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Determining the antimicrobial activity of the resin extract from the fruit of *Capsicum annuum* var. Longum

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ABSTRACT

This study is concerned with the biological testing of the resin extracts from Capsicum annuum var. longum(commonly known as siling haba in the Philippines), using the agar well method for conducting the microbial assay of the resin extractive against three different microorganisms, namely Escherichia coli for gram negative bacteria, Staphyloccus aureaus for gram positive bacteria, and Candida albicans for fungi with streptomycin used as positive control for bacteria and Nystatin as positive control for fungi.

Keywords: Antimicrobial Activity, Antimicrobial Testing, *Capsicum Annuum var. longum*, *Siling Haba*, Microbial Assay, Agar Well Method, Microorganisms.

INTRODUCTION

Capsicum annuum L. is a domesticated species of the plant genus Capsicum in the family S*olanaceae*[1]with numerous varieties. [2]The plant is native to the southern part of North America and the northern part of South America. This plant has been known since the beginning of human civilization, and used in human diet since prehistoric times.

The species *Capsicum annuum L.* has more than 200 common names including chilli pepper, paprika (sweet varieties), bell pepper, cayenne, jalapeño, chiltepin, and Christmas pepper. The varieties and cultivars of *Capsicum annuum* are classified on the basis of their fruit shapes, such as *Capsicum annuum var. glabriusculum, Capsicum annuum var. grossun, Capsicum annuum var. accuminatum, and Capsicum annuum var. longum.*This last aforementioned variety is what this study is concerned with. *Capsicum annuum var. longum*has the common names of long pepper or Spanish pepper in English, *siling haba* or *siling-sigang* in Filipino (the Philippine national language). The fruit of *Capsicum annuum var. longum*is greenish-yellow or red, oblong lanceolate narrowing to a tapering tip, up to 6 cm long and 1.5 cm across. []as shown in *Fig:1*.

Figure 1. Photo of the Fruits of Capsicum annuum var. longum (Siling haba)

The fruits with seeds are commonly used to spice food as in the traditional Filipino native dish, *sinigang*. The fruit of *Capsicum annuum var. longum*has been known to possess several medicinal properties such as antifungal, antimicrobial,[2] [3]and antioxidant.[]

1.1 Background of the Study

The present study is a follow up of the author's, previous research on theextraction and purification of resins from the fruits of *Capsicum annuum var. longum*, Frial McBride[]

1.2 Glossary

This subsection defines the various technical terms used in the study:

Agar Method. Refers to a method where an antibiotic is applied to a well that is cut into the agar.

Anti-microbial. It refers to the inhibition of the growth of microorganism. It also refers to adrug used to oppose growth of microorganism or to a drugs which preventsthe growth of microorganisms.

Assay, Microbiological. This is a quick and simple biochemical research technique employed principally for analysis of vitamins, amino acids, and other nutrients.[21]

Carminatives. Substances that have the power to relieve flatulence or colic.

Condiments. Substances that can make the food savory.

Extract. This refers to certain concentrated substances prepared from another. To pull or draw out by force or effort.

Extraction. It is the act or process of extracting, involving of processing or extraction; the separation of medicinally active portion of the plant or animal tissues, from the inactive portion of plant or animal tissues, from the inactive or inert components through the uses of selective solvent and standard extraction procedures.

Extractive. This is the dark-colored insoluble substance produced in the preparation of extract by evaporation.

Fruit. This is a seed containing part of the plant and used as food.

Local irritant. A substance that causes or gives rise to the irritation.

Maceration. This refers to the process of extraction by soaking the properly comminuted drug or substance in the menstruum until the cellular structure is thoroughly penetrated and the soluble portion are softened and dissolved without heating.

Microbiological Test. This refers to the test for the demonstration of the level of effectiveness of any added anti-microbial agent.

Microorganism. This is a minute form of life, individually, too small to be seen by naked eye.

Percolation. This is the process of the slow passage of a liquid through a filtering medium; an operation of filtration which consist in placing any substance, the virtues of which are to be extracted by a menstruum, in a funnel shaped instrument having a septum prepared with holes, or it's tube stuffed with cotton and pouring fresh portions of the menstruum upon it until all its virtues have been extracted.

