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Antibacterial Activity of Cassava *Manihot Esculenta* Leaves Extract Against *Escherichia coli*

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ABSTRACT

The purpose of this research is to determine the antibacterial activity of Cassava (*Manihot esculenta*) leaf extract against *Escherichia coli*. The experiment involved the determination of plants, extraction using the maceration method, and antibacterial testing of the fresh and dried cassava (*Manihot esculenta*) leave extracted with concentrations of 5%, 10%, 20%, and 30% ethanol against the bacterial growth of (*Escherichia coli*) and culture media using Merck Nutrient Agar. A caliper was used to measure the size of (*Escherichia coli*) growth in millimeters. The results showed that extracts of fresh and dried cassava (*Manihot esculenta*) leaves extract with ethanol concentrations of 5%, 10%, 20%, and 30% have antibacterial activity against *Escherichia coli*. This study examined the efficacy of fresh and dried cassava (*Manihot esculenta*) leaves extract against (*Escherichia coli*) in the 18th and 24th hours. The mean of fresh cassava (*Manihot esculenta*) leaves extract is 4.4, which differs considerably from the mean of dried Cassava (*Manihot esculenta*) leaves extract, which is 3.9. The fresh cassava (*Manihot esculenta*) leaves extract was the most potent therapy against (*Escherichia coli*) in the 18th hour, according to the findings of this investigation. Furthermore, at the 24th hour, the fresh cassava (*Manihot esculenta*) leaves extract was the most potent therapy against (*Escherichia coli*). Fresh cassava (*Manihot esculenta*) leaves extract has a mean of 4.4, which differs significantly from dried (*Manihot esculenta*) leaves extract, which has a mean of 3.9. According to the findings of this study's testing, the 20 percent concentration of fresh cassava (*Manihot esculenta*) leaves extract with an average of 5.75 was the most active treatment against (*Escherichia coli*). It has been proposed that cassava (*Manihot esculenta*) leaves could be turned into standardized antibacterial herbal. Future research should investigate the effects of cassava (*Manihot esculenta*) leaves used as an antibacterial agent on human health.

INTRODUCTION

Plants have long been utilized to treat people and are now recognized as a source of medicine in conventional health care systems (Meilawaty et al., 2019).

According to recent research (Shah et al., 2013), some indigenous plants offer interesting biological features that may be of interest to people all over the world. The plants include medicinal compounds, antibiotics, and microbial components that can benefit people. Many ancient herbal uses are being investigated as potential sources of natural components for novel medications. People have known for a long time that some plant-based medications can treat ailments and kill microorganisms. Many plant parts used in traditional medicine can be found in rural regions for about the same price as contemporary treatment. Rural locations have more medicine and it is less expensive than in cities. Plants produce a large number of secondary metabolites, which are essential sources of microbicides, pesticides, and many pharmaceuticals. Pharmaceutical substances used in traditional medicine are still primarily derived from plants. People are getting increasingly interested in a variety of traditional organic components.

E. coli lives and grows in the gastrointestinal tracts of many animals, including humans (ClevelandClinic, 2020) the majority of the time, this bacteria is absolutely harmless. It aids in the digestion of food. However, these

are *E. coli* strains that could enter a human's stomach and produce diarrhea, stomach ache, cramps, and a low-grade fever.

Natural resources from the Philippines are extensively used in everyday life as a culinary item in tropical countries. Cassava leaves can be used to treat ulcers, rheumatism, gout, diarrhea, fever, headache, night blindness, intestinal worms, and "beriberi" disease, in addition to consuming them. Furthermore, Cassava (*Manihot esculenta*) leaves extract demonstrated antibacterial activity against clinical isolates of *Staphylococcus epidermidis* as well as *Propionibacterium acnes*, and the findings of a related study indicated that Cassava leaves extract was effective in treating pimple-causing bacteria. (Mustarichie et al., 2020)

