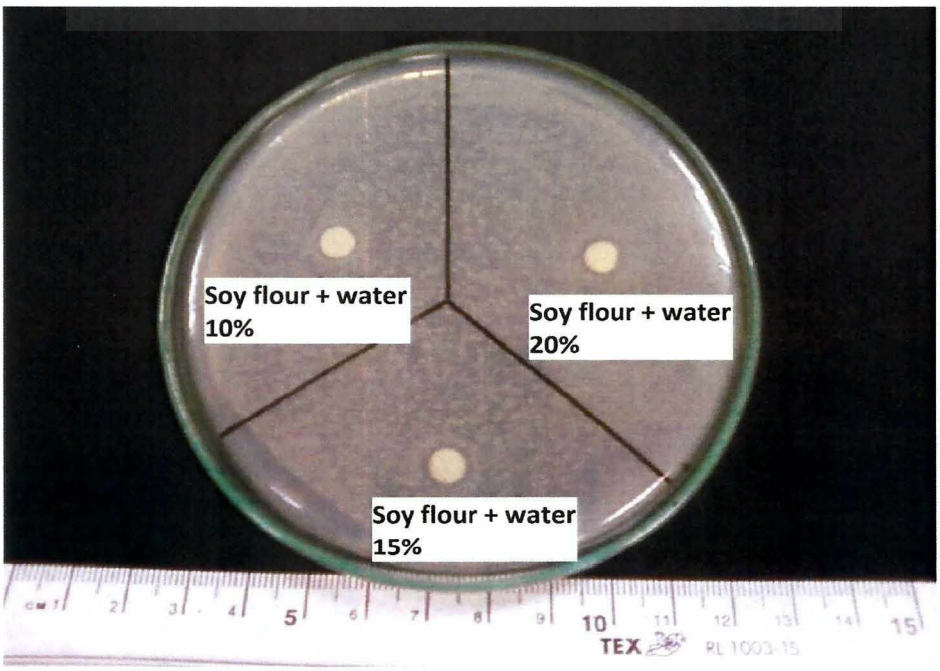
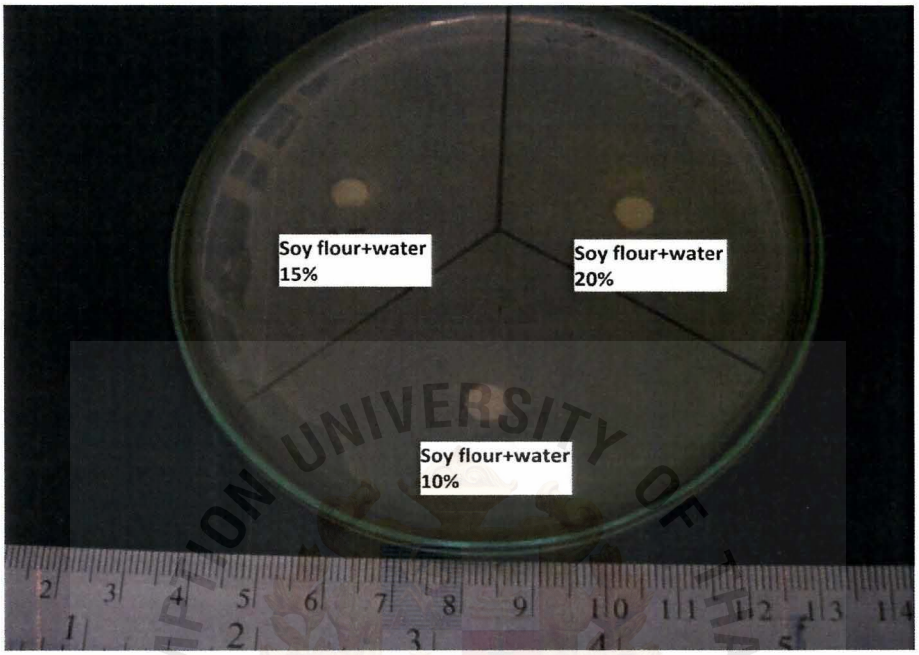
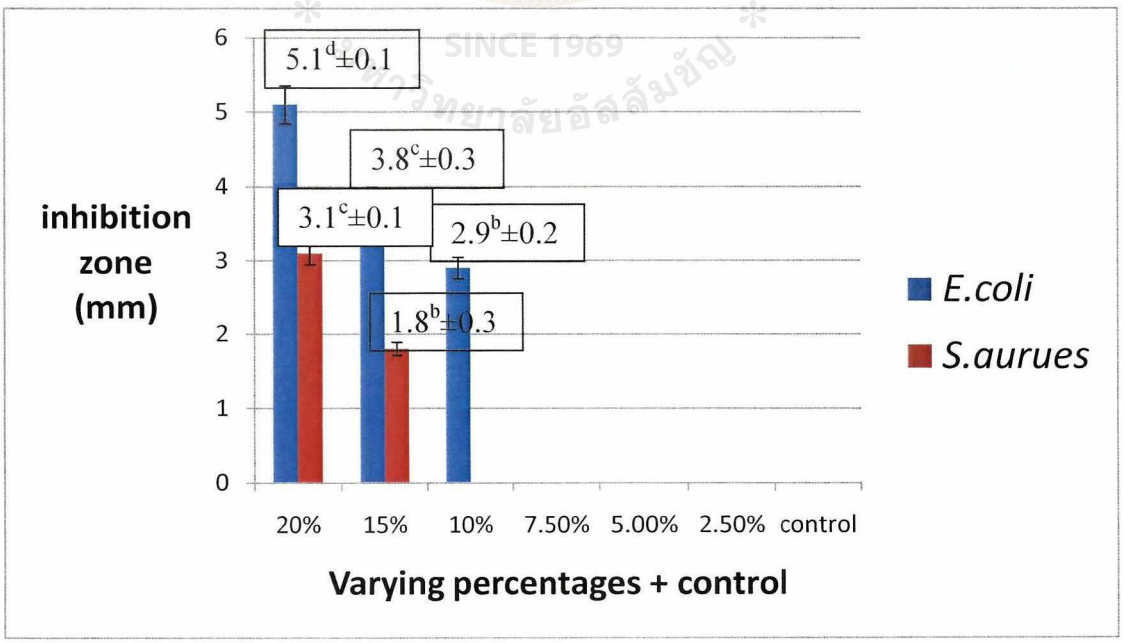


