A COMPARATIVE STUDY OF THE ANTI-BACTERIAL EFFECTS OF AMPALAYA, BAWANG, BAYABAS, DUHAT AND IKMO ON *Staphylococcus aureus*

by

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This is to certify that this undergraduate thesis entitled " A Comparative Study of the Anti-bacterial Effects of Ampalaya, Bawang, Bayabas, Duhat and Ikmo on Staphylococcus aureus" and submitted by Lawrence S. Tinio, to fulfil part of the requirements for the degree of Bachelor of Science in Biology was submitted on September, 1988.

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TABLE OF CONTENTS

ABSTRACT

This thesis is a comparative study of the anti-bacterial effects of the extracts of ampalaya, bawang, bayabas, duhat and ikmo on Staphylococcus aureus. The herbs were cut, decocted and applied to cultures of S. aureus using the KIRBY-BAUER agar disc diffusion method. The zone of inhibition, being the criteria of anti-bacterial property, the most effective extract had been derived from bawang secondarily thereafter, from bayabas, ikmo, duhat and ampalaya, in that order.

INTRODUCTION

Staphylococcus aureus is one of the most common micro-organisms that cause skin diseases and infect wounds and abrasions. The proper safeguard against infection by the above-mentioned organisms and others similar to it would therefore, be of great value to men. Impetigo, furuncles, carbuncles, pemphigus neonatorum, staphylococcal scalded-skin syndrome (SSSS), exfoliation leading to fatal systemic involvement is attributed to Staphylococcus aureus.

Ikmo, bawang, bayabas, duhat and ampalaya are very common plants in our country and found almost in every backyard. Although they are often cultivated, these plants are abundantly available in our market places. Rural folks had long recognized the medicinal properties of these plants in the treatment of skin infections, a practice which unfortunately, many doctors scoff at.

The sky-rocketting prices of imported medicines and even those produced locally by multinational companies, had forced us to look back and seriously consider a cheaper but not necessarily inferior alternative - our much maligned medicinal plants. While several hundred species of medicinal plants are known and utilized for particular cure of specified ailments, very few of them are actually pharmaceutically prepared and prescribed by physicians. In the light of this seeming indifference, Garcia (1959), in his article, emphasized the sad fact that medical practitioners do not advocate the use of medicinal plants based on their claim that the medical value of many Philippine medicianal plants are based on hearsay or empirical observations by non-competent persons.

It is the aim of this thesis, to overcome the above-stated bias of medical practitioners by undertaking preliminary scientific studies on the effects of medicinal plants as anti-bacterial agents against Staphylococcus aureus. This paper will also seek to determine which of the given medicinal plants will be most effective when prepared under local herb mixture. Furthermore, recommendations will be included, to facilitate further studies or suggest future actions on the subject.

SIGNIFICANCE OF THIS RESEARCH:

This research may pave the way for medical accreditation of medicinal plants as antibiotic agents against Staphylococcus aureus. The effectivity of the plant extracts as anti-bacterial agents will be compared, to come up with the most potent, in low and high concentrations. At the same time, we will be able to test the effectivity of the mixtures used by the herbal practitioners and thus, determine if the herbal mixture is beneficial or not.

REVIEW OF LITERATURE:

THE MEDICINAL PLANTS

I K M O

Ikmo or Piper betle, is cultivated throughout the Philippines and can be found wild in most provinces of Luzon. Quisumbing (1978) described this plant as follows:

> "This plant is a dioecious, smooth, climbing vine, reaching a height of 2 to 4 meters. The upper leaves are usually oblong-elliptic, oblong ovate or ovate, 6 to 17.5 centimeters long, 3.5 to 10 centimeters wide, 7-plinerved, and smooth on both surfaces. The male spikes are sub-pendulous, slender, 7 to 13.5 centimeters long, 2 to 3.5 millimeters in diameter. The rachis is hairy. The two stamens are stalked, 0.75 to 1 millimeter long; and the anthers reniform. The female spikes, when mature, are red, fleshy, oblong to elongated oblong, 3 to 8 centimeters long, and 0.5 to 1 centimeter thick. The rachis is hairy, and the bracts stalkless, peltate, with a smooth disk, transversely oblong to suborbicular, and about 1 centimeter wide. The fruit is coaslescing, fully embedded in the pulp and concrescent with the rachis. The seeds are smooth, oblong to globose-obovoid, 2.25 to 2.6 millimeters long and about 2 millimeters in diameter. The stigmas number 4 to 6 and rarely, 3."