Treatment for *E. coli* is entirely dependent on the administration of antibacterial agents, particularly antibiotics, which may result in more disease-causing germs, new strains of microbial resistance to antibiotics, and even renal failure. As a result, it is critical to seek out alternative methods of assistance, one of which is the usage of herbal plants, notably the cassava plant, which has phytochemical qualities such as flavonoid and rutin *E. Coli* - Diagnosis and Treatment - Mayo Clinic, 2020). Flavonoids, according to (Kumar & Pandey, 2013), are a class of plant metabolites that are hypothesized to have antioxidant and antibacterial properties via cell

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signaling pathways. Flavonoids are powerful antioxidants that promote a variety of health benefits. Aside from antioxidant action, these compounds have anti-viral, anti-cancer, anti-inflammatory, and anti-allergic properties. Rutin, on the other hand, is a pigment and a strong bioflavonoid component found in the cassava plant that is responsible for its antibacterial properties (Ullah et al., 2020).

There hasn't been a lot of research done on the usefulness of Cassava leaf extract as a treatment for disease-causing bacteria. This study compares the antibacterial activity of Cassava (*Manihot esculenta*) leaves extract against *S. epidermidis* and *P. acnes* (Mustarichie et al., 2020). As a result, our study to assess the antibacterial activity of cassava leaf extract is both contemporary and pertinent. The study examined and assessed the effectiveness of fresh and dried cassava (*Manihot esculenta*) leaves extract with ethanol concentrations of 5%, 10%, 20%, and 30% in inhibiting the growth size of (*Escherichia coli*). Finally, the goal of this study was to give scientific evidence that cassava (*Manihot esculenta*) leaf extract has the ability to treat germs (*Escherichia coli*).

What is Cassava (*Manihot esculenta*)?

Cassava is a tuberous food plant that is native to the tropics of the American continent. It is a member of the spurge family (Euphorbiaceae) and is also known as manioc, mandioca, and yuca. According to the research, the cassava leaf contains a high concentration of phytochemical components like tannins, alkaloids, steroids, flavonoids, and saponins (Babalola & Ahmed, 2005). The leaves of the cassava plant were the source of these antibacterial and antioxidant chemicals. Cassava leaf extract was studied for its potential antibacterial effects against *Staphylococcus epidermidis* and *Propionibacterium acnes* Mustarichie et al. (2021) The results of this study indicated that the cassava plant (*Manihot esculenta*) had antibacterial activity against *Staphylococcus epidermidis* as well as *Propionibacterium acnes*. In addition, it was determined that ethanol extracts of cassava leaves had antibacterial activity against both of the bacteria that were tested, with ethyl acetate being the most active component of the extract. The treatment that was found to be most successful against *Staphylococcus epidermidis* was a concentration of 5 percent with an average of 5.0. On the other hand, a concentration of 2.5 percent against *Propionibacterium acnes* was found to be the most effective treatment. The average concentration was 4.1.

According to (Landers et al., 2012) antibiotic development leads to bacterial resistance due to a variety of circumstances. These circumstances include inappropriate antibiotic usage in human and animal health and their continuous use as growth promoters at sub-clinical levels in poultry and cattle production. In addition, improper antibiotic usage in human and animal health can lead to antibiotic resistance. This research looked at the effectiveness of an extract made from

cassava leaves as an antibacterial agent in the treatment of diarrhea brought on by *E. coli* infections in both humans and animals. According to the findings of the study, the extract of cassava leaf was effective in treating diarrhea brought on by *E. coli*.