Image 17: Represents the inhibition zones of *S. aureus* obtained using the 20%, 15%, 10% concentrations of Soy flour extracted using distilled water.



Graph 7: Comparison of the inhibition zones (mm) obtained varied concentrations of soy flour extracts with negative control



CHAPTER VII

Discussion

The experiment was done to study the inhibition of the two types of bacteria *Escherichia coli* and *Staphylococcus aureus* using soy beans extractions. Raw soy beans, Roasted soy beans and soy flour were used as the three main types of samples.

In order to extract the desired crude samples certain steps and certain special reagents were used. 70% ethanol was used as the main reagent for the extraction. Main reason for this was to take out or extract isoflavones from the three types of samples. Main reason to use incubations period of 37°C was done to concentrate the sample, so that the amount of required component of the soy sample will remain in its highest concentration. Temperature of 37°C was chosen to evaporate the excess amounts of 70% ethanol from the solution.

Even after collecting residues once again ethanol was used to dissolve the components of the residue properly. This was done to make sure that all the components were used before the extraction step of the process. Main solvent used for the extraction was hexane. Hexane is a non polar solvent therefore it's perfect in dissolving non polar compounds. Because of its very high volatility makes it evaporate very quickly as well. Hexane helps to remove all the lipid components of the soy sample which is attached to the components of it. Once again the incubation process was used to concentrate the supernatant obtained after the extraction. The last part was to add 10 ml distilled autoclaved water to the residues, 10 ml was used because it was the volume required by the Minisart (single use filter unit). Minisart was used to filter the bacteria which were present in the crude residues in order to prevent contamination.