The chief constituent of the leaves is a volatile oil varying in the leaves from different countries and known as betel oil. It contains two phenols, betaphenol (Chavibetol) and chavicol. Cadinene has also been found, (Grieve, 1931 as cited by Quisumbing, 1978).

Nadkarni, (1927, as cited by Quisumbing, 1978) considered the chavicol as a powerful antiseptic, twice as strong and isomeric with eugenol. He said that the leaves also contain an alkaloid, arakene with properties allied to cocaine. The betel oil contains also, terpene and sesquiterpene.

Oncha (1987) adds that the volatile oil is from .9-1.8% of the leaves and that it is also with allyl procatechin, cineol, caryphyllene, cadine and menthone.

Quisumbing (1978) and Oncha (1987) note that Filipinos use the fresh, crushed leaves as an antiseptic for cuts and wounds and as a poultice for boils.

Betel leaves possess an antioxidant action (Sethi and Aggarwal, 1957) and Rimando (1985).

Krishnamurthi (1969, as cited by Rimando, 1985) theorizes that the presence of chavicol imparts an antiseptic characteristic to the leaf extract.

B A W A N G

Bawang, garlic or Allium sativum is rather extensively grown in Batangas and to a limited extent elsewhere in the Philippines. Nevertheless, it is very widely used by Filipinos as a spice to flavor dishes. Quisambing (1978) describes this plant as follows:

> "Garlic is a low herb, 30 to 60 centimeters high. The true stem is much reduced. The bulbs are broadly ovoid, 2 to 4 centimeters in diameter, and consist of several, densely crowded angular, truncated tubers. The leaves are linear and flat. The umbella are globose, nearly always with bulbs and with many flowers. The sepals are oblong, greenish white, or more or

less tinged with purple. The stamens are not exerted from the perianth."

Gildemaster (1916, as cited by Quisumbing, 1978) report that upon distillation of the entire plant, .005 to.009 per cent volatile oil was obtained. He quoted Semmler, who isolated a disulphide which upon examination is the bearer of the pure garlic odor.

Chopra (1946) said that the active principle of garlic is the volatile oil.

Reeds (1930, as cited by Quisumbimg, 1978) adds that the bulb contains alliin, allisin, allyl disulphide, allyl propyl- disulphide, inulin, choline, myrosinase. The leaves contain protein, 1.2% fat and 0.3% sulphide.

The garlic is reported by Bentley and Trimen (1980) and Nadkarni (1927, as cited by Quisambing, 1978) asstimulant , carminative and having the perspective of a vermifuge. Rico (1976) states that the anthelmintic action of garlic is due to its allyl disulphide content.

De Grosourdy (1949 as cited by Quismbing, 1978) wrote that garlic is considered a popular antiseptic. Guibourt (1964) considered the bulb as anthelmintic and prophylactic.

Fisher (1987) notes that garlic is reputed to have strong anti-bacterial properties and the probability is that this property is derived by its acting as an anti-oxidant, a defence mechanism against harmful substances called free radicals produced in our bodies and as a breakdown product of oxygen. He also quotes Eric Block, a Chemist at State University of New York at Albany, as saying that all supplements he tested contain little or none of the active ingredient. It seems, he added, that it is destryed in the processing stage.

