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IN-VITRO ANALYSIS AND ANTAGONISTIC PROPERTIES OF DIFFERENT KALACHUCHI (*PLUMERIAOBTUSA L.*) EXTRACTS AGAINST SELECTED HUMAN PATHOGENS

ANGELINA MENDOZA CAUZO*

* Ph.D. Science Professor, College of Education Nueva Ecija University of Science and Technology Cabanatuan City, Philippines

ABSTRACT

The medicinal use of *Plumeriaobtusa L.* or Kantutay as an inhibitor on the growth of human pathogens such *Escherichia coli, Corynebacteriumgenitalium* and *Staphylococcusaureus* was tested. Three treatments of leaves and barks of *Plumeriaobtusa L.* were prepared following scientific process.

Kalachuchi or *Plumeriaobtusa L.* leaf extracts, bark oil, and bark decoction reacted differently with the three selected human pathogens. Among the prepared treatments (except the control), the leaf extract produced the highest zone of inhibition though it is not significantly different from the bark oil and bark decoction. The implication said that the different treatment are not as effective as the control treatment. Moreover, the presence of zones of inhibition indicates that the materials could be used to inhibit the growth of the test organisms. The expected new steps in the field of health may consider the medicinal value of *Plumeriaobtusa L.* as a source of antibacterial drugs.

Keywords: ***Bacterial Seeding, Corynebacteriumgenitalium, McFarland Turbidity Standard, Plumeriaobtusa L., Staphylococcus aureus.***

INTRODUCTION

Many types of human health problems possibly originate from exposure to some pathogens such as bacteria, germs, and other disease causing agents. Skin disease for instance, colds and flu cases are possibly caused by air-borne bacteria while others were due to direct contacts with other surfaces infected with disease causing agents. In dealing with problems caused by different human pathogens, medicines and drugs are highly sought even if mostly are synthetics. Over the counter drugs are expensive, more so are those prescribed by doctors. In dealing with situation like this, an alternative medicine which can be found within the vicinity of houses in the community are highly sought and one of the most popular plant around is commonly named

Kantutay which scientifically termed as *Plumeriaobtusa L.* The pharmacological Actions of Plumeriaobtusa Linn. [4] are anti-bacterial, anti-fungal, anti-pyretic, cardiac tonic, diuretic, and Febrifuge.

As part of seemed to be normal situation, especially in remote barangays of Municipalities in the country, herbal medicines originated from plants are still the only source of medicine by less fortunate citizen. Plants have been the basis for medical treatments through much of human history. [1]While it is true that institutions of higher learning are in-charge of the training and educational growth and development of those who are enrolled in all its program offerings, it is also important to know that these institutions have duties and one of this is to assists the community members so they may survive in this time when medicines are really for the well privilege only. By subjecting Kantutay plant into test and finding out how effective it is in fighting three identified human pathogens, the community members living in selected barangays under the extension program of the University can be guided as to the helpfulness and effectivity of said plant in eradicating selected bacteria. [2]

THE PROBLEM

The study determined the antagonistic effects of Kalachuchi (*Plumeria obtuse L.*) leaves and bark extracts and oil against selected human pathogens such as *Corynebacteriumgenitalium*, *Escherichia coli*, and *Staphylococusaureus.*

The effects of the extracts were compared qualitatively and quantitatively utilizing simple Analysis of Variance.

METHODS AND PROCEDURE

Method of Research. True experimental research was applied in this study. Experimental research describes “what will be” when certain variables are carefully controlled or manipulated.[3]. It is the only method that can truly test hypothesis concerning cause and effect relationships.[5]

Materials. Alcohol lamp, Sterile funnel, Filter paper, Reagent bottles, Erlenmeyer flask, Petri dishes, Test tubes, Electric blender, Knife, casserole, Inoculation chamber, Inoculating needle, Denatured alcohol, 70-80% Alcohol, Cotton, Zonrox, Cultured media, Autoclave, Oven, Forceps, weighing scale, tap water, detergent power, sulfuric acid solution, Barium Chloride solution

Collection of *Plumeria obtuse L.* Sample Leaves and Barks.Kalachuchi (*Plumeriaobtusa L.*) leaves and barks were collected from a remote barangay in Cabanatuan City. The research made sure that the collected samples were free of any damage and healthy and they were departed from the tree using knife. The collected sample leaves and barks were rinsed using tap water and detergent powder to remove the adhering soil.