(Ref: 27)

(Ref: 28)

Table one represented the percentage yields of all the soy crude samples. The highest percentage yield was shown by soy beans. Main reason for this was the using of raw soy beans which do not pass any heat treatment. Soy beans were blended in order to reduce the particle size of the seeds, this mechanical strength must have helped to obtain higher yield. Soy flour was produced using dehulled or defatted soy beans and it was ground to smaller finer particles. After this step the soy flour is transferred through a mesh where soy flour with the highest protein content was produce. The fine particle size and high protein content explained why soy flour showed the second highest yield. Third highest yield was shown by soy beans which was the lowest as well. Compared to the other two samples heat treatment of roasted soy beans make it different from the rest. The beans were baked or roasted using oil which uses a lot of heat. Weight of the beans is much lower than the raw soy beans. This could be the reason for it to show fewer yields.

(Ref: 29)
(Ref: 30)
(Ref: 31)

The main two types of microorganisms used for the experiment were *E. coli* and *S. aureus*. In order to calculate the inoculation time for the growth of microorganisms to become stationary was very important. Therefore the growth curves were plot show by measuring the wavelength hourly. These data were shown in table 3. After 7 hours of incubation time growth of E. coli changed from log phase to stationary phase. This is represented by graph 1. For *S. aureus* even though the data were not that clear after the graph was plot which is shown by graph 2, it was clear that after 8 hours growth of *S. aureus* changed from log phase to stationary phase. Both the microbes showed value close to 0.5.

Next big task was to calculate the CFU value of the starter culture of the microbes used in the experiment. It was important to have an idea of how much of microorganisms would be there when the experiment was done. Countable number of

colonies was shown by the concentrations 10^{-4} and 10^{-5}. Below this concentrations were too low to count and concentrations above were too numerous to count. These data are represented by table 4 and 6. The cfu/ mL are shown in the table 5 and 7. Highest cfu/mL was shown by *E. coli* when compared to *S. aureus*. Growth of *E. coli* was much faster than that of *S. aureus*.

Soy beans are rich in compounds called isoflavones, genistein and daidzein. These compounds are believed that these can inhibit certain diseases, such as cancer. Some plant families do not contain isoflavones due to lack of enzyme called chalcone isomerase. This particular enzyme has the ability in converting flavones precursor to isoflavones. Luckily soy beans plants contain this enzyme therefore it has the ability to produce isoflavones on their own. These compound falls under polyphenol compounds and it function as the antioxidant flavonoids of other plant groups found in the environment.

(Ref: 32)

Many research and tests were done to test the antimicrobial property of isoflavones which is also found in soy beans. Interesting research paper was published by the international journal of antimicrobial agents volume 23, Issue 1, January 2004 on studies on the antibacterial potentiality of isoflavones. The test was done using isoflavones compounds and tested on 12 known gram positive and gram negative bacteria. Some types of the isoflavones compounds showed positive results of antimicrobial property.

(Ref: 33)

Study on antimicrobial activity of soy beans isoflavones done by Jing Legang Zhang Yongzhong Tian Lu(Harbin Normal University) (Northeast Agricultural University) (Harbin Oil Factory) is very important. This research was done using soy bean whey in three different concentrations. The results showed was very good as it proved that soy bean isoflavones were able to inhibit the growth of Escherichia coli, Staphylococcus and Saccharomyces cerevisiae.

(Ref: 34)

(Ref: 35)

In order to compare the results obtained in inhibition of *E. coli* and *S. aureus* with the three soy crude samples, Table 8 showed the obtained results after antimicrobial test was done. The results were diameters of the inhibition zones obtained. It was definitely clear that the highest inhibition zone was shown by soy flour. Second was a roasted soy bean and third was a raw soy bean respectively. Main reason for this was the processing methods of the soy samples used. Of course heat treatment was used in the production of soy flour and roasted soy beans. No heat treatment was used for the normal soy beans. Clearly the processed soy beans showed better results than that of the unprocessed one.