Similarly, a study that was carried out by Saad et al. (2014) investigated the antibacterial efficacy of five medicinal plants against five clinical pathogens. These plants were *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, and *Manihot esculenta*. *Plectranthus ambonicus* was also included in this study (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*). It was shown that an extract of the leaves of the cassava plant (*Manihot esculenta*) has antibacterial activity against five clinical isolates of pathogens. These pathogens included *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. However, research conducted by Sari (D. B. 2019) evaluated the antibacterial activity of three different varieties of cassava leaf (*Manihot esculenta* Crantz.) against the germs *Bacillus cereus*, *Shigella dysenteriae*, and *Vibrio cholerae*. Maceration with varying concentrations of ethanol, a positive control, and negative control was used in an experiment to investigate the antibacterial properties of an ethanol extract of three different kinds of cassava leaves. Based on the findings, it was determined that an ethanol extract of cassava (*Manihot esculenta*) leaves was the antibacterial substance that was the most efficient against *Bacillus cereus*.

What is *Escherichia coli*?

According to Wikipedia, *E. coli*, sometimes known as *Escherichia coli*, is a type of bacteria that can be found in people's intestines (Felson, 2020). It has also been found in the gastrointestinal tracts of other mammals. In addition to having food poisoning from *E. coli*, a person may develop pneumonia or a urinary tract infection. In fact, *E. coli* is the causal culprit in 75 to 95 percent of all instances of urinary tract infections. Because *E. coli* is a common colon dweller, it frequently travels through the bowel to enter the urinary tract. Several strains of *E. coli* have been related to diarrhea, including the kind that causes watery travelers' diarrhea. Antibiotics have been demonstrated to lessen the amount of time spent experiencing symptoms and may be recommended to people with moderately severe illnesses. Inadequate antibiotic use may expedite the development of germs resistant to the drugs used to treat them.

However, (Riley, 2014) emphasized that extraintestinal pathogenic *E. coli*, the specialized strains of *E. coli* that cause the bulk of extraintestinal *E. coli* infections, pose a significant yet underappreciated hazard to public health. These *E. coli* strains cause the majority of extraintestinal *E. coli* infections. Despite the fact that the roots of their evolution are unknown, *E. coli* has a unique ability to cause disease in places of the host body other than the intestinal tract. The many virulence characteristics

that *E. coli* possesses illustrate this potential. Effective preventive interventions against the morbid and costly infections caused by *E. coli* may be possible if there is a better understanding of the existence and significance of *E. coli*, as well as the distinct virulence mechanisms, reservoirs, and transmission pathways associated with these bacteria.

According to Gomes et al. (2016) study, *E. coli* causes digestive diseases, the most prevalent of which is diarrhea. Diarrhea is a major cause of morbidity and mortality in disadvantaged countries. Hemorrhagic colitis is caused by *E. coli* cytotoxins or Shiga-like toxins, which are responsible for the disease. It is currently relevant to public health since it is easily isolated from waste items left behind by humans and animals, both of which contribute to the pollution of the environment (Hernandez-Cortez et al., 2017). Because of the frequency of food and water contamination in the environment, people, particularly newborns and the elderly, pose a substantial risk to their health. Human and animal feces can contain contaminants as well as infectious organisms such as *E. coli*.

Tannins, flavonoids, and rutin are just a few phytochemical compounds that can be found in a variety of plant extracts. These chemicals are capable of treating bacterial infections. This study aimed to provide antimicrobial effects by utilizing a natural antibacterial agent with suitable extraction parameters and concentrations. The antibacterial activity of cassava (*Manihot esculenta*) leaves extract against *Escherichia coli* will give future researchers baseline data, changing it into a standardized antibacterial herb plant (Mujeeb, et al., 2014).

Phytochemical Compounds of Cassava (*Manihot esculenta*) Leaves

The presence of tannins, alkaloids, steroids, flavonoids, and saponins in *M. esculenta* leaves extract was discovered by a phytochemical investigation (Clemen-Pascual et al., 2022). Plant phytochemical compounds assess the medicinal value of plant leaves. Antimicrobial and antioxidant effects of flavonoids and tannins Alkaloids, on the other hand, have significant physiological effects, particularly on the neurological system.

The presence of these three phytochemicals in the leaves of *M. esculenta* revealed that this plant is physiologically active and has the capacity to inhibit germs that cause diseases. This investigation supported the antibacterial activity of *M. esculenta* leaves extract. Future research should focus on isolating, identifying, and purifying these phytochemicals, as well as determining their antibacterial potencies and toxicity evaluation in order to formulate antibiotics.