Percolator. This is an apparatus for extraction of drugs with liquid solvent by downward displacement.

Reagent. This refers to the substances used either as such or as constituents of solutions, a substance used to produce a chemical reaction.

Reflux. This is the process of boiling so that a vapor is liquefied and returned to the boiler.

Resins. These are amorphous substances products with a complex chemical nature. Physically, they are usually hard, or translucent and when heated, they soften and finally melt.

Rubefacient. This refers to any substance that causes redness of the skin; an agent causing redness of the skin, producing a local congestion, the vessel becoming dilated and simply the blood increased.

Solvent. It is any substance where the solute is dissolved.

Stimulant. It is any substance that can cause an increase in the activity of some parts of the brain and spinal cord.

Agar. This refers to a solidifying agent, desirable in microbes which needs a solid medium. This is a complex polysaccharides derived from a marine algae.

Antibiotic. It is an anti-microbial agent produced naturally by a bacterium fungus.

Autoclave. This is an equipment for sterilization steam under pressure, usually operated at 15psi and 121 degree Celsius.

Antifungal. This is the act of destroying or killing of the fungi.

Aseptic. It is free from or doing away with microorganism that produce disease or putrefaction.

Aseptic Technique. It is used in microbiology to exclude contamination.

Candida Albicans. It is an acute or chronic superficial disease producing lesion in mouth, vagina, skin or nails. It can be a systemic disease, sometimes by hematogenic.

Dissemination, involving lungs, heart, kidney, brain and other organ.

Culture. The microbes that grow and multiply in culture medium.

Culture Media. It is any material in which microorganism find nourishment in which they can reproduce.

Escherichia coli. It is a bacteria normal in habitat of large intestine of vertebrates, including human presence is beneficial because it help produce certain vitamin and break down under stable food stuff, the manifestation of E. coli cause bloody diarrhea when it grows in the intestine.

Incubation Period. This is the time interval between the actual infection and first appearance of any sigh and symptoms of disease.

Nutrient. The medium commonly used liquid complex medium that is media for which the exact chemical composition veins slightly from batch.

Nystatin. It is a polyene anti-fungal antibiotic. It was isolated from Streptomycin, noursci in 1950 and originally named "Fungicidin" (Haun & Brocin, 1951). It is used for skin infection, oral candidiasis (thresh) vagina candidiasis, candidiasis of GIT.

Purification. The act or operation of removing impure noxious or foreign matter.

Resistant. The organism that are not inhibited by microbes.

Staphylococcus aureus. This are gram positive, non-motile, non-spore forming spherical cells found on the skin, anterior nares and mucous membrane of the majority of healthy adults.

1. Statement of the Problem

The problem that this study sought to answer waswhether the resin extracted from *Capsicum annuum var. longum*has antimicrobial properties.

2. Significance of the Study

The search for and use of drugs from plants have expanded and accelerated in recent years especially in developing countries because the modern pharmaceutical industry is dominated and controlled by big multinational corporations (MNCs) like Pfizer and GSK making pharmaceuticals prohibitively expensive for most of the world's population. Many previous studies have pointed to the potential antimicrobial properties of *Capsicum annuum var. longum*. The author has therefore decided to confirm such previous studies by investigating the antimicrobial properties of resins extracted from the fruits oflocally grown*Capsicum annuum var. longum.* It is hoped that this study will be able to provide consumers in the developing world with a cheaper and effective alternative to today's MNC-produced antibiotics.

3. Review of the Literature

The taxonomic features and fruit morphology of five varieties of *Capsicum annum L. Solanaceae* have been discussed by D.A Zhigila et al.