Graph 3 and graph 4 represented the results much clearer, peak of soy flour much higher than that of the roasted soy beans and raw soy beans. Main reason for this is the isoflavones found in soy beans. Principle soy isoflovones are genistein, daiazein and their metabolites. Actually isoflavones are naturally occurring non steroidal compound. Substitute derivative of isoflavones are produced by the replacement of two or three hydrogen atoms by hydroxyl groups. As shown is the figure Genistein has two hydroxyl groups and Daidzein contain one hydroxyl group and one hydrogen atom. This shows that these compounds are chemically bound to each other.

Usually isoflavones are produced through a branch of the main phenylpropanoid pathway, the amino acids phenylalanine and naringenin are converted to genistein using two main types of enzymes. The two legume specific enzymes are isoflavone synthase and dehydratase. Same way daidzein is produced using narinsenin chalcone by the help of three enzymes, chalcone reductase, type II chalcone isomerase and isoflavone synthase.

According to different research done on isomerase structure in raw soy beans these chemicals are bound to the parent isomerase making it highly stable. But when the heat treatment is used to the productions of soy flour and roasted soy beans these chemicals which are bound to the isomerase get degraded. Isomerase alone is heat stable which will be discussed in the latter part of the discussion. When the chemical around are destroyed isomerase can be easily extracted. As in this research this was the main target. Therefore above results were possible. Reasons soy beans to show results was the usage of mechanical strength used to make it to finer particles and the heat used in the extraction procedure. This is the reason for raw soy beans to show positive results as well.

When soy flour and roasted soy beans were compared, highest results was shown by soy flour. Soy flour was produced by grounding the soy beans to very fine particles unlike roasted soy beans. Grounding mechanism produces a lot of heat. But for roasted soy beans the soy beans not crushed. These are just baked or fried in oil.

(Ref: 36)

Various different concentrations of crude soy extracted were used to test the inhibition effect on bacterial growth. 75%, 50%, 25%, 12.5%.6.25% and 3.125% were the concentrations made using the soy crude extracts. All three samples were tested using the 6 concentrations on the growth of *E. coli* and *S. aureus*. When the overall results are concerned highest inhibition zone was shown by the highest concentration which was 75%, when compared with other concentrations. There is a gradual decrement in the inhibition zones from 50% to 3.125% respectively. Most possible assumption is that as the concentration of the crude sample decreases the effect of isoflaovones decreases, therefore the antimicrobial activity decreases.

As the three types of crude samples are compared best results were shown by soy flour, then roasted soy beans and last was raw soy beans. Effect of soy flour was really good as it showed positive result up to the concentration of 6.25%. Roasted soy beans were effective until the point of 25% and raw soy beans 50% respectively. These results further more proves the point which was discussed earlier part. Due to the heat treatment used in the process of making soy flour and roasted soy beans, showed better results than raw soy beans.

Six different concentrations were used to determine the minimum inhibition concentration (MIC) of the extracted soy crude samples. In order to inhibit the growth of *E. coli* at least 6.25% of soy flour crude sample was required. For roasted soy beans, in order to show antimicrobial property it requires at least 25% of crude sample. But the lowest was raw soy beans which required 50% concentration soy crude extract. In order to inhibit *S. aureus,* the 25% concentration of soy flour crude sample was required. For roasted soy beans, in order to show antimicrobial property it requires at least 25% of crude sample. But the lowest was raw soy beans which required 75% concentration soy crude extract. Even though the required the same concentration for soy flour and roasted soy beans the inhibition zone of soy flour results was higher than that of the other, still makes soy flour better than roasted soy beans.

Overall results of two types of microorganisms showed same results presenting soy flour best soy crude sample, then roasted soy bean crude sample and at last raw soy beans. When both were compared together inhibition on *E. coli* showed better results than of *S.aureus*. Main difference between the two microbes is that *E. coli* is gram negative and *S.aureus* is gram positive. *E. coli* being gram negative has a thinner peptidoglycan layer. Lipoproteins which are covalently linked lipids bind the peptidoglycan laler of *E. coli*.

