PHILIPPINE SCIENCE HIGH SCHOOL WESTERN VISAYAS
Dona Lawa-an H. Lopez Campus
Iloilo City

COMPARATIVE STUDY ON THE ANTIBACTERIAL EFFECTS OF LOCAL HERBAL LEAF EXTRACTS AND ALCOHOL ON *Eschirichia coli*

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Science Research II

by

Abe Jun F. Clarito
Gynette Pearl M. Templonuevo

February 2000

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APPROVAL SHEET

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Abstract

Experiments were conducted to determine if the leaf extracts of aloe vera, hierba buena, kamote, and alcohol have antibacterial effects on *Eschirichia coli*. It was hypothesized that there exist no significant differences in the antibacterial effects of aloe vera, hierba buena, kamote and alcohol on *E. coli*. Filter paper discs were soaked in the leaf extracts and in alcohol. Then these discs were applied to the culture plates, which were incubated within 15 minutes aerobically and without carbon dioxide for 24 hours. The results were analyzed using the One Way Analysis of Variance (ANOVA) as inferential statistical tool was set at 0.05 alpha level of significance. It was found out that there are no significant differences in the antibacterial activity of aloe vera, hierba buena, kamote and alcohol upon *E. coli*.

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COMPARATIVE STUDY ON THE ANTIBACTERIAL EFFECTS OF LOCAL HERBAL LEAF EXTRACTS AND ALCOHOL ON *Eschirichia coli*

Chapter 1

Introduction to the Study

Background of the Study

The Philippines, being a tropical country, is rich in natural resources. Different species of flora, especially herbs, are known to exist in the wild and in some backyard gardens. Among Filipinos, certain plants in their immediate environment have been known to have some curative values (Quisumbing , 1987).

Although herbs have been used to treat a variety of ailments, reliable information on herbal medicine has been hard to come by. In relation to this, the researchers decided to conduct a study on the antibacterial activity of aloe vera (Aloe vera L.), hierba buena (*Mentha x cordiopolia* Opiz.), and kamote (*Ipomoea batatas* (L.) Lank.) on *Eschirichia coli*.

By using the leaf extracts of these herbs, the researchers determined if antibacterial activity is eminent among these herbs on the test organisms.

The relationship between the variables in the study was presented in Figure 1.

Statement of the Problem and the Hypotheses

This study was conducted in order to determine the antibacterial effects, of the leaf extracts of aloe vera, hierba buena, kamote and alcohol on *Eschirichia coli*.

Specifically, it sought answers to the following questions:

1. Do (a) aloe vera, (b) hierba buena, (c) kamote, and (d) alcohol have antibacterial effects on E. coli?

2. Is there a significant difference on the antibacterial effects of (a) aloe vera, (b) hierba buena, (c) kamote, and (d) alcohol on *E. coli*?

Based on the questions presented, it was hypothesized that:

1. There is no significant difference in the antibacterial effects of (a) aloe vera, (b) hierba buena, (c) kamote, and (d) alcohol on *E. coli*.

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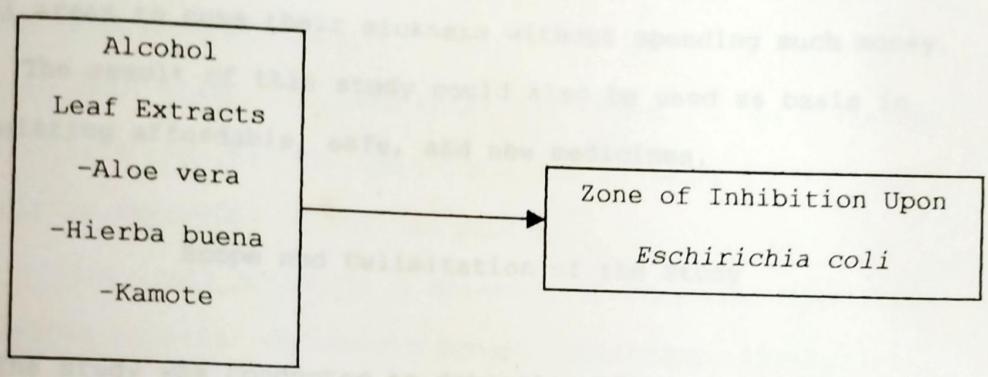

Figure 1. Antibacterial effects of the different leaf extracts on *E. coli*.

Significance of the Study

With the continuing economic crisis and the rising costs of basic commodities, many people, especially those living in the rural areas, could not afford to buy commercial drugs or medicines. The results of this study could help people living in rural areas to cure their sickness without spending much money.