An excellent overview of *Capsicum annum L.* has also been written by Sunil Pandey et.al, who discussed its constituents, uses, and activities and indicating its important medicinal uses,while a very good review of the antimicrobial properties of Chili peppers has been given by Morrine A. Omolo et al.[ ]The antimicrobial property of plant oils and extracts has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using methods that are directly comparable. In a study by K.A. Hammer, 52 plant oils and extracts were investigated for activity against *Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serotype *typhimurium, Serratia marcescens and Staphylococcus aureus,* using an agar dilution method. Lemongrass, oregano and bay inhibited all organisms at concentrations of $\leq$ 2.0% (v/v). Six Oils did not inhibit any organisms at the highest concentration, which was 2.0(v/v) oil for apricot kernel, evening primrose, macadamia, pumpkin, sage and sweet almont. Variable activity was recorded for the remaining oils. Twenty of the plant oils and extracts were investigated, using a broth microdilution method, for activity against *C. albicans, Staph.aureus* and *E. coli.* The lowest minimum inhibitory concentrations were 0.03% (v/v) thyme oil against *C. albicans* and *E. coli* and 0.008% (v/v) vetiver oil against *Staph.aureus.* These results support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives.[20]

Sarah Shayan and Saeide have investigated the antibacterial and antibiofilm activities of an extract for *Capsicum annum L.* on the growth and biofilm formations of common pathogenic strains. In their study, the antibacterial and antibiofilm activities of extracts from*Capsicum annuum L.* on the growth and biofilm formation of common pathogenic strains that were isolated from the urinary tractinfection were examined. All 6 strains (2 *Klebsiella pneumoniae*, 2 *Pseudomonas aeruginosa* and 2 E.coli) isolated from the urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, Southeastern Iran) suffering from urinary tract infections during a period of one month were evaluated. The ESBL producing *Klebsiella pneumoniae,* Pseudomonas aeruginosa and Ecoli were evaluated by disk diffusion test and growth and biofilm formation of common pathogenic strains were determined using the microtiterplate method. The results showed that different concentrations of extract plant had significant effects on the bacterial growth and even at 5and 10mg/ml showed the most restraint in the biofilm formation of the isolate. Furthermore, the data demonstrate the biocontrol potential of *Capsicum annuum L.* on the planktonic growth and biofilm formation of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Ecoli* and can suggest it as suitable biocide for the recirculating urine systems.[21]

More recently (2014), Morrine A. Omolo et al. undertook a review of the antimicrobial activities of Chili peppers against human pathogens.

Earlier (1996), Roberto H. Cichewicz and Patrick A Thorpe made a study of the antimicrobial properties of chili peppers and their uses in Mayan medicine.

Likewise, S. Soetarno (1997) et al. investigated the Antimicrobial Activity of the Ethanol Extracts of *Capsicum annuum* fruits with different levels of pregnancy in Indonesia.

Furthermore, according to Liljana et al. (2013), several types of *capsaicinoids* can be present in the oleoresin extracted from hot peppers *Capsicum annuum L. (Solanaceae)* a major component of which is capsaicin (69%). The extraction of the oleoresin from the fruit of hot pepper can be made in many ways. The most appropriate, in their experiments was extraction by Soxlet, and this procedure was compared to extractions by means ofvacuum filtration. They found that ethanol was appropriate for extraction and quantification of *capsaicin* for food and pharmaceutical grade. The content of *capsaicin* in the three different varieties of hot peppers with Macedonian origin was measured spectrometrically and the results were compared with the sweet pepper variety as a control. Results of quantification measurements made for Soxlet oleoresins were different in a really special way from the results for vacuum filtration oleoresins, and it was due to conditions used for the procedure of extraction. These results showed that for extraction of *capsaicin* for different purpose, conditions should be always adjusted.[22]

Moreover, M.A Calvo et al. have described the techniques that allow evaluating the antimicrobial activity of plants extracts and their essential oils against bacteria and fungi. The different methodologies were differentiated in terms of techniques on broth culture media, techniques on solid culture media, microwell techniques, aromatogram technique and bioauthographies. These techniques also allowed establishing the Minimum Inhibitory Concentration (MIC) of the assayed products.[23]

MATERIALS AND METHODS

This section discusses the methods and procedures utilized to undertake the biological testing of the resin extract from the fruits of *Capsicuum annuum var. longum*.