This layer is located in the space between outer and inner cytoplasmic membrane. This particular space is called periplasm. There are no acids found in *E. coli* to facilitate the transfer of ions in and out of the cell. Therefore the cell walls are very easy break due to the low amount of peptidoglycan. But it has the ability to survive in extreme environmental conditions due to lipopolysaccaharides, phospholipids and lipoproteins. Unfortunately thinner layer makes it easier to break through the cell wall, therefore crude samples were able to inhibit the growth of *E. coli*.

But in the case of *S.aureus* being gram positive bacteria has a thick peptidoglycan layer over cytoplasmic membrane. It is thick because several peptidoglycan layers joined together. It is difficult to break though this rigid, thick layer. There are two main types of acids lipoteichoic acid and wall teichoic acid found inside the cell. Actually lipoteichoic acid is connected to plasma membrane but teichoic acid connected to the peptidoglycan layer alone. Both acids are negatively charged because of the phosphate groups. Therefore it has the ability to take positive ions in and out of the cell. Due to this reason *S.aureus* cells are able to stop excessive wall damage and cell lysis. The concentration of soy crude sample required was more in order to inhibit the growth of *S. aureus*.

(Ref: 37)

Next part was to find the inhibition ability of soy flour extracted using distilled water, soy flour was chosen because in earlier tests done by using soy crude extracts extracted by 70% ethanol soy flour crude extracted showed the best result. Both microbes were tested in order to compare the antimicrobial activity. Concentrations were chosen different according the dissolving ability of soy flour in water. Mild hot distilled was used for better dissolving of soy flour. The concentrations were varied from 20%, 15%, 10%, 7.5%, 5% and 2.5%.

When compared overall results of soy crude extract extracted by 70% ethanol and soy flour extracted by distilled water better results were shown by 70% ethanol. 70% ethanol facilitates to take out isoflavones more prominently than that of distilled water. The amount of extracted isoflavones using 70% ethanol was much higher than that of extracted water soluble isoflavones produced by distilled water. Inhibition zones of 70% ethanol was much higher than that of distilled water.

According to research done, in order produce water soluble isoflavones, cyclodextrin is added to the soybean extract. This indicates that soy products contain water soluble isoflovones. Cyclodextrin is added to produce an inclusion solution with the desired isoflavones. Then the solution is heated to a temperature ranging from 40°C to 100°C. in order for the isoflavones to dissolve it requires a pH of 8- 13. As the soy flour crude sample tested for pH, it showed a reading close to that of 7-8, which helped in taking out the isoflavones to inhibit the growth of microbes. Not only this, the usage of warm water triggered the isoflvones as well. According to previous research composition of water soluble of isoflavone is said to be 20-90%. This was the reason why positive results were shown by soy flour extracted by distilled water.

(Ref: 38)

When water was used as the solvent, amount or volume should be adjusted according the moisture content of soy food type used. As moisture content of soy flour was very low the volume was adjusted to each concentration. Polarity of isoflavones affect the solubility of the solvent either it is ethanol or water. Ethanol is

straight chain alcohol with a molecular formula of C_2H_5OH. It is polar due to the OH group on the second carbon (CH3, CH2OH). Because of the OH group ethanol is slightly negative. Isoflavones show polar effect, due to this reason, the positive pole and negative pole come into contact and a net dipole movement it created. Because of this dipole movement both molecules get attracted to each making it easier to extract. Bit when water and isoflavones come in contact both are polar molecules. Water contains positive and negative poles making it difficult to attract to each other very easily. Positive pole of one molecule repels the other molecule with positive pole. Therefore heat is required in order reduce the effect of polarity of water.

(Ref: 39)

Same way inhibition of the growth of *E. coli* was more prominent than *S. aureus*. This is due to the reason *E. coli* being gram negative bacteria and *S. aureus* being gram positive bacteria. At least 15% concentration of soy flour sample was required in order to inhibit *S. aureus* but for E. coli 10% of concentration was enough.