Research from Chaiareekitwa et al. (2022) investigated the antioxidant components and chlorophyll content, as well as the antioxidant activity of cassava leaf flour extract from different plant ages and groups. The antioxidant content (vitamin C, polyphenols, and carotene) was considered high and increased as the plants grew. The presence of these antioxidant compounds on the Cassava leaf flour

extract demonstrates its efficacy as an antibacterial agent. The mature the leaf is, the larger the level of antioxidants. According to the findings of this study, Cassava leaf flour extract is high in antioxidants, particularly flavonoids. Cassava leaf flour extract's flavonoid level suggests its antibacterial activity.

According to (Shrestha et al., 2021, Kabra et al., 2019, and Saini et al., 2013)) *M. esculenta* leaves were reported to have antibacterial action, and anti-oxidant, anti-tyrosinase, anti-inflammatory, and hepatoprotective. The high flavonoid content of *M. esculenta* leaves was responsible for these actions. According to a recent study (Chahyadi & Elfahmi, 2020), seven glycoside flavonoids are present in cassava leaves, with Rutin being the most abundant. According to the findings, *M. esculenta* leaves are a rich source of Rutin, making extraction the best method for obtaining a high yield of bioflavonoids. According to this study, the maceration procedure with ethanol was the best extraction method for obtaining rutin.

According to the evaluations linked to this work, the antibacterial efficacy of Cassava (*Manihot esculenta*) leaves extract can deter *Escherichia coli*. Cassava leaves' phytochemical characteristics enable researchers to create antibacterial drugs from their extract. Fresh and dried Cassava (*Manihot esculenta*) leaves were macerated with 95 percent ethanol. In this study, varying percentages of concentration (5 percent, 10%, 20%, and 30%) were observed to assess the level of effectiveness of fresh and dried Cassava (*Manihot esculenta*) leaves and their concentration. Concentrated extracts of fresh and dried Cassava (*Manihot esculenta*) leaves were tested for antibacterial activity against *Escherichia coli*, a bacteria often found in human and animal intestines. This study will give baseline data on the efficacy of Cassava (*Manihot esculenta*) leaves extract against *E. coli* infections. This study also found that cassava (*Manihot esculenta*) leaf extract has antibacterial activities that are useful not only in treating pimple-causing bacteria (*Staphylococcus epidermidis* and *Propionibacterium acnes*), but also in infections caused by *Escherichia coli*.

MATERIALS

The present study was conducted to evaluate the antimicrobial activity of Cassava (*Manihot esculenta*) leaves extract against clinical isolate of *Escherichia coli*. This experimentation tested the effectiveness of Treatment-A using fresh Cassava (*Manihot esculenta*) leaves extract and Treatment-B using dried Cassava (*Manihot esculenta*) leaves extract.

Research Design. This study manipulates clinical isolates of *Escherichia coli* where procedures and experimentation were done in the Biological Laboratory of Notre Dame of Midsayap College. Thus, this study adopts an experimental research design using a randomized complete block design (RCBD).

Instrumentations. The experimental method was used in this study to test the antibacterial activity of Cassava (*Manihot esculenta*) against *Escherichia coli*. Randomized

Complete Block Design was the method used in this study, in which experimental units were grouped into fresh and dried setups. RCBD was the method used since experimental units are grouped into blocks or replicates. A caliper was used in measuring the bacterial growth size and was measured in millimeters. The treatment of the extracts was done between 18 and 24 hours at room temperature.

The results of the experimentation were recorded and analyzed using ANOVA and t-test tools.

Beaker, Erlenmeyer flasks, graduated cylinder, stirring rod, inoculating loop, alcohol lamps, petri-dishes, mortar and pestle, electric oven, pressurized cooker, inoculating chamber, and the caliper was used in this study.