Surface Disinfection. Ethyl alcohol was sprayed on the sample leaves and barks of Kantutay (*Plumeriaobtusa L.*) to disinfect the plant parts that were used in the concentrated crude extract preparation. After which, the plant parts were chopped into pieces about 2-3cm long.

Extract Preparation. One hundred grams (100gm) of leaves and bark parts were used in preparing concentrated crude extract (CCE). The weighed plant materials were rinsed three times in distilled water.

Leaf Extract. The chopped and sterilized leaves were placed in a disinfectant electric blender. The blender ran for two (2) minutes until a homogenous mixture was formed and placed in a reagent bottle. The bottle was submerged into 80% ethyl alcohol with 1:2 dilution. The solution was filtered using sterilized funnel with double-layered sterile ordinary filter paper. The extracts were placed separately in tightly stoppered amber bottle and then refrigerated.

Bark Decoction. The sterilized bark was placed in a casserole with distilled water following the ratio of 500-mL water is to 1000 gram of *Plumeriaobtusa L.* barks. The mixture was heated for 30 minutes until the bark extract got concentrated in the distilled water.

Bark Oil. The sterilized bark was placed in an aluminum container and oil was added following the ratio 500-mL oil to 1000 gram plant barks. The aluminum container with sterilized bark and oil was placed in a steamer and again the steamer was placed on top of the casserole with distilled water in it. The mixture was heated for 30 minutes with the steamer fully covered until the bark got concentrated in the oil.

Table 1 shows the various treatments prepared in triplicates and applied to selected human pathogens.

Table 1: Kalachuchi (*Plumeriaobtusa L.*) Extracts on Selected Human Pathogens

Human Pathogens	Treatments	Identification
Escherichia coli	T I	Leaf Extract
	T II	Bark Decoction
	T III	Bark Oil
	T IV	Control (Amoxicillin)
Corynebacteriumgenitalium	T I	Leaf Extract
	T II	Bark Decoction
	T III	Bark Oil
	T IV	Control (Amoxicillin)
Staphylococcus aureus	T I	Leaf Extract
	T II	Bark Decoction
	T III	Bark Oil
	T IV	Control (Amoxicillin)

Bacterial Culture Preparation. Pure culture of *Escherichia Coli (E.coli.) Corynebacteriumgenitalium,* and *staphylococcus aureus* were obtained from Microbial Culture Collection and Testing Laboratory of PhilRice in Munoz, Nueva Ecija.

Procedure in Testing. Ten (10)mm of nutrient broth were dispensed to sterilized test tubes and sterilized in an autoclave at 15 psi for 15 minutes. A loopful of six bacterial cultures grown for 24 hours was separately inoculated on the prepared nutrient broth. The test tubes were incubated at ambient room temperature for 6 hours from 24 hours old cultures before turbidity were adjusted using 0.5 McFarland Turbidity Standard (MTS).

MacFarland Standard Preparation. Ninety nine point five (99.5) mL of 1% sulfuric acid (H_2SO_4) and 0.5mL of 1% Barium Chloride ($BaCl_2$) was prepared. The solutions were placed in a sterilized scre-capped test tubes and was used as the turbidity standard. The approximate standard cell was 1.5 x 10^8 cell/mL.

Seeding of Disc Extracts. Filter paper discs measuring 6mm were prepared by suing a puncher. They were placed in vials, sterilized in an autoclave at 15 psi for 30 minutes, oven-dried at 45^0 C for 2 hours, impregnated with the *Plumeriaobtusa L.* leaves and bark extracts and bark oil for 30 minutes, oven dried again at 45^0C for 1 hour. Paper discs for the negative control were soaked in distilled water and ethyl alcohol for 30 minutes and then the discs were oven-dried.

Nutrient agar (NA) was melted. Twenty-five (25) mL of it were poured into the plates aseptically and were allowed to solidify. Disposable syringe was used to transfer 0.1 [Rillion, 2003] prepared bacterial inoculum in broth into the plates containing solidified nutrient agar. The bacterial cells was evenly distributed to the entire nutrient agar surface using a sterilized aluminum L-rod. The oven-dried discs containing the extracts and oil were simultaneously seeded equidistantly and randomly with two control discs at the middle using separate sterilized forceps. Three (3) trials with three (3) replicates were prepared for each treatment.