5.1 Collection and Preparation of the Resin Extract

The resin extract from the fruits of *Capsicuum annuum var. longum* was obtained and purified using the procedure and methods shown in Fig. 2 and described fully in an earlier study.[]

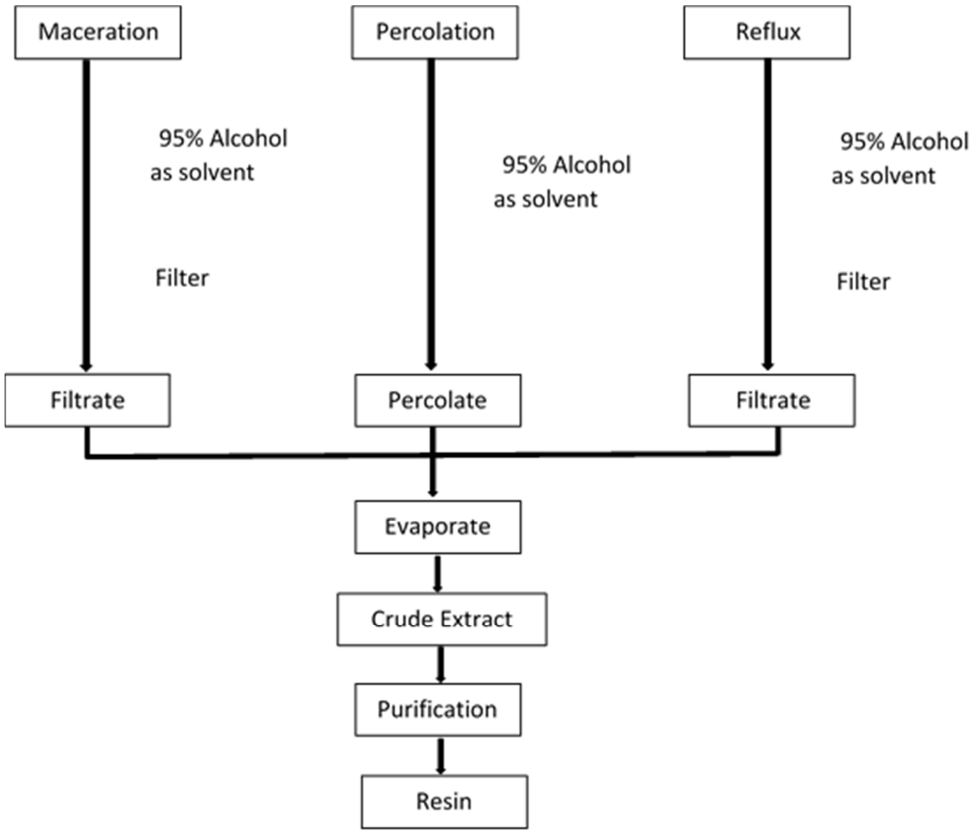

Figure 2: Flow Chart of the Resin Extraction Procedure

5.2 Preparation of the Materials for the Microbial Testing of the Risen Extract

5.2.1 Preparation of the Resin Extract with Different Concentrations

Each of the following one hundred, two hundred, and three hundred milligrams of resin extractive was weighed in an evaporated dish separately. Each of them was diluted with one milliliter of ninety-five percent alcohol.

5.2.2 Preparation of the Reagent

Hydrochloric acid (1%) – This was prepared by mixing 23.6 milligrams of HCL with water to make one (1) liter.

Phloroglucinol Test Solution – about 0.5 grams of phloroglucinol was dissolved in water to make one (1) liter.

Resorcinal Test Solution – About one (1) gram of resorcinol was dissolved with hydrocholoric acid in sufficient water to make 100 milliliters.

5.2.3 Preparation of the Inoculum

The recently grown stock culture of the organism contained in surfaces of the agar slants in three test tubes were inoculated. With the aid of platinum loop, the suspension was spread evenly on the surface of the agar and incubated at specified temperature. A stock solution was prepared by collecting the surface growth in about ten milliliters of the sterile saline solution. (See **Appendix A**).

5.2.4 Preparation of the Culture Media

Two media were used – Mueller Hinton Agar and Sabourauds's Dextrose Agar. Mueller Hinton Agar was prepared by sterilizing and autoclaving at 15 lbs. Pressure (121°C) for 15 minutes. After sterilizing, thirty-eight grams of the agar was suspended to one liter of distilled water. It was then boiled to completely dissolve the medium. For Sabouraud's Dextrose Agar, it was prepared by sterilizing first at 121 - 124°C for 15 minutes. Sixty five grams of the agar was then suspended in one liter of distilled water. It was next boiled for a complete dissolution of the agar as detailed in **Appendix B**.