Manihot esculenta leaves were obtained at San Mateo, Aleosan, Cotabato and determined using PictureThis-Plant Identification Application.

Chemicals. Distilled water, and 95% ethanol were used in the experimentation.

Medium for Bacterial Growth. Merck Nutrient Agar was used.

Microbial Test. Clinical isolates of *E. coli* from the University of Southern Mindanao were used in this study.

Procedures

Collection of Cassava (*Manihot esculenta*) leaves.

The study began with material collection by selecting Cassava (*Manihot esculenta*) leaves, which were not affected by pests, collected and sorted into 2 setups. Setup 1 was freshly picked cassava leaves, while Setup 2 was dried Cassava (*Manihot esculenta*) leaves. Both were macerated and extracted at room temperature for 48 hours.

Preparation and Sterilization of Media

28g of Merck Nutrient Agar was dissolved in a beaker with 1 liter of distilled boiling water. Then, the medium, petri-dishes, beaker, and flask were sterilized using an autoclave at 121°C psi for 15 minutes. The autoclave process will eliminate unwanted microorganisms to ensure decontamination of the culture media. The sterilized medium and laboratory apparatus was placed on a disinfected incubator to cool off.

Extraction

Fresh and Dried *Manihot esculenta* leaves were macerated using 95%. This method was selected to prevent the occurrence of damage to the thermolabile chemical compounds contained in the Cassava (*Manihot esculenta*) leaves. The samples were mashed using mortar and pestle, then soaking it in the petri dishes with different concentrations. In each dish, 30g of mashed Cassava (*Manihot esculenta*) leaves was soaked with concentrations of 5% (95ml distilled water and 5ml ethanol), 10% (90ml distilled water and 10ml ethanol), 20% (80ml distilled water and 20ml ethanol), and 30% (70ml distilled water and 30ml ethanol) of 95% ethanol. The maceration was

carried out for 48 hours at room temperature.

Antibacterial Activity Test

The clinical isolate of *E. coli* was suspended using inoculating loop into the sterile petri-dishes with 20ml of sterile Merck nutrient agar (solidified at room temperature). In each of the dishes, fresh and dried Cassava (*Manihot esculenta*) leaves extract were added with concentrations of 5%, 10%, 20%, and 30% using a sterile dropper. The test media were then incubated for 18-24 hours. In the 18th hour, the diameter of the test bacteria was measured using a caliper. And in the 24th hour, the diameter of the test bacteria was measured using a caliper.

Data Gathering Procedures

Extraction

The efficiency of Cassava (*Manihot esculenta*) leaves extract against *E. coli* was investigated in this experimentation. In this design, Cassava (*Manihot esculenta*) leaves were collected and assigned to the two groups (Fresh and Dried Cassava (*Manihot esculenta*) leaves) and was extracted with concentrations of 5%, 10%, 20%, and 30% of 95% ethanol. The varying concentration of Fresh and Dried Cassava (*Manihot esculenta*) was done to test its level of effectiveness against *E. coli*. The responses of the growing size of *E. coli* to Fresh and Dried Cassava (*Manihot esculenta*) on the 18th and 24th hours were the data to be gathered and analyzed.

Antibacterial Treatment

Fresh and dried *Manihot esculenta* leaves extract with concentrations of 5%, 10%, 20%, and 30% of 95% ethanol were applied against the colony formed by cultured *E. coli* for 18 and 24 hours of treatment. The diameters of the treated *E. coli* was measured in the 18th and in the 24th hour respectively using a caliper. After close observation, both groups were tested to measure the degree of change in the growth size of *E. coli*.

Statistical Tools and Treatment of Data

Using a t-test, both fresh and dried *Manihot esculenta* leaves extract were compared according to their level of effectiveness against *E. coli* during 18 and 20 hours of treatment. ANOVA test was used in analyzing the effectiveness of the concentrations between 5%, 10%, 20%, and 30% of both Fresh and Dried *Manihot esculenta* leaves extract. A conclusion was drawn after the analysis of the results.

