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Antibacterial Activity of the Aloe vera, *Aloe barbadensis* Leaf Extracts Against *Escherichia coli*

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ABSTRACT

This study aimed to determine the antibacterial activity of the different leaf parts of *Aloe barbadensis*, specifically aloe vera leaf peel, aloe vera leaf gel, and whole leaf, as well as their medicinal properties. Ethanol was the solvent used in extracting the bioactive compounds present in each leaf part. Each leaf part had an ethanolic concentration of 100% each. The disc diffusion assay was employed to determine the antibacterial activity of *Aloe barbadensis.* The appearance of zones of inhibition measured the antibacterial effect. Ampicillin served as the positive control, while distilled water served as the negative control. Antimicrobial tests showed that among the three leaf parts, the aloe vera leaf gel was the only extract that inhibited the growth of *Escherichia coli* with a mean zone of inhibition of 8.56mm. The results suggest that aloe vera gel contains certain bioactive compounds that can suppress the growth of *Escherichia coli*.

Keywords: Antibacterial, aloe vera, bioactive, ethanol, and ampicillin

INTRODUCTION

Herbal plants are widely spread around the world. They are known to possess medicinal properties and serve as a good source of potent and powerful drugs with antibacterial properties (Chopra et al., 1992; Iyengar, 1985). The use of medicinal plants to treat infections is a traditional practice in many parts of the world. These are most common in rural countries that rely solely on natural medicines to maintain the good health of humans and animals (Redda et al., 2014). Extracts of plants represent a means of discovering new phytochemical compounds against pathogens (Thiruppathi et al., 2010). According to the study by Dhanabal et al. (2012), a significant number of people from Asia and Africa rely on traditional medicines for their healthcare maintenance. Even the World Health Organization has estimated that 80 percent of the population uses and relies on natural forms of medicine, also known as herbal plants, for the treatment of diseases (Arunkumar and Muthuselvam, 2009). One of the medicinal plants utilized by people today is the *Aloe barbadensis*. *Aloe barbadensis* is recognized as one of the biologically active Aloe species (Bozzi et al., 2007). This is well dispersed in the Philippines. Locally, people use them as a source of good nutrients for healthy hair growth. For others, aloe vera is beneficial in treating damaged skin through its wound-healing properties (Ghazanfar, 1994). Extracts from aloe vera leaves have been proven to be antimicrobial agents against pathogenic microorganisms, including bacteria (Kumar et al., 2012). Bacteria, as well as viruses, fungi, protozoa, and certain multicellular parasites, are considered pathogenic microorganisms that cause infectious diseases. Infections and diseases, such as diarrhea, urinary tract infections (UTIs), and sepsis, are primarily caused by human pathogenic bacteria. One of these is the *Escherichia coli* (Kaper et al., 1994). Different types of *Escherichia coli* are resistant to other antimicrobials (Rahman et al., 2004; Li et al., 2007). Studies have shown that leaf extracts of Aloe vera inhibit the growth of bacteria, including that of *Escherichia coli.* Aloe vera has a long history as a therapeutic agent, possessing medicinal properties, including antibacterial components (Cock, 2007). Antimicrobial activities against infectious agents have been associated with Aloe vera (Grover et al., 2011). The term “antimicrobial” refers to natural or synthetic substances that can kill or inhibit the growth of various microorganisms (Iyer et al., 2012). It would be very interesting to know which part of the *Aloe barbadensis* leaf exhibits an inhibitory effect against the human pathogenic bacterium *Escherichia coli*, benefiting humanity and expanding knowledge in both the scientific and medical worlds. Thus, this study was proposed. Generally, this study aimed to test the antibacterial properties of the different parts of the leaf extracts of *Aloe barbadensis* (Aloe vera) against the human pathogenic bacterium *Escherichia coli* This study can contribute to the addition of knowledge on the therapeutic properties of plant species, especially *Aloe barbadensis.* By conducting an antibacterial activity test on the different parts of the Aloe vera leaves, we can determine its potential as an antimicrobial agent against a specific harmful bacterium, *Escherichia coli*, especially since the plant species is abundant in the locality. Conducting antibacterial experiments using the whole aloe vera leaf, aloe vera leaf peel, and aloe vera leaf gel helped us determine which can be best used as an antibacterial agent.

MATERIALS AND METHODS

Study area

The collection of fresh leaf samples took place at the herbal garden of Dawan Central Elementary School (DCES), Dawan, Mati City, Davao Oriental. Dawan Central Elementary School is located 20 kilometers away from the downtown area of Mati City. The laboratory experiment was conducted at the Microbiology Laboratory of Davao Oriental State College of Science and Technology (DOSCST). The study was conducted over four months, from October 2015 to February 2016. The collection of samples was conducted in October 2015 in the herbal garden of Dawan Central Elementary School, Dawan, Mati City, Davao Oriental.