5.2.5 Microbial Test of the Extract

The determination of the anti-microbial activity of the crude extract was performed using the following procedure shown in *Fig: 3*

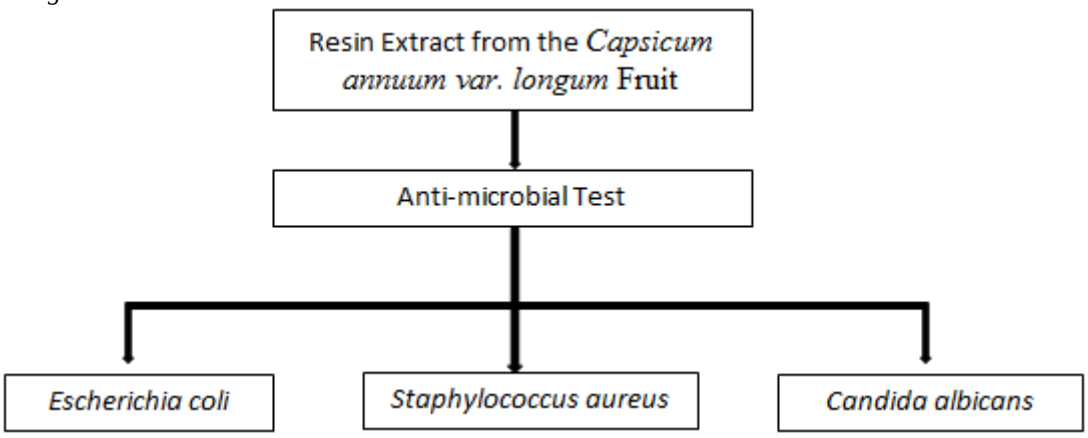

Figure 3: Design Showing the Microbial Assay Performed

1. The assigned culture was aseptically swabbed on the appropriate plate. It was swabbed on the appropriate plate. It was swabbed into three directions to ensure complete plate coverage/
2. Plant extractive with different concentration was placed into the agar well using a dropper.
3. The plates were incubated at 35° C for 48 hours for bacteria and one week for fungi.

Disc diffusion is a qualitative method. The recommended medium for this method is Mueller-Hinton agar. This medium demonstrates good batch-to-batch reproducibility, and supports the growth of most non-fastidious bacterial pathogens (Jorgensen and Turnrnidge, 2003). However, the researcher used the Well-variant of the diffusion method, which were considered as more sensitive and best conditions for the determination of the zone of inhibition. (Valgas *et al*., 2007).

The agar well diffusion method, using Mueller-Hinton agar plates, was employed to screen for antimicrobial activities. The *Staphylococcus aureus* which are the gram-positive *cocci; Escherichia coli* represents the gram-

negative bacilli; and the *Candida albicans* as a dimorphic fungus. From positive control, Streptomycin was used for *Staphylococcus aureus* and *Escherichia coli;* and Nystatin for *Candida albicans.* Ninety five percent (95%) alcohol was used as a negative control for test organisms. Subsequently, the inhibition zones formed around the agar well were measured in millimeter.

For accurate results, trial 1 and trial 2 was done for the extracted resins from the fruits of *Capsicum annuum var. longum*. The results for anti-microbial test were shown in **Tables 1, 2, and 3**. And **Plates 1, 2, and 3)**

5.2.6 Measurement of Zone of Diameter

The zone of inhibition was measured in millimeters with the use of a ruler under the plate after the incubation period. Results were interpreted based on the values given as susceptible, intermediate, or resistant as shown in **Appendix C** and **Fig. 4**.