RESULTS

Table 1 presents the bacterial growth response of *Escherichia coli* to the Fresh Cassava (*Manihot esculenta*) with concentrations of 5%, 10%, 20%, and 30% ethanol. Caliper was used in measuring the diameter of treated *Escherichia coli* in the 18th and 24th hours. The data gathered in this study was analyzed using a t-test and ANOVA test.

Table 1: Fresh Cassava (*Manihot esculenta*) leaves extract with concentrations 5%, 10%, 20%, and 30% ethanol treatment against *Escherichia coli*

Diameters (millimeters)					
Time	Control	5%	10%	20%	30%
18th hour	4 mm	4 mm	6 mm	4 mm	9 mm
24th hour	2 mm	3.5 mm	5.5 mm	3 mm	8 mm

Table 2: Dried Cassava (*Manihot esculenta*) leaves extract with concentrations 5%, 10%, 20%, and 30% ethanol treatment against *Escherichia coli*.

Diameters (millimeters)					
Time	Control	5%	10%	20%	30%
18th hour	4 mm	4 mm	4 mm	6 mm	5 mm
24th hour	3 mm	4 mm	3 mm	5.5mm	4 mm

Table 2 present the s bacterial growth response of *Escherichia coli* to the Dried Cassava (*Manihot esculenta*) with concentrations of 5%, 10%, 20%, and 30% ethanol. The diameter was measured in millimeters using a caliper. Bacterial growth sizes were measured in the 18th and 24th hours. The data gathered in this study were analyzed using a t-test and ANOVA test.

Table 3: The t-test result for fresh Cassava *Manihot esculenta* extracts in comparison to 18 and 24 hours t-test paired two samples for means.

Time	Observations	Mean	df	t-value
18 hours	5	5.4	4	0.01*
24 hours	5	4.4		

*Significant at $\alpha=0.05$

Table 3 compares the effects of fresh *Manihot esculenta* leaves extract after 18 and 24 hours of therapy. Fresh *Manihot esculenta* leaves exhibit a significant difference, with the 18-hour having a mean of 5.4 and the 24-hour having a mean of 4.4. The fresh *Manihot esculenta* leaves extract is most active around the 18-hour mark of treatment, according to this finding. Table 4 compares

Table 4: The t-test for dry *Manihot esculenta* extracts in comparison to the 18 and 24 hours t-test paired two samples for means

Time	Observations	Mean	df	t-value
18 hours	5	4.4	4	0.05ns
24 hours	5	3.9		

*Not significant at $\alpha=0.05$

the effects of dried *Manihot esculenta* leaves extract after 18 and 24 hours of therapy. There is no significant difference in the dried *Manihot esculenta* after 18 and 24 hours of treatment. According to this finding, dry *Manihot esculenta* leaves were effective for both 18 and 24 hours.

In 18 hours of therapy, Table 5 compares the effectiveness of fresh and dried *Manihot esculenta* leaves extract. With a mean of 5.4, the fresh *Manihot esculenta* leaves extract differs significantly from the dried *Manihot esculenta* leaves extract, which has a mean of 4.4. Fresh *Manihot*

Table 5: The t-test result for fresh and dried *Manihot esculenta* leaves extracts in 18 hours in t-test paired two samples for means.

Type of leaf extract	Observations	Mean	df	t-value
Fresh	5	5.4	4	0.19*
Dried	5	4.4		

*Significant at $\alpha=0.05$

Table 6: The t-test result for fresh and dried *Manihot esculenta* extracts in 24 hours t-test paired two samples for means.

Type of leaf extract	Observations	Mean	df	t-value
Fresh	5	4.4	4	0.19*
Dried	5	3.9		

*Significant at $\alpha=0.05$

esculenta leaves extract was the most effective treatment against *Escherichia coli* after 18 hours.