A M P A L A Y A

Ampalaya, or Momordica charantia, is found throughout the Philippines. It is cultivated and is also thoroughly naturalized in thickets, waste places, at low and medium altitudes. It is pantropic in distribution. Quisumbing (1978) describes this plant as follows:

> "This vegetable is a climbing, nearly or quite smooth, annual vine. The simple tendrils are up to 20 centimeters long. The leaves are rounded, 2.5 to 10 centimeters long diametrically, cut nearly to the base into five or seven, oblong-ovate, variously toothed and lobed, and heart shaped at the base. The flowers are axillary, long-peduncled, and yellow. The male flower is about 12 millimeters long, and is peduncled, with a rounded, green and about 1 centimeter-long bract approximately at the middle. The female flower is similar to the male one, but long peduncled. The fruit in contrast, is oblong, cylindric, 15 to25 cm. in length, pointed at both ends, ribbed, and wrinkled; white in wild forms, it is ovoid, and 2to 4 centimeters long. The seeds are oblong compressed, 10 to 13 millimeters long, and corrugated on the margins."

Greshoff (1898, as cited by Quisumbing, 1978) reported way back in 1898 about the presence in the leaves of a bitter alkaloid and a glucoside. Peckolt (1904, as cited by Quisumbing, 1978) had isolated from the leaves and fruit a bitter principle - momordicin.

Rivera (1941) conducted a preliminary chemical investigation of the drug, Momordica charantia and as Oncha (1987 found out that it contains bitter principle, momordicin; sterol, charatin, iron, phosphorus, calcium and Vitamin B.

Salas (1945) observed that the extract of th fruit was used in Brazil for wound treatment. Morton (1966) stated

that in Central Venezuela, the roots, the over-ground plant seeds and fruit were used in folk medicine as cathartic, abortive, aprodisiac, analgesis, anti-pyretic, anti-rheumatic anti-gout, anti-diarrheal, vermifuge, anti-hemmoroidal, emetic, digestant, anti-ulcerogenic and anti-malarial.

Burkill(1935, as cited by Quisumbing, 1978) reported that it is enough and common just to pound the leaves and apply them to skin diseases in India, Malaya and elsewhere. Burkill and Haniff,(1930, as cited by Quisambing, 1978) stated that they are applied in cases of burns and scalds.

Lejano, (1976) attempted to isolate charatin from the fruit, but managed only to get .024% yield.

B A Y A B A S

Bayabas, or Psidium guajava, is found throughout the Philippines and is usually very common in thickets and secondary forests. It is pantropic in distribution. Again, Quisumbing (1978) desccibes this plant as follows:

> "This plant, which is somewhat hairy, reaches a height of 8 meters. The young branches are 4-angled. The leaves are opposite, oblong to elliptical, and 5 to 12 centrmeters long, the apex, being pointed and the base usually rounded. The peduncles are 1 to 3-flowered. The flowers are white, 3 to 3.5 centimeters across, solitary or two to three together. The fruit is rounded, ovoid or obovoid, 4 to 9 cm. long, and green, but yellowish when ripe, and contains many seeds embedded in aromtic, pink, edible pulp."

Bayabas is one of the most common and best known fruit in the Philippines. A wild tree, it grows abundantly in settled areas.

Wehmer (1931, as cited by Quisumbing, 1978) recorded that the leaves contain fixed oil - 6% and volatile oil-.365%.

The essential oil contains eugenol, mallic acid, and tannin 8 to 15%. The fruit contains "glykosen" 4.14 to 4.3%, Saccharose 1.62 to 3.4%, protein .3%, etc; and the ash yields 75% of $CaCO_3$. The bark contains 12 to 30% of tannin.

Oncha (1987) noted that guava contains fixed oil 6%, volatile oil .365%, eugenol, beta-caryophyllene, cineote, benzaldehyde, limonene, tannin 8-15%.

Quisumbing (1978) says that the leaves, in the form of a decoction, are used for washing ulcers and wounds. Oncha (1987) added that the decoction of unripe fruit as wash or poultice on affected parts is a treatment for skin ulcers. This is also confirmee by Nadkarni (1927, as cited by Quisumbing, 1978) and Medina (1959).