There are different processed food types in the market rich in isoflavones. Isoflavones are naturally found in soy beans. In order to produce soy flour, roasted soy beans, soy milk, tofu etc, different methods are used. But for some heat treatment is necessary. Genuine isoflavone pattern will be altered due to heat treatment but does not affect its activity. Now HPLC-DAD analysis is used to determine the stability of daidzein, genistein flavones etc; From this method structure related stability can be determined. There were no decay observed by research at pH 7 and 5.6, but showed degradation at pH 3.1.

This factor was helpful because all the soy crude samples were pH 7- pH 8, which indicated that isoflavones remain in the crude samples. According to the research daidzein showed the most labile compound after any time interval. According to this observation it is clear that the soy crude sample contained the desired isoflaovones to test its antimicrobial activity.

Chapter VIII

Conclusion

- Modified ethanol-hexane extraction was able to isolate the inhibition compound of soy samples.

- Water was able to isolate inhibition compound from soy flour.

- Inhibition ability
  Soy flour> Roasted soy beans> Soy beans

- In case efficiency of the two methods, soy flour Ethanol-hexane extraction is better than distilled water for extraction.

- Inhibition effect on *E. coli* was higher than *S. aureus* after using various soy crude extracts.

- Ability of inhibition decreases as the concentration decreases.

Chapter IX

Recommendation

- Determination of concentrations of Isoflavones in the form of aglycone and glusoside , from soy crude extracts are required.

- Study the inhibition of bites in plants which has become a great threat to many plant species, using soy crude extracts in order to produce efficient pesticides

Reference:

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Chapter X

Appendix

Calculation of the weight of the crude extract obtained.

Weight of the Erlenmeyer flask used for each soy sample extraction

- Soy flour – 128 grams
- Soy beans – 128 grams
- Roasted soy beans – 128.3 grams

Weight of the Erlenmeyer flask with crude extract

- Soy flour – 136.8 grams
- Soy beans – 137.3grams
- Roasted soy beans – 135.2 grams

Soy samples	Weight of crude extract (grams)
Soy flour	136.8 – 128 = 8.8
Soy beans	137.3 - 128 = 9.3
Roasted soy beans	135.2 – 128.3 = 6.9

1. Percent yield of crude soy samples after extraction

Table 1: Percent yield of dried crude soy samples

% yield (w/w) = Weight of crude extract/ Weight of soy bean sample initially

Initial weight of the soy samples:

- Soy flour – 200 grams
- Soy beans – 200 grams
- Roasted soy beans – 200 grams

Weight of the crude samples obtained

- Soy flour – 9.3 grams
- Soy beans – 6.9 grams
- Roasted soy beans – 8.8 grams

Soy samples	% Yield of soy sample crude extract (w/w)
	70% ethanol
Soy flour	(8.8/200) * 100 = 4.4
Soy beans	(9.3/200) * 100 = 4.65
Roasted soy beans	(6.9/200) * 100 = 3.45

2. Colony forming unit count for bacterial starter cultures

1. Calculation of CFU value for dilution of 10^{-4} of *E. coli*

$C = (N \text{ cfu})/(0.1 \text{ mL} \times 10^{-4})$

$= 10^5 \times N \text{ cfu/mL}$ ($N = 98 \pm 8$)

$C = 10^5 \times (98 \pm 8)$ cfu/mL

$= (10.6*10^5 \pm 9*10^6)$ cfu/mL

2. Calculation of CFU value for dilution of 10^{-5} of *E. coli*

$C = (N \text{ cfu})/(0.1 \text{ mL} \times 10^{-5})$

$= 10^6 \times N \text{ cfu/mL}$ ($N = 21 \pm 4$)