In 24 hours of treatment, the efficiency of fresh and dried *Manihot esculenta* leaf extracts against *Escherichia coli* is compared in Table 6. With a mean of 4.4, the fresh *Manihot esculenta* leaves extract differs significantly from the dried *Manihot esculenta* leaves extract, which has a mean of 3.9. After 24 hours, fresh *Manihot esculenta* leaves extract was the most effective against *Escherichia coli*.

Table 7 examines the effectiveness of fresh *Manihot*

Table 7: The ANOVA result for the concentration between the control, 5%, 10%, 20%, and 30% for Fresh *Manihot esculenta* leaves extract.

Source of Variation	SS	df	MS	F	P-value
Rows	73.57	2	36.78	21.71	0.00*
Columns	22.12	3	7.37	4.35	0.05*
Error	10.16	6	1.69		
Total	105.86	11			

*Significant at $\alpha=0.05$

esculenta leaves extract at concentrations of 5%, 10%, 20%, and 30%. With a P-value of 0.00, the fresh Manihot esculenta leaves extract demonstrates a significant difference. With an average of 0.16, the control extraction of fresh Manihot esculenta leaves extract is the least active therapy against Escherichia coli. With an average of 5.75, the 20% concentration is the most effective therapy against Escherichia coli, while the 5% concentration is the second most effective treatment against Escherichia coli, with an average of 5. Table 8 examines the

Table 8: The ANOVA result for the concentration between the control, 5%, 10%, 20%, and 30% for dry Manihot esculenta leaves extract.

Source of Variation	SS	df	MS	F	P-value
Rows	46.20	2	23.10	52.23	0.00
Columns	5.57	3	1.85	4.19	0.06
Error	2.65	6	0.44		
Total	54.43229	11			

*Significant at $\alpha=0.05$

effectiveness of dry Manihot esculenta leaves extract at concentrations of 5%, 10%, 20%, and 30%. With a P-value of 0.00, the dry Manihot esculenta leaves extract shows a significant difference. With an average of 0.16, the control extraction of dry Manihot esculenta leaves extract was the least active treatment against Escherichia coli. The 20% concentration is the most active treatment with an average of 4.5, while 10% was the second most active treatment with an average of 4.125.

Findings

Based on the results and findings of this study, the Fresh and Dried Cassava (Manihot esculenta) leaves extract with concentrations 5%, 10%, 20%, and 30% of ethanol had effective antibacterial activity against the bacterial growth size of Escherichia coli.

In the 18th and 24th hour mark of treatment, both Fresh and Dried Cassava (Manihot esculenta) leaves extract with concentrations 5%, 10%, 20%, and 30% of ethanol treatments were active against Escherichia coli. The Fresh Cassava (Manihot esculenta) leaves extract exhibited a significant difference and was most active treatment against Escherichia coli on both 18th and 24th hour mark of treatment. In addition, according to the results of the study the Fresh Cassava (Manihot esculenta) leaves extract with concentrations 5%, 10%, 20%, and 30% of ethanol was most active on the 18th hour mark of treatment against Escherichia coli.

On the other hand, Dried Cassava (Manihot esculenta) leaves extract with concentrations 5%, 10%, 20%, and 30% of ethanol treatments against Escherichia coli had no significant difference in the changes of bacterial growth size. According to the findings of this study, the Dried Cassava (Manihot esculenta) leaves extract was least active on the 18th and 24th hour mark of treatment. The findings of this study concluded that Fresh Cassava

(Manihot esculenta) leaves extract with concentration of 20% of ethanol was most active treatment against the bacterial growth size of Escherichia coli. This concentration was most active in both 18th and 24th hour mark of treatment. According to (Santos, 2013), the Fresh Cassava leaves extract contains rich amount of tannins, bioflavonoids and rutins. The presence of this phytochemicals indicates its active antibacterial ability in suppressing the bacterial growth size of Escherichia coli. On the other hand, the most active concentration of Dried Cassava (Manihot esculenta) leaves extract was 20% of ethanol concentration. The results of the study affirmed that the optimal concentration added to the Cassava (Manihot esculenta) leaves extract was 20% ethanol. It was supported by (Chatterjee, 2006) that 20% ethanol was a high concentration and was bacteriostatic. This concentration prevents the growth of bacteria.