The disc-seed plates were incubated for 18-24 hours at ambient room temperature before microbial effect is determined by means of identifying zones of inhibition. The diameter of the "zone" expressed in millimeter (mm) was measured every 12 hour interval.

Appearance of the zone of inhibition meant the effectiveness of Kalachuchi (*Plumeriaobtusa L.*) leaves, bark extracts, and bark oil as growth inhibitor of the three sample human pathogens. Any of the treatment or the treatment that displayed zone of inhibition was further used for the second part of the testing of *Plumeriaobtusa L.* leaves and bark extracts and bark oil as protectant and eradicant of the sample skin causing bacteria namely *Escherichia Coli (E.coli.) Corynebacteriumgenitalium,* and *Staphylococcus aureus.*

Test as Eradicant. Filter paper discs were prepared and were sterilized in an autoclave at 15 psi, oven-dried, and then soaked into the plant treatment. Paper discs for the negative controls will be soaked in distilled water and the other in 80% ethyl alcohol respectively for 30 minutes and then oven-dried. Bacterial inoculum was prepared and adjusted to 05 McFarland Turbidity Standard (MTS). The adjusted bacterial cultures were transferred to plated nutrient agar measuring 0.1mL using disposable syringe, then spread into the nutrient agar surface using sterilized aluminum L-rod and incubated for 24 hours at ambient room temperature in inverted position. The disc extracts were seeded onto the swabbed incubated plates. The disc-seeded plates were incubated again for 24 hours at ambient room temperature in inverted position. Zones of inhibition were observe within the identified time interval. Evidence of zone of inhibition meant that the extracts eradicated the bacteria.

STATISTICAL TREATMENT OF DATA

Split-plot Factorial in Randomized Complete Block Design (RCBD) was used to determine the difference among the three factors involved in the research at 5% level of significance with trials for every replicate. The three factors are the cultivars, the plant parts, and the time. For the significant result in the Analysis of Variance (ANOVA), Duncan's Multiple Range Tests was employed to determine which treatment combination have significant difference.

RESULTS AND DISCUSSION

The first trial involved the organism *Escherichia coli*. The prepared treatments from Kalachuchi (*Plumeriaobtusa L.*) extracts were applied together with the control treatment which the synthetic medicine is named amoxicillin. Table 2 shows the results of the trials.

Table 2: Effect of the *Plumeriaobtusa L.* Extracts on the Zone of Inhibition of *Escherichia coli* 12 Hours, 24 Hours, 36 Hours and 48 Hours After Application

Treatments	12 Hours		24 Hours		36 Hours		48 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
T I (Leaf)	8.6mm	8.7mm	8.4mm	8.3mm	8.2mm	8.2mm	7.8mm	7.9mm
T II (Bark Decoction)	8.6mm	8.5mm	8.3mm	8.3mm	8.1mm	8.1mm	7.6mm	7.6mm
T III (Bark oil)	8.6mm	8.5mm	8.3mm	8.2mm	8.1mm	8.2mm	8.1mm	8.0mm
T IV (control)	10.5mm	10.4mm	10.5mm	10.4mm	10.5mm	10.5mm	10.4mm	10.4mm

As revealed on Table 2, all the treatments shown zones of inhibition. An indication that all the extracts can control and inhibit the growth of organism Escherichia coli.

Table 3: Effect of the *Plumeriaobtusa L.* Extracts on the Zone of Inhibition of *Corynebacteriumgenitalium* 12 Hours, 24 Hours, 36 Hours and 48 Hours After Application

Treatments	12 Hours		24 Hours		36 Hours		48 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
T I (Leaf)	9.4mm	9.1mm	9.0mm	9.2mm	8.9mm	872mm	7.0mm	7.3mm
T II (Bark Decoction)	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition
T III (Bark oil)	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition
T IV (control)	18.9mm	18.9mm	17.8mm	17.6mm	15.0mm	15.4mm	13.04mm	13.2mm

Table 3 shows that Treatment I (leaf extracts) and Treatment IV (control) were the only treatments which produced zones of inhibition against organism *Corynebacteriumgenitalium*. The treatments which originated from barks of *Plumeriaobtusa L.* did not show any indication of potential to inhibit growth of bacteria. Furthermore the strength of Treatment I and IV in inhibiting the subject organism slowed down as it takes longer period of observation.