D U H A T

Duhat, or Syzygium cumini, is found throughout the Philippines, either planted or spontaneous in growth. Quisumbing (1978) gives the following description:

> " This is a smooth tree 4 to 5 meters in height. The leaves are leathery, oblong-ovate to elliptical or obovate-elliptic, and 6to12 centimeters long, the tip being broad and shortly pointed. The panicles are borne mostly from the branchlets below the leaves, often being axillary or terminal, and are 4 to 6 centimeters long. The flowers are numerous, scented, pink or nearly white, without stalks, and borne in crowded fascicles on the ends of the branchlets. The calyx is funnel-shaped, about 4 millimeters long and 4-toothed. The petals cohers and fall all together as a small disk. The stamens are very numerous and about as long as the calyx. The

> fruit is oval to elliptic, 1.5 to 3.5 cm. long, dark-purple or nearly black, luscious, fleshy and edible; it contains a single large seed."

Wehmer (1931, as cited by Quisumbing, 1978) records that early chemical studies showed the presence of tannin (19%) and gallic acid (1.67%) in the bark.

Bacquillon-Limosin (1895, as cited by Quisumbing, 1978) quoted Gerard, who has isolated a crystalline substance and named it jambosine ($C_{10}H_{15}AZO_3$).

Power and Callan (1912, as cited by Quisumbing, 1978) subjected the plant seeds to more critical analysis which showed no alkaloid or enzyme, but revealed a considerable amount of gallic and tannic acids, together with sugar which yielded d-phenyl-glucosazone and a small amount of a phenolic substance, which was obtained in much larger quantity from the resin. This phenolic substance is named jambulol. Hart and Heyl (1916, as cited by Oncha, 1987) obtained the same results.

Duhat also contains anti-mellin, olein, linolein, palmitin, stearin, phytosterin, myricyl alcohol, hentriacontane (Oncha, 1987).

Duhat is currently under investigation for its anti-diabetic properties, but in Oncha (1987), it was mentioned in passing, that it is also used for the treatment of wounds using the decoction of the bark.

Very little investigation is done on its anti-bacterial or anti-fungal properties and only through Oncha that this medical property was mentioned. Goodman (1965) classifies phenols as potent antiseotics and since duhat has a good amount of jambulol, it should prove to be a good anti-bacterial agent

THE MICROORGANISM : Staphylococcus aureus

Staphylococcus aureus is an ubiquitous pathogenic microorganism and is the most common cause of localized supperative infections and is among the longest recognized of the pathogenic bacteria. It has been charactrized in the early 1880's largely through the work of Rosenbach (Elek, 1959). Cruickshank (1973) describes this microbe as follows:

> "The coccus form tends to be more uniform in size than the other morphological types of bacteria and the other staphylococci are constantly slightly less than 1 micrometer in diameter and typically are more nearly perfect spheres than many cocci. Their most obvious morphological characteristics is their marked tendency to occur as masses of cells. This is a consequence of cell division occurring in three planes, coupled with a tendency of the daughter cells to remain in close proximity to give the characteristic appearance. These irregular clusters are three-dimentional: this isapparent on examination of wet-mounts, but in the usual stained smear preparations, the clusters are flattened out to give the appearance of irregular sheets of cells.While some cells may be found singly, in pairs, or even very short chains, this charcteristic morphology serves to identify The staphylococci except under special circumstances: e.g.,it would be extemely difficult to differentiate staphylococci and streptococci admixed in a smear. These bacteria stain readily and deeply with the usual basic dyes in the simple staining procedure and are strongly gram-positive. They do not form spores, apparently do not have capsulesexcept in the case ofrare mucoid variants, and although motile forms have been described are mostly invariably non-motile."

This microbe is attributed to causing several diseases. They are:

1. Superficial infections: skin pustules, boils, carbuncles, impetigo, pemphigus neonatorum, sycosis barbae, paronychia, styes, blepharitis, and conjunctivities; infections of accidental and surgical wounds and burns.

2. Subcutaneous and submucus abscesses;

3. Osteomyelitis, broncho-pneumonia, particularly post-influenzal and pyelo-nephritis;

4. Lymphangitis, lymphademitis, bacteriaemia, septicaemia, pyaemia, and acute bacterial endocarditis;

5. Staphylococcal food poisoning (Cruickshank,1973)

Staphyloccocci aureus is relatively more resistant to heat and to a certain extent , disinfectants, than the vegetative forms of most pathogenic bacteria (Burrows, 1975). Sensitive strains of S. aureus are susceptible to the anti-bacterial activity of the sulfonamides, penicillin, the tetracycline, chloroamphenicol, erythromycin and other anti-biotics active on gram positive bacteria (Burrows, 1975) and (Brock, 1974).