Field collection

Fresh, healthy, and mature leaves of the aloe vera plant were collected from the herbal garden of Dawan Central Elementary School (DCES). Plant identification was confirmed through its description based on books and journals.

Leaf peel

Aloe vera leaf peel was collected by using a clean, sterile knife. The aloe vera leaves were carefully washed with running water. The aloe vera leaves were gently peeled to remove the gel. A 2 kg aloe vera leaf peel was obtained. The 2 kg aloe vera leaf peel was placed in a clean container. Air drying of the aloe vera leaf peel then followed.

Leaf gel

Aloe vera leaf gel was collected using a clean, sterile knife. The leaves were carefully washed with running water. It was cut longitudinally, and the aloe vera leaf gel was removed by scraping it out using a sterile spatula. 2 kg of aloe vera gel was collected. The scraped gels were placed in a clean container, and the aloe vera leaf gel was then air-dried.

Whole leaf

2 kg of aloe vera leaves were collected and washed carefully with running water. They were placed into a clean container and then air-dried.

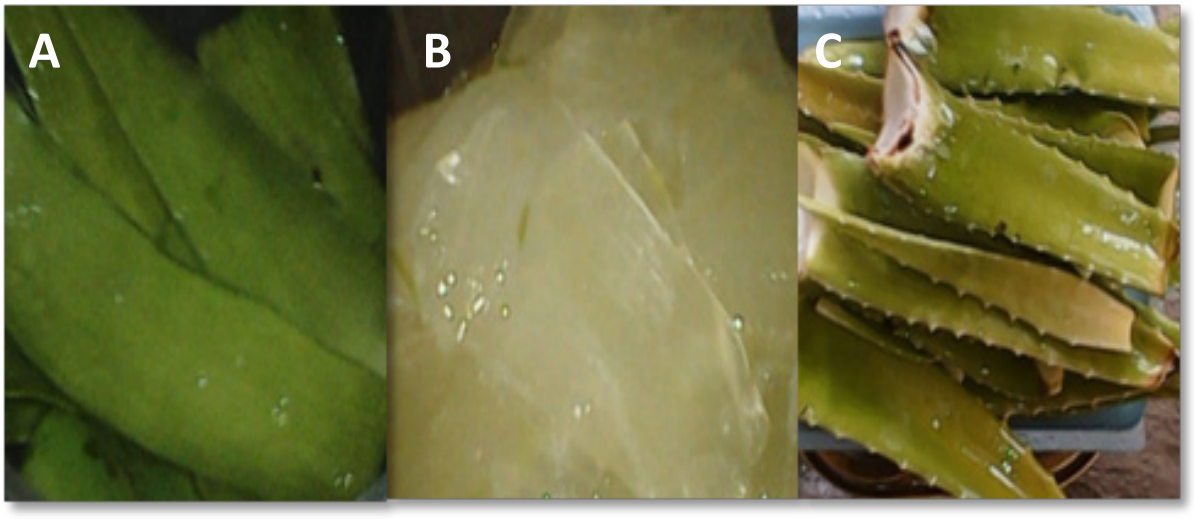

Figure 1. Plant collection of the different aloe vera leaf parts.

Preparation and sterilization of laboratory area and apparatus

The experiment was conducted in the Microbiology Laboratory of Davao Oriental State Colege of Science and Technology. The laboratory room was cleaned to prevent contamintion. Laboratory apparatus and equipment were carefully washed with running water. The glass ware used, including beakers, test tubes, Erlenmeyer flasks, pipettes, and graduated cylinders, was thoroughly washed with detergent and water. Several clean glassware materials were packed in paper for sterilization in an oven at 121°C for 15 minutes (Figure 2) (Cheesebrough, 2000). After experimenting, the used Petri dishes were packed and subjected to an oven at a temperature of 150ºC to kill the bacteria, as well as the other apparatus used. The materials used were washed with detergent and water, and the disposable petri dishes were disposed of. The contents were then buried carefully for proper disposal.

Figure 2. Preparation of microbiology laboratory area and sterilization of apparatus.