Table 4: Effect of the *Plumeriaobtusa L.* Extracts on the Zone of Inhibition of *Staphylococcus aureus* 12 Hours, 24 Hours, 36 Hours and 48 Hours After Application

Treatments	12 Hours		24 Hours		36 Hours		48 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
T I (Leaf)	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition
T II (Bark Decoction)	9.2mm	9.1mm	9.1mm	9.3mm	8.9mm	8.8mm	7.7mm	7.mm
T III (Bark oil)	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition	No Zone of Inhibition
T IV (control)	19.9mm	19.5mm	18.8mm	18.56mm	18.1mm	18.0mm	15.04mm	15.3mm

Table 4 shows that Treatment I1 or bark decoction and Treatment 4 (control) had produced zones of inhibition against *Staphylococus. aureus*. The other treatments, leaf and bark oil did not show any antagonistic effect. It is important to note that the effectivity of Treatment 1I (leaf extract) and the control diminished as the length of period of observation kept longer.

Using SPSS 11.5 output with significant value (<0.05) indicated differences among the four treatments applied to the three sample human pathogens. Tables 5 and Table 6 shows the results of the statistical analysis.

Table 5: ANOVA Summary Table on Effect of Four Treatments as Eradicants

TREATMENT	N	Subset for alpha = 0.5	Subset for alpha = 0.5
		1	2
Bark oil	24	2.7458	
Bark Decoction	24	5.1250	
Leaf Extract	24	5.6125	19.2125
Control	24		1.0000
Significance	0.098		

On Table 5 shows the ANOVA summary on the effects of the four prepared treatments as eradicants. Statistical test showed that there were significant differences among the treatments in the study. Comparison among means indicated that the control produced the highest zone of inhibition as compared to the other treatments. On the other hand, among the three prepared treatments, the leaf extract produced the highest zone of inhibition (5.6125mm) but it is not significantly different from the bark oil and bark decoction. This implies that although the different treatments were not as comparable as the control antibiotics, still the presence of zone of inhibition indicates that the sample extracts could be used to control or inhibit the growth of the test organisms.

Table 6: ANOVA Summary Table on Exhibited Zones of Inhibition of Escherichia *Coli (E.coli.)* *Corynebacteriumgenitalium,* and *Staphylococcus aureus.*

TREATMENT	**N**	**Subset for alpha = 0.5**
		1
Escherichia Coli	32	6.1906
Corynebacteriumgenitalium	32	8.7687
Staphylococcus aure	32	9.5625
Significance	0.138	

Table 6 shows another factor used in the study. Statistical test showed that as to the test organisms used, no significant difference was observed. This means that all the organisms were susceptible to the treatments.

Table 7: ANOVA Summary Table on Exhibited Zones of Inhibition of Escherichia *Coli (E.coli.) Corynebacteriumgenitalium,* and *staphylococcusaureus* in Relation to Time Intervals

TIME	N	Subset for alpha = 0.5
		1
48 Hours	24	7.6375
36 Hours	24	8.0667
24 Hours	24	8.3875
12 Hours	24	8.6042
Significance	0.730	

The above Table 7 shows the last factor used in the study. Statistical test showed that as to the time, no significant differences was observed. This result indicates that regardless of time, the effect of the test materials were the same.

CONCLUSIONS

1. The different extracts of *Plumeriaobtusa L.*namely leaf, bark decoction, and bark oil can be used as an anti-bacterial medicine.
2. Different *Plumeriaobtusa L.* extracts have different zones of inhibition on selected human pathogens. In *Escherichia coli* all of the treatments had shown zones of inhibition. In *Corynebacteriumgenitalium,* the only treatment that had shown zone of inhibition was the leaf extract. The only treatment that had shown zone of inhibition when applied to *Staphylococcus aureus* was the bark decoction.
3. The prepared treatments of *Plumeriaobtusa L.*were not as effective as the control antibiotics, but the presence of zones of inhibition indicate that the materials can be used to control or inhibit the growth of the selected human pathogens.

4. There will be a new step of progress in the fields of education and health in a way that knowledge about the medicinal values of *Plumeriaobtusa L.* and this will open doors for others plants to be studied and be used as based anti-bacterial medicine.

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