5.2.7 Drug for Routine Susceptibility and Zone Diameter

Different antimicrobial agents have different effects on different organisms. Some organisms may be completely resistant to a specific antimicrobial while others are highly susceptible. The Kirby-Bauer or disk diffusion test is used to determine if an organism is susceptible or resistant to a selection of antimicrobial agents. When the test is run in a very specific manner, it can even be used to determine how susceptible an organism is to a specific antimicrobial agents. This is a very useful procedure when trying to determine a therapeutic course against a particular infection. It can also be used the test the efficacy of a new antimicrobial agents. The interpretation of the drug susceptibility and zone diameter is shown in **Appendix D.**

5.2.8 Computation for Percentage Yield

The percentage yield of the extracted resin resulting from maceration, percolation and reflux method was computed based on the actual yield of the resin extracted over the weight of sample multiplied by 100. Computation for all methods were shown on **Appendix E.**

5.2.9 Computation of Average Zone of Inhibition

To measure the effectiveness of the resin extractive as an antimicrobial agents, the average zone of inhibition were computed based on the two trials done for each bacterial culture plates on different concentrations. The computation of average Zone of Inhibition were shown in **Appendix F.**

RESULTS AND DISCUSSION

The results for the anti-microbial test show that the resin extract possesses antimicrobial activity, as summarized in **Tables 1, 2, and 3** and depicted in **Plates 1, 2, and 3**.

The highest significant antimicrobial activity was observed with *Candida albicans* with a zone of inhibition of 28mm on both trials at 300 mg/ml concentration against the standard which does not show inhibitory effect on higher concentration. For gram positive and gram negative bacteria, the standard larger zone of inhibition compared to the resin extract of different concentration. The extracted resin from the fruit of *Capsicum anuum var. longum* was found to be resistant to *E.coli* and *S. aureus as shown in* **Tables 1 and 2**) but found to be susceptible to *C.albicans as shown in* **Table 3.**

Table 1: Results obtained from Anti-microbial Test using *Escherichia coli*

Concentration (mg/mL)	Zone of Inhibition (mm)			
	T1	T2	(+)	(-)
100 mg/mL	0	0	26mm	0
200 mg/mL	0	0	27mm	0
300 mg/mL	7mm	5mm	25mm	0

Table 2: Results obtained from Anti-microbial Test using *Staphylococcus aureus*

Concentration (mg/mL)	Zone of Inhibition (mm)			
	T1	T2	(+)	(-)
100 mg/mL	9mm	10mm	20mm	0
200 mg/mL	11mm	14mm	24mm	0
300 mg/mL	9mm	15mm	24mm	0

Table 3: Results obtained from Anti-Microbial test using Candida albicans.

Concentration (mg/mL)	Zone of Inhibition (mm)			
	T1	T2	(+)	(-)
100 mg/mL	15mm	20mm	10mm	0
200 mg/mL	21mm	20mm	7mm	0
300 mg/mL	28mm	28mm	0	0

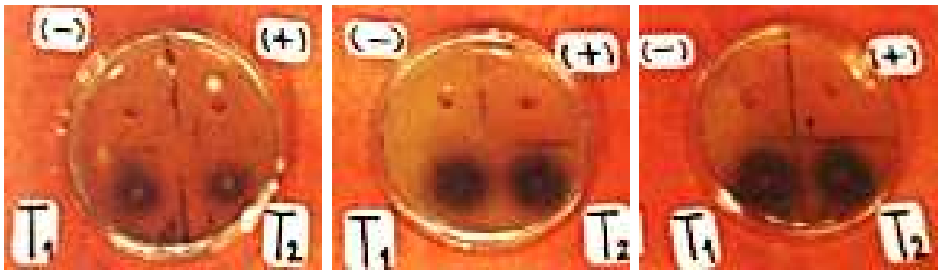

Plate 1:*Escherichia coli* plates showing the zone of inhibition with different concentrations

Plate 2:*Staphylococcus aureus* plates showing the zone of inhibition with different concentrations