Burrows (1975) notes that they are especially prone to develop drug resistance. The increasing frequency of multiple resistance in hospital-acquired strains has led to a changed therapeutic strategy. He adds that resistance to new anti-biotics develops rapidly as they come to use.

Topical disinfectants are now recommended, rather than anti-biotics, for the treatment of accessible skin infections (Norton, 1981). Burrows (1975) said that when the atrain is sensitive to an available chemotherapeutic agent, chemotherapy of staphylococcus bacteremia is strikingly successful.

M E T H O D O L O G Y

MATERIALS:

1. Autoclaver
2. Bunsen Burner
3. 3# Pipettes (100 ml.)
4. 3# Pipettes (1 ml.)
5. 7# Petri Dishes
6. 7# Erlenmeyer Flask (200 ml.)
7. 7# Beakers (200 ml.)
8. Filter Paper
9. Forceps
10. Magnifying Glass
11. Micrometer Ruler
12. Nutrient Agar (50 gr.)
13. Nutrient Broth (30 gr.)
14. Inoculating Needle
15. Glass Marker

METHODOLOGY:

I. Preparation of Test Culture

A. Obtain a stock culture of S. aureus

B. With an inoculating needle, transfer a considerable amount of S. aureus into a test tube of nutrient broth and incubate for 24 hours. (see Appendix I)

C. Autoclave the clean Petri dish along with the nutrient agar in an Erlenmeyer flask which is properly wrapped with paper and plugged with a cotton stopper.

D. Pour the nutrient agar (see Appendix II) with the aid of a sterile pipette into the Petri dishes.

E. Allow to solidify on a level surface.

F. Prepare four cubic centimeters of seeded nutrient agar, by suspending 2 cubic centimeters of the S. aureus from the 24 hr. broth culture in 100 ml. melted nutrient agar at 45°C.

G. Distribute this evenly on the surface of the solidified agar in the dish.

H. Incubate at 37°C for 45 minutes.

II. Preparation of the Extracts

A. AMPALAYA

1. Obtain clean, healthy ampalaya leaves and cut them into small pieces.
2. Weigh 5, 10, and 20 grams of the leaves
3. Mix them separately in beakers with 100 ml. of distilled water.
4. Boil for 15 minutes and let stand for an hour, with ocassional stirring.
5. Filter the extract into the Erlenmeyer flask and use immediately in testing.

B. BAYABAS

Repeat the instructions above-enumerated but instead of Ampalaya leaves, use fresh Bayabas leaves.

C. DUHAT

Repeat the instructions above for Ampala- but replace the leaf with Duhat bark.

D. BAWANG

Follow the instructions for Ampalaya, except that now, utilize the bulb of Bawang to prepare the extracts.

E. IKMO

Follow the instructions for Ampalaya, but this time, use crushed Ikmo leaves before boiling, to achieve the extract.

RESULTS AND OBSERVATIONS:

After carefully following the methodology presented hereinbefore, I came up with the following results:

Bawang has the widest zone of inhibition with the mean of 7.58 mm. after two trials. It is also the most potent anti-bacterial agent at low and high ..... concentrations. Bayabas produced a zone of inhibition with a mean of 6.88 mm. to rank second. Its zone of inhibition is complete. Ikmo follws, with its zone of inhibition averaging 6.29 mm. Duhat comes in fourth with the zone of inhibition having a mean of 5.46. Finally, Ampalaya has a zone of inhibition with a 4.98 mean. Its zone of inhibition is only partial, meaning that some S. aureus colonies still exist within the zone of inhibition.

In analyzing the data, using the factorial ANOVA design (see Appendix II), the following has been arrived at:

1. There is a significant difference between the results of the treatments.
2. There is a significant difference between the results of the treatments - meaning that the higher the concentration, the more effective is the inhibitory action.