Extract Preparation

Each desired air-dried sample was sliced and chopped into smaller pieces using a clean knife and then pulverized using a blender. These were conducted in the microbiology laboratory of Davao Oriental State College of Science and Technology. One hundred grams of the different leaf parts were mixed in a 200 mL solution of 100% ethanol solvent for 24 hours. The super natants were separated through a funnel and filter paper and then placed in a clean glass bottle. The bottles were labeled accordingly (Appendix B). The filtrates were evaporated using a rotary evaporator (Appendix A) in the laboratory of the Department of Science and Technology in Davao City (Table 1).

Table 1. The concentration of the different leaf extracts.

Different leaf parts	Pulverized leaf (g)	Solvent (ml) (High-grade ethanol)	Concentration (%)
Leaf peel	100	200	100
Leaf gel	100	200	100
Whole leaf	100	200	100

Bacterial Strain

Cultured *Escherichia coli*, a potent gram-negative bacterium, was purchased from the Department of Science and Technology Regional office in Davao City.

Culture Media and Petri Plate Preparation

The culture medium used in the experiment was Pronadisa nutrient agar. The following illustrates the preparation of Pronadisa culture media.

Base Agar

1. Suspend the 23 g of powder in 1000 mL of purified water and mix thoroughly.
2. Heat with frequent agitation and boil until the powder is completely dissolved.
3. Sterilize in the oven for 15 mins at a temperature of 150 ºC.

After preparation, the culture medium was cooled. The culture medium was then transferred into individual sterile Petri dishes using a sterilized pipette and rubber aspirator, with a volume of 10 mL per plate (Jeyaseelan & Jashothan, 2012; Saguibo & Elegado, 2012), and allowed to cool for 3 to 5 minutes.

Soft Agar

1. Suspend the 18 g of powder in 1000 mL of purified water and mix thoroughly.
2. Heat with frequent agitation and boil until the powder is completely dissolved.
3. Sterilize in the oven for 15 mins at a temperature of 150 ºC.

After preparation, the soft-agar culture medium was inoculated with *Escherichia coli*. Inoculation was done by scraping the bacteria from the stock culture using a sterilized cotton swab. The inoculated soft-agar culture media was then shaken to distribute the bacteria evenly. Using a sterilized pipette and rubber aspirator, 7 mL of soft agar was poured on top of the base agar (Jeyaseelan & Jashothan, 2012; Saguibo & Elegado, 2012).

The paper disc diffusion method

Whatmann filter paper No.1 with a diameter of 6mm was impregnated with 100% ethanolic extracts of the aloe vera leaf peel, leaf gel, and whole leaf. This was then transferred in sterile petri dishes, which were inoculated with *Escherichia coli*. Each of the Petri dishes contained three paper discs impregnated with 100% ethanolic extract. Cultures were incubated for 24 hours to observe the inhibitory effect. The antibacterial activity was performed in triplicate for each leaf part at the same concentration. The zones of inhibition were measured in millimeters, and the mean values of the growth inhibition were recorded. The use of control groups in this experiment served as a standard basis for determining the difference in the inhibitory effect of the different leaf parts. Ampicillin was used as the positive control substance, while sterilized distilled water served as the harmful control substance. The control groups carefully followed the paper disc diffusion method.

Data analysis

The results of the conducted experiment were recorded in a tabular form to facilitate a more precise and better understanding of the comparison of zone inhibition obtained by using a 100% ethanolic concentration of the extract on different parts of the leaves. The same procedure was followed on the results of the control groups. A table was set to display the results of the average zone of inhibition for 100% ethanolic concentration across different leaf parts, as well as the positive and negative controls, for further discussion and interpretation of the data.

RESULT AND DISCUSSION

The study utilized ethanol as its solvent to extract the possible antibacterial compounds present in the different parts of *Aloe barbadensis* leaves, specifically aloe vera leaf peel, gel, and whole leaf. The type of solvent used in extracting the medicinal components from plant extracts helps determine the antibacterial activity of a plant species (Niranjan et al.,2013). In a study by Malini et al. (2013), it was reported that ethanol was the most effective solvent for screening medicinal plant activity. With this, a 100% concentration of each leafpart was used to determine its antibacterial activity against *Escherichia coli*. Table 2 presents the data obtained from each medium, including the control groups from the experiment. Presented here were the average zones of inhibition in every petri dish. Among the three leaf extracts of *Aloe barbadensis* used in the experiment, a 100% ethanolic concentration of the aloe vera leaf gel extracts was found to be an effective growth inhibitor of *Escherichia coli*. The leaf gel extract showed an average mean inhibition of 8.56 mm. At the same time, aloe vera leaf peel and aloe vera whole leaf extract with 100% ethanolic concentration showed an inability to suppress the growth of the bacteria. According to Redda et al. (2014), one of the essential factors that help determine the antimicrobial activity of a plant is the type and composition of its extract.