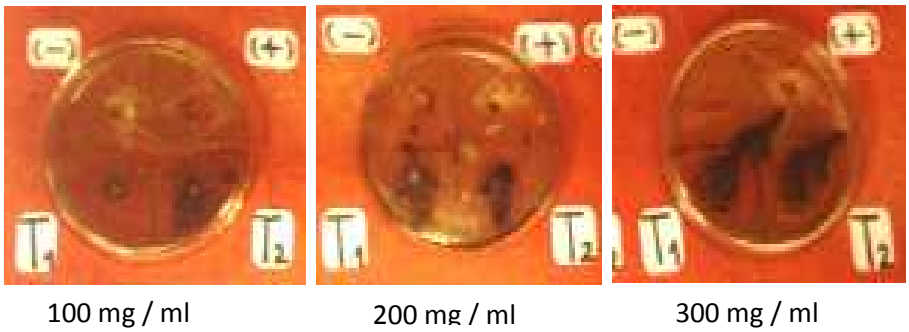

Plate 3: *Candida albicans* plates showing the zone of inhibition with different concentrations

CONCLUSION

Based on these aforementioned results, the researcher concluded that the extracted resin from the fruits of *Capsicum anuum var.longum* can destroy and kill fungi and, therefore, possesses antimicrobial property particularly as an antifungal. The current work can provide new reference data for the development of new drugs from natural plants that possesses the ability to inhibit fungi. Further studies, however, need to be done, using higher resin extract and other microorganisms, to determine the extent of the antimicrobial property of the resin extracts from the fruits of this plant.

8. Recommendations

Based on the results of this study, the researcher recommends the following:

1. It is recommended that the resin extract from the fruits of *Capsicum annuum var. longum be investigated further* to test its anti-microbial property.

2. The extraction and isolation of the resin constituents using different solvents and methods of extraction shouldalso be tried.
3. The cultivation and propagation of the *Capsicum annuum var. longum* fruit should be increased to make the fruits more accessible and available for experimental use.
4. Dosage formulation of *Capsicum annuum var. longum* fruit should be established.
5. A more sophisticated method of analysis should be tried such as the use of instruments like an infrared spectroscopy, to determine the functional group present in the fruit sample.
6. Higher concentrations of the resin extract should be used in the antimicrobial test.
7. Other microorganisms should be used to determine the antimicrobial property of resins from *Capsicum annuum var. longum.*

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APPENDICES

APPENDIX A

Preparation of Reagent used in the Study

Hydrochloric acid (1%). This was prepared by mixing twenty-three point six milligrams of HCL with water to make one liter.

Phloroglucinol Test Solution. About point five grams of phloroglacinol was dissolved in water to make one liter.
Resorcinol Solution. About one gram of resorcinol was dissolved with hydrochloric acid in sufficient water to make one hundred milliliters.

APPENDIX B

Preparation of Agar Used in Biological Test

Preparation of Media

1. Mueller Hinton Agar

Formula:	g/liter
Ingredients	300
Beef infusions	17
Case in Acid Hydrolyze	1.5
Starch	17

Final pH (at 35 degree Celsius) 7.4 0.2

Directions:
Suspend 38g in 1000ml distilled water. Boil to dissolved the medium completely. Sterilized by autoclaving at 15 lbs., pressure (121 degree Celsius) for 15 minutes. Mix well before pouring.

1. Sabourauds Dextrose Agar:

Formula:	
Ingredients	g/liter
Dextrose (glucose)	40
Peptone	10
Agar	15

Final pH 5.6 0.2 at 25 degree Celsius

Directions:
Suspend 65g in 1000 ml or deionized water and boil to dissolved completely. Sterilized at 121 – 124 degrees for 15 minutes.

Preparation of inoculum

Incubation condition

	Temp C	Time	Amount per ml
Escherichia coli	33 to 35	24 hrs	0.7
Staphylococcus aureus	32 to 35	24 hrs	0.05
Candida albicans	25 to 37	46-168 hrs	1.0

Procedure:
Inoculate from a recently grow stock culture of the organisms contained in surfaces of agar slant in 3 test tube. Spread the suspension evenly over the surface of the agar of sterile glass beads or by streaking platinum loop and inoculate at the specific temperature.

At the end of this period, prepare the stock solution by collecting the surface growth in about 10 ml of sterile saline T.S.

APPENDIX C

Measurement of Zone Diameter

Although zone of inhibition may be set as early as 16 to 18 hours after inoculation, the zone diameter are measured 24 to 36 hours to obtain complete growth of inhibition. Using a ruler, the zone of complete growth of inhibition around each other disks are measured to within the nearest millimeter of the disk in included in this measurement.