3. There is a significant difference between the results of the interaction - meaning that not all the types of treatment produced the same increase in anti-bacterial action as the concentration is increased.

DISCUSSION:

Judging from the results presented, we can readily state that the anti-bacterial properties of the plant extracts in decreasing order are bawang, bayabas, ikmo and ampalaya. I also used three controls: water, isopropyl alcohol and mercurocrome.

The first control - water - served as the negative control. Since this water (distilled) was used as the medium in decocting the plants and in the preparation of the nutrient agar and nutrient broth, testing it for any inherent anti-bacterial properties is necessary, if the study conducted is to remain credible. As shown in the data obtained, water causes no inhibition and therefore, eliminating one source of error and produces the possibility that the medium has, in itself, anti-bacterial properties.

The second control - isopropyl alcohol - serves as a positive control. Isopropyl alcohol is a widely used disinfectant. Its mode of action is the coagulation of cellular protein. As shown in the results and observations, alcohol (at 70%) exhibits only a mild anti-bacterial property, since it showed a zone of inhibition underneath. It would be immediately noticeable that 3 out of 5 plant extracts, namely bawang, bayabas and ikmo, managed to out perform this control, considering the concentrations of the latter is 4 to 14 times more than the plant extracts (70% compared to 5, 10, and 20%).

The third control - mercurocrome - proved to be a very powerful anti-bacterial agent. It registered the largest zone of inhibition, clearly showing that the plant extracts still need some improvements to be at par with the commercial antiseptics. Mercurocrome has a number of disadvantages, one of which is a mercury content, which is regarded as poisonous by most physicians.

Among the plant extracts, bawang or garlic, has the most potent anti-bacterial property. Its area of inhibition is complete. There are theories that garlic has a strong anti-bacterial property derived from its acting as an anti-oxidant, a defense mechanism against harmful substances called free radicals, produced in our bodies and a breakdown product of oxygen. It is not yet fully understood as to which compound this property of garlic can be attributed to, but it is believed that the volatile oil content of garlic is responsible for this.

The second most potent anti-bacterial plant extract is that of bayabas or guava. Its area of inhibition, like the garlic, is complete. I believe that the anti-bacterial action of guava is mainly derived from the phenol and phenol derivative contents. P. guajava contains up to 8-15% tannin and engenol, an aromatic phenol. Tannins form insoluble compounds with other metallic salts, with alkaloids, with proteins etc. This precipitation leads to astringent actions and antiseptics, the latter derived chiefly by depriving the bacteria of food. However, tannin is non-toxic, except when applied to burns or parenterally.

Next, is Ikmo or betel leaf, which contains the third most potent anti-bacterial extract. Like the first two extracts, the zone of inhibition of P. betle is complete.

This bacteriacidal property can be attributed to two phenols, betaphenol (chavibetol) and chavicol. Chavicol, which is relatively more abundant in leaves, is a powerful antiseptic, twice as powerful and isomeric with engenol.

Fourth, is duhat or S. cumini, with a complete zone of inhibition. This can be attributed to a number of substances found in duhat. First, is the large amount of tannin, 19%, which as I have discussed earlier, has astringent and antiseptic actions. Next is garlic acid (1.63%) which has a reputed astringent effect, but other authors would like to disagree with this theory. They point out that the said property is brought out by a small quantity of tannin which is usually present. And lastly, a phenotic substance known as jambulol, which is a potent antiseptic, that exists in large quantity in the resin.

Finally, ampalaya produced the weakest anti-bacterial action against all tested extracts. Its zone of inhibition is only partial. This mild inhibitory effect can be attributed to the weak anti-bacterial of momordicin, the bitter principle. A cursory examination of its other components, quickly discounts any other substances from contributing to this anti-bacterial property.

The study conducted by the author has several limitations and such being the condition, a degree of error may have unintentionally altered some of the results in this experiment. First, the temperature in which the S. aureus culture and the test culture were incubated, tended to fluctuate. This is attributed to the inavailability of proper facilities at the time this thesis was conducted. In effect, the optimum growth temperature of the organizms was not maintained. Next, is the measurement of the zone of inhibition. There is an irregularity in the border of

the zone of inhibition. This forced the author to get the average of the extremes, creating some degree of error. Finally, human error and instrumental error cannot be discounted, for these can also provide a substantial degree of error.