Summary

The primary objective of this research was to examine the antibacterial activity of Fresh and Dried Cassava (Manihot esculenta) leaves extract against the Escherichia coli colonies. The antibacterial efficiency of Fresh and Dried Cassava (Manihot esculenta) leaves extract was added with varying concentrations of 5%, 10%, 20%, and 30% ethanol. These concentration was added to test the Fresh and Dried Cassava (Manihot esculenta) leaves extract level of effectiveness in treatment against Escherichia coli.

The maceration method was used in the extraction process, which required utilizing 95 percent ethanol and keeping it at room temperature. This method was selected to prevent the occurrence of damage to the thermolabile chemical compounds contained in the cassava leaves (Mustarichie, 2021). The maceration method was done by soaking the powdered cassava leaves into the macerator with concentrations of 5%, 10%, 20%, and 30% ethanol and was left for 48 hours with occasional stirring.

The bacterial growth size of Escherichia coli that was treated with Fresh and Dried Cassava (Manihot esculenta) leaves extract with concentrations of 5%, 10%, 20%, and 30% ethanol, was measured in the 18th and 24th hour using a caliper. The data results were gathered and analyzed using t-test and ANOVA-test tools.

CONCLUSION

The results of this study concluded that Fresh and Dried Cassava (Manihot esculenta) leaves extract demonstrated antibacterial activity against Escherichia coli. This study compares the effectiveness of Fresh Manihot esculenta leaves extract at concentrations of 5%, 10%, 20%, and 30%, as well as Dried Manihot esculenta leaves at concentrations of 5%, 10%, 20%, and 30%. The varying concentrations added to the Fresh and Dried Cassava (Manihot esculenta) leaves extract will examine the level of efficiency as an antibacterial agent against Escherichia coli.

The fresh Cassava (Manihot esculenta) leaves extract at a concentration of 20% has the most active treatment in

suppressing the colony size of *Escherichia coli*, with an average of 5.75. In addition, in the 18th hour of treatment, the 20% concentration of fresh Cassava (*Manihot esculenta*) leaves extract was the most active. Taking the control out of the evaluation, the 5% concentration of fresh *Manihot esculenta* leaves was the least effective treatment in reducing the bacteria's growth size. In the 18th and 24th hours of treatment, the dried Cassava (*Manihot esculenta*) leaves extract exhibits no significant difference. The dried Cassava (*Manihot esculenta*) leaves extract was found to be efficacious in both the 18th and 24th hours. The 20 percent concentration of dry Cassava (*Manihot esculenta*) leaves extract was the third most active therapy against *Escherichia coli*, with an average of 4.5. Then there's a 10% concentration with an average of 4.125.

As a result, it can be stated that a 20 % concentration of fresh Cassava (*Manihot esculenta*) leaves extract was most effective in reducing *Escherichia coli* growth size.

RECOMMENDATION

With the results presented, the cassava plant can be used not only as a good source of food, but also in microbiology and medicine. Future research should look into the impact of cassava leaves on human health after they've been used as an antibacterial agent. Future researchers also could conduct a study regarding to effectiveness of Cassava (*Manihot esculenta*) in treating infections and diseases caused by *Escherichia coli*.

In addition, recommendations have been made to educate the community with the antibacterial efficiency of Cassava leaves to provide immediate response and treatment on common bacterial infections. Additional research into the Cassava plant's nutrient content and application as an antibacterial agent against various microbial infections that cause disease is highly recommended. Future researchers especially in the field of medicine must further evaluate the effectiveness of Cassava (*Manihot esculenta*) leaves in treating bacterial infections and developed it as a standardized antibacterial agent.

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