Table 2. Average zone of inhibition of ethanolic concentration (100%) to the different aloe vera leaf parts as well as its positive and negative controls.

Leaf Part/ Positive and Negative Control		Zone of Inhibition (mm)	Mean (mm)
Aloe vera leaf peel	**R1**	**0**	**0**
	R2	**0**	
	R3	**0**	
Aloe vera leaf gell	**R1**	**9.67**	**8.56**
	R2	**9.5**	
	R3	**6.5**	
Aloe vera whole leaf	**R1**	**0**	**0**
	R2	**0**	
	R3	**0**	
Ampicillin (+ control)	**R1**	**28**	**30.22**
	R2	**30.67**	
	R3	**32**	
Distilled Water (- control)	**R1**	**0**	**No zone of inhibition**
	R2	**0**	
	R3	**0**	

The results could be responsible for the antimicrobial compounds found in aloe vera leaves. According to Davis et al. (1989), aloe vera leaves possess bioactive compounds such as anthraquinones/anthrones, amino acids, lignins, saponins, monosaccharides/polysaccharides, minerals, and vitamins. Each of the bioactive components performs its functions. Bashir et al. (2007) noted that the antibacterial activities of aloe vera were dependent on the dose of anthraquinone, a bioactive compound present in its leaf parts. In addition, according to Agarry et al. (2005), most of the bioactive components are found in the gel and not in the peel, making the gel more active (Plate C-B). Moreover, Karpagam & Devaraj (2011) stated that aloe vera gel contains bioactive compounds, such as

anthraquinones and phenolic compounds, including aloes, aloin, aloe-emodin, and barbalo in, which act as painkillers and function as antibacterial and antiviral agents. Additionally, these compounds exhibit potent antimicrobial activity against bacteria, viruses, fungi, and yeast (Karpagam & Devaraj, 2011). Tian et al. (2003) reported that the compounds aloe-emodin and aloin altered the morphological cell structure of their test organism, *Escherichia coli*, and damaged its outer cell membrane, causing suppression of bacterial growth. In a similar study by Agarry et al. (2005) on the antimicrobial activities of *Aloe barbadensis*, the Aloe vera leaf gel inhibited the growth of Trichophyton mentagrophytes, whereas the Aloe vera leaf peel did not affect the organism. The same result was observed in this study, where the leaf peel was unable to show an inhibitory effect against the test organism *Escherichia coli* (Plate C-A). The result suggests that although the leaf gel and leaf peel share specific components, they remain distinct from each other (Foster, 1999). In the third treatment, the aloe vera whole leaf showed the same results as the aloe vera leafpeel, which performed no inhibitory effect against *Escherichia coli*. The probable reason can be the low concentration of the extracts on the aloe vera whole leaf. The entire leaf was not air-dried properly due to the limited time available for air-drying the plant part, which was only 8 days. The water inside the whole leaf was not extracted, causing less concentration of the bioactive compounds found in the aloe vera whole leaf. A less concentrated bioactive compound may have a lesser effect on suppressing the bacterial growth of *Escherichia coli* or may have no effect at all on the bacteria (Figure 3).