The result of this study could also be used as basis in formulating affordable, safe, and new medicines.

Scope and Delimitation of the Study

The study was conducted to determine if the leaf extracts of aloe vera, hierba buena, and kamote have antibacterial activity on *Eschirichia coli*. The leaf extracts were extracted by boiling. The plant leaves were taken from Antique while the test organism was taken from the University of San Agustin.

The study was conducted from January to February at the Science Research Laboratory of Philippine Science High School Western Visayas.

The plants whose leaves were used were aloe vera, hierba buena and kamote while the controls were distilled water and 80% ethyl alcohol.

Definition of Terms

For the purpose of clarity and single-mindedness, the following terms were given their conceptual and operational meanings:

[Antibacterial] effect- the quality or state of being active (Webster's Dictionary, 1986).

In this study, the term meant the effect of the different leaf extracts against the test organisms in terms of the zone of inhibition exhibited in culture plates.

[Crude leaf] extracts- a solution of essential constituents of complex material (Webster's School Dictionary, 1994).
In this study, the term meant leaf extracts of herbs obtained by boiling.

Herbs- are annual, biennial, or perennial plants that does not develop persistent woody tissue but dies down at the end of a growing season (Webster's School Dictionary).

In this study, the term referred to the kind of plants used. These plants were aloe vera, hierba buena and kamote.

Eschirichia coli- is a Gram-negative, rod-shaped bacterium used for testing the antibacterial activity of herbs as mentioned by the resulting zone of inhibition in culture plates.

In this study, the term meant the healing properties of herbs in terms of antibacterial activity.

[Zone of] inhibition- the act of inhibiting (Webster's School Dictionary, 1994).

In this study, the term meant the area in culture plates around the antibacterial discs.

Chapter 2

Review of Related Literature

Medicinal Properties of Common Herbs

Aloe vera Linn.

Sabila or aloe is a herbaceous plant introduced from Africa, whose stems grow from thirty to forty centimeters in height. The leaves are fleshy, mucilaginous and succulent, twenty to fifty centimeters long, five to eight centimeters wide. It gradually narrows from the base, pale green in color, irregular, white-blotched, and the margins having weak prickles. The inflorescence is erect, and usually twice the height of the plant. The flowers are from two to three centimeters long, yellow, with segments about equaling the oblong tube (Quisumbing, 1978).

It is common knowledge that aloe vera is used for a variety of purposes. The Filipino women, to prevent falling of hair and cure baldness, usually mix gogo with the juice of the fleshy leaves. It is also used to treat burns and as a purgative (Quisumbing).

According to de Sta. Maria (1885, in Quisumbing), its juice with milk cures dysentery and pains in the kidney. Guerrero (in Quisumbing, 1978) reports that the leaves are used by Filipino herbalists to poultice edema of beriberi patients.

The alcohol tincture of this inpissated juice is used in India and in the Antilles to cure bruises or contusions and eechymosis.

Ipomoea batatas (L.) Lank.

Sweet potato or kamote is cultivated widely in temperate and tropical regions worldwide (Grolier's Encyclopedia, 1995). It is a spreading prostate, herbaceous, glabrous, or somewhat hairy vine from small or large, red, white or yellow tubers. Its leaves are ovate to oblong-ovate, six to fourteen centimeters long subentire, angular and lobed, acuminate and basecordate. Peduncles are axillary, about as long as petioles, erect, and several flowered. Sepals are about one centimeter long, greenish in color, and the corolla is campanulate, four to five centimeters long, and pink-purple and whitish in color (de Padua et al., 1998).

It usually grows from planting sprouts or cuttings from kamote. Crude leaf extracts have been found out to have anticancer activity. Leaf infusion is antidiarrhetic, root

decoction is vermifuge and is also a remedy for toothache, and is said to be purgative (de Padua et al.).

Its root sprouts or cuttings could grow into stems of not less than three meters. It is usually grown for its large edible roots, which is rich in Vitamin A, that when properly stored could remain edible for six to seven months (Grolier's Encyclopedia).

Mentha x cordiopolia Opiz.

Hierba buena, also known as peppermint, is widely scattered in cultivation in the Philippines having introduced by the Spaniards. It is a native of Europe, and is now cultivated or naturalized in most countries (Govil and Sugh, 1993).

The plant is prostrate, much branched, with stems growing up to forty centimeters long, and the ultimate branches ascending. The leaves are elliptic to oblong-ovate, one and one-half to four centimeters long, short-stalked, toothed in the margins and rounded or blunt-tipped. The flowers are borne in axillary, head-like whorls. The calyx teeth are triangular to lanceolate, and hairy, the corolla also being hairy (Quisumbing, 1978).