$C = 10^6 \times (21 \pm 4)$ cfu/mL

$= (25*10^6 \pm 17*10^6)$ cfu/mL

3. Calculation of CFU value for dilution of 10^{-4} of *S. aureus*

$$C = (N \text{ cfu})/(0.1 \text{ mL} \times 10^{-4})$$
$$= 10^5 \times N \text{ cfu/mL} \quad (N = 111 \pm 10)$$

$$C = 10^5 \times (111 \pm 10) \text{ cfu/mL}$$

$$= (12.1*10^5 \pm 10.1*10^5) \text{ cfu/mL}$$

4. Calculation of CFU value for dilution of 10^{-5} of *S. aureus*

$$C = (N \text{ cfu})/(0.1 \text{ mL} \times 10^{-5})$$
$$= 10^6 \times N \text{ cfu/mL} \ (N = 16 \pm 4)$$

$$C = 10^6 \times (16 \pm 4) \text{ cfu/mL}$$

$$= (2*10^7 \pm 12*10^6) \text{ cfu/mL}$$

Statistical Analysis

Dun can test done in order to compare results obtained by three types of soy crude extracts with the negative control

1. E. coli

Inhibition zone (mm)						
Comparing inhibition of *E. coli* by three crude extracts with negative control water		N	Subset			
			1	2	3	4
Duncan[a,b]	Water as negative control	3	.0000			
	Raw soy bean crude extract	3		3.9000		
	Roasted soy bean crude extract	3			6.7667	
	Soy flour crude extract	3				14.8000
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .020.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

2. S. aureus

Inhibition zone (mm)						
Comparing inhibition of *S. aureus* by three crude extracts with negative control water		N	Subset			
			1	2	3	4
Duncan[a,b]	Water as negative control	3	.0000			
	Raw soy bean crude extract	3		2.6333		
	Roasted soy bean crude extract	3			4.8667	
	Soy flour crude extract	3				6.5667
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .066.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

Dun can test done in order to compare results obtained by varying concentrations of raw soy bean crude extract

1. E. coli

Inhibition zone (mm)					
Inhibition of *E. coli* using Soy Bean crude extract		N	Subset 1	Subset 2	Subset 3
Duncan[a.b]	25% Soy Bean crude extract	3	.0000		
	12.5% Soy Bean crude extract	3	.0000		
	6.25% Soy Bean crude extract	3	.0000		
	3.125% Soy Bean crude extract	3	.0000		
	50% Soy Bean crude extract	3		2.7667	
	75% Soy Bean crude extract	3			4.9000
	Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .011.					
a. Uses Harmonic Mean Sample Size = 3.000.					
b. Alpha = 0.05.					

2. *S. aureus*

Inhibition zone (mm)				
Inhibition of *S. aureus* using Soy Bean crude extract		N	Subset 1	Subset 2
Duncan[a,b]	50% Soy Bean crude extract	3	.0000	
	25% Soy Bean crude extract	3	.0000	
	12.5% Soy Bean crude extract	3	.0000	
	6.25% Soy Bean crude extract	3	.0000	
	3.125% Soy Bean crude extract	3	.0000	
	75% Soy Bean crude extract	3		.9000
	Sig.		1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .002.				
a. Uses Harmonic Mean Sample Size = 3.000.				
b. Alpha = 0.05.				

Dun can test done in order to compare results obtained by varying concentrations of roasted soy bean crude extract

1. E. coli

Inhibition zone (mm)						
Inhibition of *E. coli* using Roasted Soy Bean crude extract		N	Subset			
			1	2	3	4
Duncan[a,b]	12.5% Roasted Soy Bean crude extract	3	.0000			
	6.25% Roasted Soy Bean crude extract	3	.0000			
	3.125% Roasted Soy Bean crude extract	3	.0000			
	25% Roasted Soy Bean crude extract	3		2.0000		
	50% Roasted Soy Bean crude extract	3			3.9000	
	75% Roasted Soy Bean crude extract	3				4.9167
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .003.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