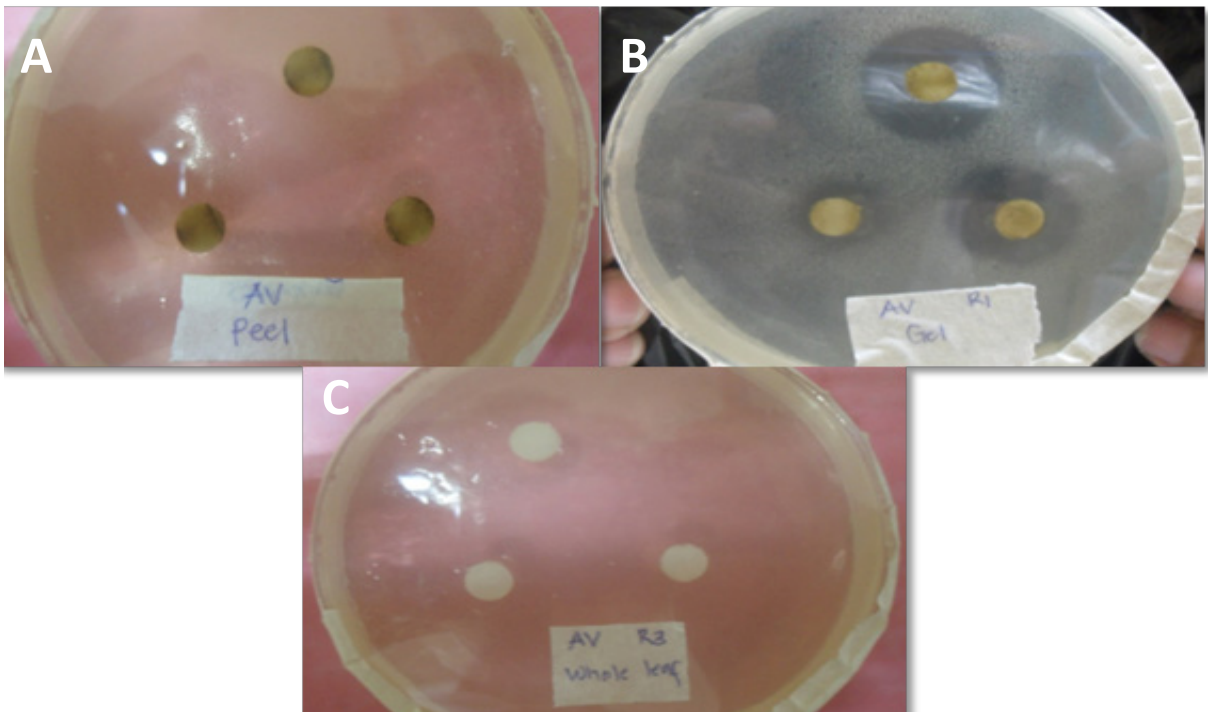

Figure 3. Antibacterial activity of aloe vera leaf peel (A), gel (B) and whole leaf extracts (C).

Figure 4 below presents the clear zones found in the positive control (ampicillin) and aloe vera leaf gel samples but none in the negative control (distilled water). The clear zones observed in the leaf gel extract are smaller than those in the positive control. Ampicillin almost completely inhibited the growth of *Escherichia coli*, giving an average mean of 30.22 mm (Table 2). This may imply that ampicillin is a more effective growth inhibitor for the bacteria compared to the leaf gel extract. It was even mentioned by Ashnagar & Naseri (2007) that

ampicillin is a good antibiotic against gram-negative bacteria, including *Escherichia coli*. Nevertheless, there is also a possibility that the amount of antibacterial compound extracted from the leaf gel is lower than the concentration in ampicillin, resulting in a smaller zone of inhibition.

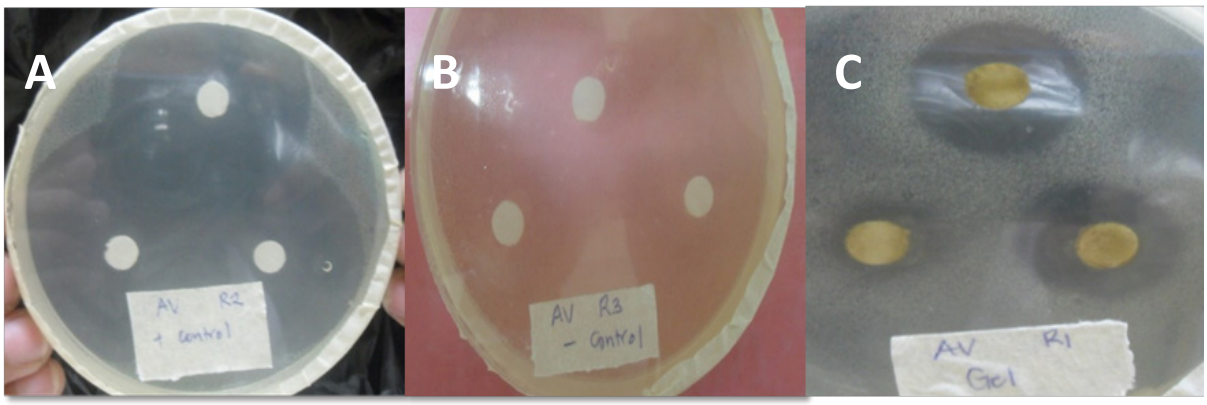

Figure 4. Antibacterial activity of the positive (Ampicillin (A), negative (dH2O) controls, and Leaf gel extract (C).

CONCLUSION

The study utilized ethanol as a solvent for extracting bioactive compounds from the different parts of *Aloe barbadensis* leaves. Among the three ethanolic extracts of aloe vera leaf parts (aloe vera leaf peel, aloe vera whole leaf with 100% concentration each), only the aloe vera leaf gel performed antibacterial activity against *Escherichia coli*. This is due to the high concentration of bioactive compounds, which are mostly found in aloe vera gel. Thus, aloe vera leaf gel can be effective against *Escherichia coli*.

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