In the Philippines, the peppermint oil is largely used in pharmaceutical preparations to remove or diminish the unpleasant taste of drugs. It is also domestically used as a flavoring in confections and mouthwash preparations. The mint is also a valuable remedy for neuralgic affections, renal and eye calculus, weakness of the stomach, diarrhea, vomiting, and asthma. It is also recognized as an antiseptic and anaesthetic. The pounded leaves are used as antidote for stings of poisonous insects and as remedy for indigestion (Govil and Sugh).

Dymock, Warden and Hooper (1980, in Quisumbing, 1978) recommended an alcoholic or ethereal as local anaesthetic in affections of the nose, pharynx and larynx. The leaves and stems are infused, used as carminative, and antispasmodic and a sudorific. Menthol produces local anesthesia in cases of headache and facial neuralgia. Its decoction, vapor from the former, is largely used with lemon grass as febrifuge. It is also given in hiccups. Kirkitar and Basu (1918, in Quisumbing, 1978) add that the plant has emmenagogue properties. It is used in jaundice. The powdered, dried plant is used as dentrifice.

Description of the Test Organism

Eschirichia coli

E. coli is a Gram-negative, rod-shaped bacterium with flagella, fimbriae and in some strains, pili. It does not form spores or any form of differentiation during or after growth. Nor does it produce secondary metabolites in any quantity. As might be expected from its normal habitat, it is a heterotroph, which grows by breaking down complex organic molecules, and a facultative anaerobe, that is, it can grow in the absence of oxygen by fermentation. Because of its ease of cultivation, *E. coli* is readily isolated from natural resources. It can be obtained nearly pure in one step on simple media and was thus isolated and described early in 1885 (Kendrew and Lawrence, 1994)

It is one of the predominant facultative anaerobic bacteria of the intestinal tract and so is used as an indicator organism in determining the amount of local fecal contamination in water and food. Many strains are used in experimental work in cell research. Much of the current work in molecular biology uses *E. coli* because more is probably known about this organism than any other bacterium. Certain strains of *E. coli,* called *Enterotoxigenic E. coli* (ETEC), is the most frequent cause of

infectious diarrhea, often referred to as "traveler's diarrhea," considered one of the nuisances of international travel. Other infections that are commonly due to *E. coli* include urinary tract infections, bacteremia, and neonatal meningitis (Jensen and Wright, 1993).

Chapter 3

Research Design and Methodology

This study determined the antibacterial effect of the leaf extracts of aloe vera, hierba buena, and kamote on *E. coli*.

It was hypothesized that there was no significant difference in the antibacterial effects of aloe vera, hierba buena, and kamote on *E. coli* and when compared with each other and alcohol.

Methods

Collection of Specimens

Collection of test organism. The strains of *Eschirichia coli* were taken from the University of San Agustin.

Collection of leaf specimens. The leaves of aloe vera, hierba buena, and kamote were taken from Antique. They were washed thoroughly after gathering and were brought to the laboratory immediately. The leaves will be thoroughly washed with running water and rinsed with distilled water again at the laboratory.

Sterilization of the Equipment

All the equipment were thoroughly washed with soap and water, sterilized in the autoclave at 120 degrees Centigrade or 15 psi pressure for 30 minutes.

Preparation of Leaf Extracts

The wet weight was determined by using the weighing scale, and 200 grams. of each species of leaves were cut into pieces. Each species was boiled in a flask containing 300 ml of 80% ethyl alcohol. The plant residue was discarded.

Soaking of Antibacterial Discs in Leaf Extracts

Filter paper discs (Whatman #42) were1 soaked in the aqueous extracts of each plant, distilled water, and alcohol for at least ten minutes.

Inoculation of Streaking Plates

The method used was Kirby-Bauer Single Disc Agar Diffusion Test. The agar used was Meuller-Hinton agar, prepared accordingly. Sixty millimeters of the medium was poured into 8 separate 150-mm diameter Petri dishes so they had a depth of 4-mm. Before using the plates, they were first incubated in 25 to 35 degrees Centigrade for 30 minutes. When the plates were not

used, they were placed in the refrigerator, after the test organism.

Eight colonies of the organism was touched with a loop and were seeded into 4 ml of trypticase soy broth and were incubated for 2 to 3 hours at 37 degrees Centigrade.