An interpretative correlate (susceptible, intermediate, or resistant) is provided by reference to publish guidelines (see below) Zones which fall into the intermediate range should be considered equivocal.

Zone Diameter Interpretative Standards

Resistant	Zone diameter (mm)
	Less than equal to 14
Intermediate	Between 15 and 18
Susceptible	Greater than equal to 19

APPENDIX D

Drug for Routine Susceptibility and Zone Diameter

Disk Symbol	Chemotherapeutic Agent	Disk Content	Resistant	Intermediate	Susceptible
P	Penicilin	10	11 or less	12-21	22 or more
PB	Polymyxin G	300	8 or less	9-11	12 or more
S	Streptomycin	10	11 or less	12-14	15 or more
G	Sulfathiozole	250	12 or less	13-16	17 or more
T	Sulfamethoxazole	250	10 or less	11-15	16 or more
T	Tetracycline	30	14 or less	15-18	19 or more
VA	Vancomycin	30	9 or less	10-11	12 or more

Interpretation:
Resistant – is meant that organism is not inhibited.
Intermediated – is meant that special consideration need to be followed of the agent is to be need.
Susceptible – is meant that the organism is inhibited by the antimicrobial at chemically attainable concentration.

APPENDIX E

Computation of Percentage Yield

The percentage yield of the resin extractive by maceration was calculated:

Data:

Weight of sample	=	100g
Weight of evaporated dish	=	48.5718g
Weight of evaporated dish + extract	=	53.1843g
Weight of resin extractive	=	4.6125g

$$\% \text{ yield of resin extractive} = \frac{\text{Weight of resin extractive}}{\text{Weight of sample}} \times 100$$

$$= \frac{4.6125\text{g}}{100\text{g}} \times 100$$

$$= 4.6125\%$$

The percentage yield of the resin extractive by reflux method was calculated:
Data:

Weight of sample	=	100g
Weight of evaporated dish	=	42.287g
Weight of evaporated dish + extract	=	44.652g
Weight of resin extractive	=	2.365g

$$\% \text{ yield of resin extractive} = \frac{\text{Weight of resin extractive}}{\text{Weight of sample}} \times 100$$

$$= \frac{2.365g}{100g} \times 100$$

= 2.365%

The percentage yield of the resin extractive by reflux method was calculated:
Data:

Weight of sample	=	100g
Weight of evaporated dish	=	48.5718g
Weight of evaporated dish + extract	=	51.3541g
Weight of resin extractive	=	2.7823g

$$\% \text{ yield of resin extractive} = \frac{\text{Weight of resin extractive}}{\text{Weight of sample}} \times 100$$

$$= \frac{2.7823g}{100g} \times 100$$

= 2.7823%

APPENDIX F
Computation of Average Zone of Inhibition
Escherichia coli
300 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1 + Trial 2}}{2}$$

$$= \frac{7mm + 5\ mm}{2}$$

$$= 6\ mm$$

Staphylococcus aureus

100 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1 + Trial 2}}{2}$$

$$= \frac{9\ mm + 10\ mm}{2}$$

$$= 9.5\ mm$$

200 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1 + Trial 2}}{2}$$

$$= \frac{11\ mm + 14\ mm}{2}$$

$$= 12.5\ mm$$

300 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1 + Trial 2}}{2}$$

$$= \frac{9\ mm + 15\ mm}{2}$$

$$= 12\ mm$$

Candida albicans
100 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1} + \text{Trial 2}}{2}$$

$$= \frac{15 \text{ mm} + 20 \text{ mm}}{2}$$

$$= 17.5 \text{ mm}$$

200 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1} + \text{Trial 2}}{2}$$

$$= \frac{21 \text{ mm} + 20 \text{ mm}}{2}$$

$$= 20.5 \text{ mm}$$

300 mg/ml

$$\text{Average zone of inhibition} = \frac{\text{Trial 1} + \text{Trial 2}}{2}$$

$$= \frac{26 \text{ mm} + 28 \text{ mm}}{2}$$

$$= 27 \text{ mm}$$