CONCLUSION:

The results and the data on hand has led me to conclude that Allium sativum or bawang produced the most potent antibacterial action among the five plant extract tested. Psidium guajava or bayabas, Piper betle or ikmo, Syzygium cumini or duhat and Momordica charantia or ampalaya follws respectively. Allium sativum, Psidium guajava and Piper betle have strong antibacterial properties and their use as antiseptics has justification. Syzygium cumini exhibits only a mild anti-bacterial action and use of this plant as an antiseptic or an anti-bacterial or anti-staphylococcal agent is not recommended. Use of Momordica charantia as an antiseptic or as an agent against S. aureus must be discouraged if not completely stopped.

RECOMMENDATIONS:

1. Chemical assay of the various components of the plant extract, especially that of garlic, necessary, to fully understand its effects.

2. In vivo experiment of the plant extracts must be done, before actual accreditation for human use.

3. Further refinement of the methodology, to produce more potent extracts, is imperative.

4. Stop the use of duhat bark and ampalaya leaves/ fruits as antiseptic as actually practiced by herbalists.

5. Plant extracts should be applied to other bacteria and fungi strains, to see if they are also useful in fighting other micro-organisms.

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LIST OF PLATES

LEGEND

S ----- *Staphylococcus aureus* colonies
F ----- filter paper disk
A ----- 5% concentration
B ----- 10% concentration
C ----- 20% concentration
Z ----- zone of inhibition
W ----- water
I ----- isopropyl alcohol
M ----- mercurochrome