Within 15 min. of diluting the broth culture, the organism was streaked evenly on the medium in the three directions using a cotton swab. Excess suspension was removed from the swab by rotating it firmly against the side of the tube before seeding the plate.

Antibacterial Testing

Application of Discs. After the inoculum has dried from 3 to 5 min., the discs were placed on the plate. The discs were applied with a flamed forceps. The plates were incubated within 15 min., aerobically and without carbon dioxide for 24 hours at 37 degrees Centigrade.

Data Collection. After incubation, the diameter of the zones of inhibition were measured using a ruler. The mean of the results was solved for each treatment. To determine if there is a significant difference among the treatments the One Way Analysis Of Variance(ANOVA)was computed using the SPSS program.

Replicates. There were 3 replicates for this testing for the independent variables, namely trial 1, 2, and 3.

Statistical Data Analysis

Certain statistical tools were used in this study. The mean and standard deviation were used as descriptive statistical tools. The One Way Analysis of Variance (ANOVA) was used as inferential statistical tool.

Descriptive Statistical Tools

The mean was used to determine the average of the diameter zone of inhibition in three trials. The standard deviation determined the homogeneity of the data in every trial from their means.

Inferential Statistical Tool

The One Way ANOVA, set at 0.05 alpha level of significance, determined the significant difference in the antibacterial activity of the leaf extracts on the test organism.

Chapter 4

Results and Discussions

This study determined the antibacterial effects of the leaf extracts of aloe vera, hierba buena, and kamote on *E. coli*.

It was hypothesized that there was no significant difference in the antibacterial effects of aloe vera, hierba buena, and kamote on *E. coli* and when compared with each other and alcohol.

Antibacterial effects of leaf extracts on *E. coli*

Results showed that all the plants except hierba buena possess antibacterial activity against *Eschirichia coli*. This was deduced after the zones of inhibitions were measured. The leaf extracts of aloe vera, kamote and alcohol possess antibacterial activity against *E. coli* as shown by the mean zones of inhibition which were 0.67 and 0.33 respectively. On the other hand, hierba buena did not exhibit any activity at all as shown by the 0 zone of inhibition.

Table 1 shows the data.

Table 1

Diameter Of Zone Of Inhibition(mm) Of Leaf Extracts On Eschirichia coli

Treatment	Replicates 1	2	3	Mean(mm)	S.D.
Aloe vera	2	0	0	0.67	1.15
Hierba Buena	0	0	0	0.00	0.00
Kamote	1	0	0	0.33	0.33
Ethyl alcohol	1	0	0	0.33	0.33

Table 2.

The One Way Analysis Of Variance(ANOVA) Table

Results	Sum of Squares	Degrees Freedom	Mean Square	F	Significance
Between groups	0.933	4	0.233	0.350	0.838
Within groups	6.667	10	0.667		
Total	7.600	14			

Comparing the effects of the treatments

Table 2 shows that there existed no significant differences in the zones of inhibition among the treatments, as reflected by $F(10) = .838$, $p > .05$.

Chapter 5

Summary, Findings, Conclusions and Recommendations

Summary

This study was conducted in order to determine the antibacterial effects, of the leaf extracts if aloe vera, hierba buena, kamote and alcohol on *Eschirichia coli*.

Specifically, it sought answers to the following questions:

1. Do (a) aloe vera, (b) hierba buena, (c) kamote, and (d) alcohol have antibacterial effects on E. coli?

2. Is there a significant difference on the antibacterial effects of (a) aloe vera, (b) hierba buena, (c) kamote, and (d) alcohol on *E. coli*?

Findings

According to the results of the study, aloe vera, hierba buena, kamote, and alcohol had antibacterial effect against *Eschirichia coli*.

It was also found out that aloe vera, hierba buena, kamote and alcohol had no significant differences in their antibacterial effects against *E. coli* when compared with each other.

Conclusions

The results showed that, alcohol and all of the leaf extracts, except hierba buena, had antibacterial activity against *Eschirichia coli*. It was also found out that there were no significant differences in the antibacterial activity of aloe vera, hierba buena, kamote, and alcohol upon *E. coli*. This only means that the leaf extracts of the herbs had almost the same effect on the test organism and could be interchangeably used.

Recommendations

After the conduct of the study, it is recommended that any of the treatments could be used against *E.* coli.

The researchers recommend that other plants leaf extracts or other plant parts may also be used to benefit other researchers. For the purpose of diversity and also to combat other diseases it is recommended that studies be conducted using different and more pathogenic test organisms. Other ways of measuring the antibacterial activity could also be used so that the results of the study would be more accurate.

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