2. *S. aureus*

Inhibition zone (mm)						
Inhibition of *S. aureus* using Roasted Soy Bean crude extract		N	Subset 1	Subset 2	Subset 3	Subset 4
Duncan[a,b]	12.5% Roasted Soy Bean crude extract	3	.0000			
	6.25% Roasted Soy Bean crude extract	3	.0000			
	3.125% Roasted Soy Bean crude extract	3	.0000			
	25% Roasted Soy Bean crude extract	3		1.7000		
	50% Roasted Soy Bean crude extract	3			3.0000	
	75% Roasted Soy Bean crude extract	3				6.9000
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .014.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Dun can test done in order to compare results obtained by varying concentrations of soy flour crude extract

1. E. coli

Inhibition zone (mm)						
Inhibition of *E. coli* using Soy flour crude extract		N	Subset			
			1	2	3	4
Duncan[a,b]	3.125% Soy Flour crude extract	3	.0000			
	6.25% Soy Flour crude extract	3		2.6000		
	12.5% Soy Flour crude extract	3		2.8333		
	25% Soy Flour crude extract	3		3.0000		
	50% Soy Flour crude extract	3			4.9000	
	75% Soy Flour crude extract	3				7.2000
	Sig.		1.000	.088	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .061.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

2. *S. aureus*

Inhibition zone (mm)						
Inhibition of *S. aureus* using Soy Flour crude extract		N	Subset			
			1	2	3	4
Duncan[a,b]	12.5% Soy Flour crude extract	3	.0000			
	6.25% Soy Flour crude extract	3	.0000			
	3.125% Soy Flour crude extract	3	.0000			
	25% Soy Flour crude extract	3		4.8000		
	50% Soy Flour crude extract	3			6.7333	
	75% Soy Flour crude extract	3				8.9000
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .019.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

Dun can test done in order to compare results obtained by varying concentrations of soy flour extracted with distilled water

1. E. coli

Inhibition zone (mm)						
Inhibition of *E. coli* using Soy Flour extracted by using distilled water		N	Subset			
			1	2	3	4
Duncan[a,b]	7.5% Soy Flour extracted by distilled water	3	.0000			
	5% Soy Flour extracted by distilled water	3	.0000			
	2.5% Soy Flour extracted by distilled water	3	.0000			
	10% Soy Flour extracted by distilled water	3		2.8667		
	15% Soy Flour extracted by distilled water	3			3.8000	
	20% Soy Flour extracted by distilled water	3				5.1000
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .015.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

2. *S. aureus*

Inhibition zone (mm)					
Inhibition of *S. aureus* using Soy Flour extracted by using distilled water		N	Subset 1	Subset 2	Subset 3
Duncan[a,b]	10% Soy Flour extracted by distilled water	3	.0000		
	7.5% Soy Flour extracted by distilled water	3	.0000		
	5% Soy Flour extracted by distilled water	3	.0000		
	2.5% Soy Flour extracted by distilled water	3	.0000		
	15% Soy Flour extracted by distilled water	3		1.8000	
	20% Soy Flour extracted by distilled water	3			3.1000
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .014.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Dun can test done in order to compare results obtained by positive and negative controls

1. E. coli

Inhibition zone (mm)						
Positive and Negative controls-*E. coli*		N	Subset			
			1	2	3	4
Duncan[a,b]	Ethanol from lab (+ve control)	3	.0000			
	Water (-ve control)	3	.0000			
	Hexane (+ve control)	3		4.2667		
	Ethanol from store (+ve control)	3			6.6000	
	Ampicillin (+ve control)	3				44.4333
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .311.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						

2. *S. aureus*

Inhibition zone (mm)						
Positive and Negative controls- *S. aureus*		N	Subset			
			1	2	3	4
Duncan[a,b]	Ethanol from lab (+ve control)	3	.0000			
	Water (-ve control)	3	.0000			
	Hexane (+ve control)	3		4.1333		
	Ethanol from store (+ve control)	3			6.2667	
	Ampicillin (+ve control)	3				39.7667
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .104.						
a. Uses Harmonic Mean Sample Size = 3.000.						
b. Alpha = 0.05.						