PLATE 1

Piper betle, test plate

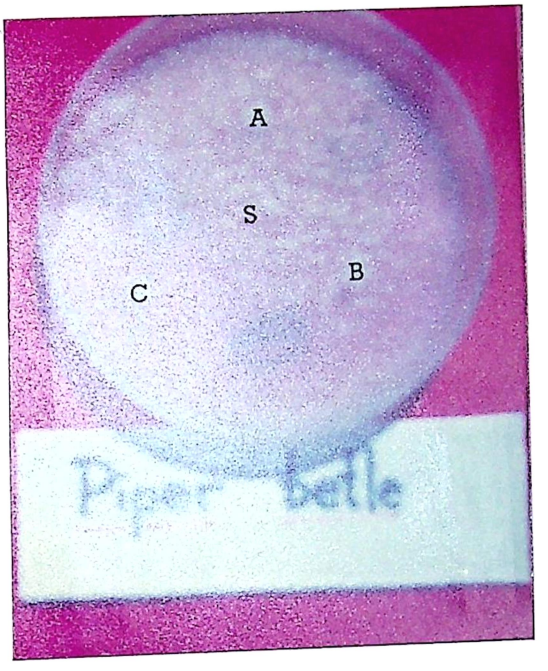

PLATE 2

Allium sativum, test plate

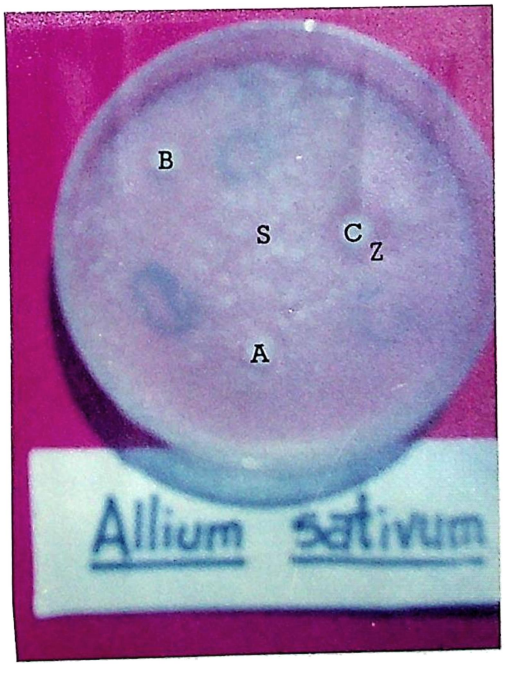

PLATE 3

Psidium guajava, test plate

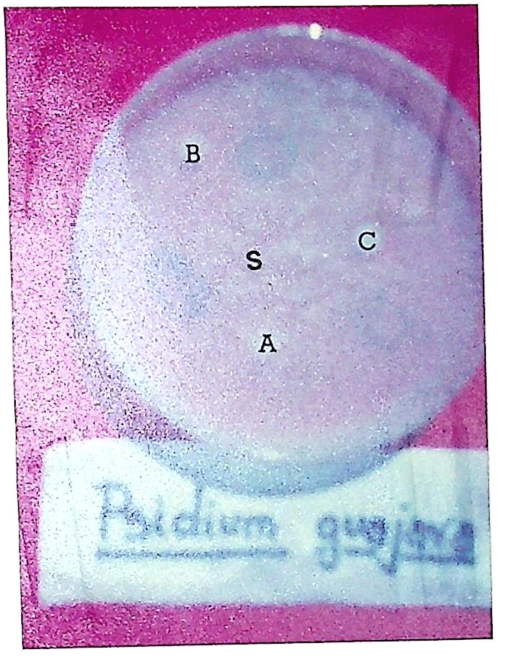

PLATE 4

Syzygium cumini, test plate

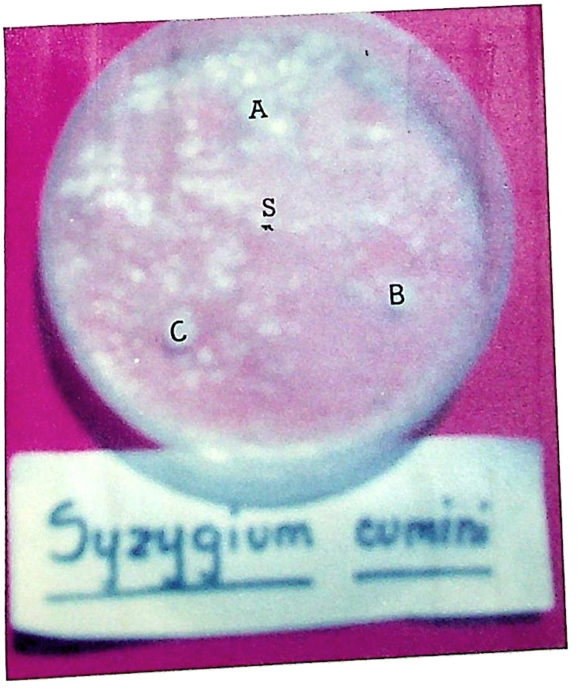

PLATE 5

Momordica charantia, test plate

PLATE 6

Control test plate

APPENDIX " 1 "

NUTRIENT AGAR

Meat Extract	5.0 gr.
Peptone from Meat Extract	3.0 gr.
Agar-Agar	12.0 gr.
Distilled Water	1 liter
pH of Prepared Solution	7.0 ± 0.2

NUTRIENT BROTH

Meat Extract	5.0 gr.
Peptone from Meat Extract	3.0 gr.
Distilled Water	1 liter
pH of Prepared Solution	7.0 ± 0.2

Note: Both are available in dehydrated form

ANALYSIS OF THE DATA USING
3×5 FACTORIAL ANOVA 3 WAY DESIGN:

CONCENTRATION

TREATMENT	5 %	10 %	20 %	TOTAL	MEAN
AMPALAYA	5.0 4.5	5.0 5.0	5.0 5.0	29.5	4.92
BAWANG	6.25 6.0	7.5 7.0	10.0 8.75	45.5	7.58
BAYABAS	6.0 6.0	6.75 7.0	8.0 7.5	41.25	6.88
DUHAT	5.0 5.0	5.5 5.75	5.5 6.0	32.75	5.46
IKMO	5.5 6.0	6.0 6.0	7.25 7.0	37.75	6.29
TOTAL	55.25	61.5	70.0	186.75	
MEAN	5.53	6.15	7.0		