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Phytochemical analysis and microbial inhibition activity of Serpentina (*Rauvolfia serpentina*) leaves syrup on *Escherichia coli* causing Diarrhea

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ABSTRACT

Diarrhea is among the leading global causes of child morbidity and death worldwide (WHO, 2018), and this disease also kills 1.8 million people yearly. Contaminated food and water resources are the most common causes of diarrhea. 1 out of 10 people still doesn't have improved water sources, leading to people being infected by diarrhea, WHO, 2019.

Moreover, this study is aligned with SDG number 3, or good health and well-being, as this study was done to determine the content and the microbial inhibition of Serpentina leaves syrup against *Escherichia coli* causing diarrhea. Tests were: I. Phytochemical analysis (Alkaloids, Flavonoids, and Tannins) II. Disk Diffusion method on 4 different concentrations. III. Shelf life determination. IV. One-way ANOVA.

For phytochemical analyses, serpentina leaves showed chemicals that can inhibit E. coli causing diarrhea, with implications such as pharmacological and microbial inhibition effects. Specifically, it showed the presence of alkaloids, flavonols, a subclass of flavonoids, and tannins. The disk diffusion method showed that serpentina leaves syrup has intermediate to susceptible efficiency against *E. coli* causing diarrhea (50% to 70% interprets that it's intermediate effective while 75% and above interprets that it's susceptible effective) as results showed that it is 65% effective with 25% and 50% concentrations, 70% effective with 75%concentration, and 80% effective with 100% concentration, which clearly states that the syrup can be used as a potent remedy against diarrhea due to its effective inhibition. After the shelf-life determination, it was observed that serpentina syrup could last for up to 2 weeks, as the syrup changes in color on the 13th day and both in color and appearance on the 14th day. Following the one-way ANOVA, the results indicated a significant difference between the 4 concentrations and the positive control group, with F (73.344) > 3.48.

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INTRODUCTION

Many people are suffering because of the harmful effects of *Escherichia coli*, especially the children of this planet, Earth. In comparison to the present problems, many people may not think diarrhea to be an example of a dangerous illness. But many people need to be made aware that this is among the world's most serious problems that we must pay attention to.

As stated by the World Health Organization (2017), Diarrhea is a leading cause of child morbidity and death around the world. It is primarily the result of contaminated food and water resources. As mentioned by Palawan News, 2018, DOH announced that water is the leading cause of local diarrhea infections, as Southern Palawan also experienced a diarrhea outbreak in 2018 (Philippines News Agency).

The World Health Organization (2019) also states that out of 10 people, 1 person still does not have access to improved water sources, leading to many diseases, one of the most common being diarrhea. In accordance to the Department of Health, this disease kills 1.8 million people yearly around the world. At the same time, 137,468 people around the country were recorded to experience acute watery diarrhea, and 20,290 people in the Ilocos Region have experienced acute watery diarrhea (2018).

Serpentina (*Rauvolfia serpentina*) comes from the milkweed family Apocynaceae (Rather et al., 2022), which has many good health-beneficial effects and has a potent antimicrobial activity, Alshahrani et al. (2021). Furthermore, one chemical in Serpentina (*Rauvolfia serpentina*) is similar to a prescription drug called reserpine. According to the same article, reserpine is prevalently used to help treat schizophrenia, high blood pressure, etc. According to Shivalika et al., serpentina (*Rauvolfia serpentina*) leaves have a lot of therapeutic effects, some examples are antidiarrheal, anti-inflammatory, antioxidant, etc. With the plant's antidiarrheal properties, it can be used as an alternative medicine to Diarrhea.

However, there are solutions to diarrhea that you can try at home. According to Kandola A. (2019), 5 examples you can try at home are Rehydrating, eating a recovery diet, avoiding certain foods, taking probiotics, and trying medicine. But why does the researcher still research a serpentina leaves-based syrup where there are remedies such as these five? Well, according to this same article, medications are not appropriate sometimes. Metronidazole is a good remedy but still has side effects. According to an article by NHS UK, Metronidazole can also cause diarrhea, one of its most common side effects, as the good bacteria in the gut suffer collateral damage when the antibiotic fights the pathogens that are sickening you. Loose, watery stools may be the outcome if that balance shifts, as the Mayo Clinic notes. Another mentioned alternative that you can try at home is taking probiotics, but this still has effects. According to Cleveland Clinic, 2022, taking probiotics can cause allergic reactions. Other than that, it may also trigger an upset stomach (mild), passing of gas, Diarrhea, and causes you to be bloated in the initial days after taking probiotics; people that have a weakened immune system, have a critical illness, and people that have had recent surgery are not advised to take probiotics. Although unlikely, the possible risks from taking probiotics include infection, antibiotic resistance, and potentially harmful byproducts from the supplement if you have an immune system that is weakened.

In comparison, this serpentina leaves-based syrup is also more affordable than other remedies. It is much easier to get than medicine, fixes hormones and metabolism, has natural healing, gives stronger immunity, has fewer side effects, and is cost-effective. It has also been proven to be effective against Escherichia coli. It has been proven that serpentina has antimicrobial activity against Escherichia coli, Naidu et al., 2016. Other than that, Serpentina (*Rauvolfia serpentina*) leaves have potent antidiarrheal properties and solidify serpentina's traditional uses in medicine, according to Ezeigbo et al., 2012. It is always better to care for our health; prevention is always better than cure.

Moreover, following the conduct of this study, it can quickly help many parts of the community that are experiencing the harmful effects of Diarrhea, as this study is also connected with the Sustainable Development

Goals, or SDG by the United Nations; specifically SDG 3, or good health and well-being, which tells that the health and well-being of everyone is essential, as this was done to determine the content and the microbial inhibition of Serpentina leaves against *Escherichia coli* causing Diarrhea; serving as a good alternative for the disease.

OBJECTIVES OF THE STUDY

Generally, this paper's main objectives are to know the content and the microbial inhibition activity of the serpentina *(Rauvolfia serpentina*) leaves-based syrup against *Escherichia coli* causing diarrhea. The specific objectives are:

1. To determine the serpentina leaves' phytochemical contents that can inhibit *Escherichia coli*'s growth.
2. To determine the microbial inhibition of serpentina leaves-based syrup on *Escherichia coli.*
3. To determine the shelf life of serpentina leaves-based syrup.
4. To determine the significant difference between the 4 concentrations, which uses the serpentina leaves-based syrup, and the control group, which uses a commercial product.

MATERIALS AND METHODS

This study used the Post-Test Only Control Group Design (The subjects are chosen at random and placed into 2 groups, namely the experimental group and control group, and only the experimental group receives the treatments.) to determine the syrup's effectiveness using *Rauvolfia serpentina.*

Gathering and preparations of materials

The researcher gathered 10 grams of Serpentina leaves at the researcher's Barangay at Barangay C.Lichauco, Tayug Pangasinan. The honey was bought at the town market in the researcher's town.

Laboratory analyses

Alkaloids, Flavonoids, and Tannins Determination

Screening for alkaloids, tannins, and flavonoids was conducted at a certified laboratory testing site with the help of a qualified scientist. The screening for alkaloids was based on the standard reagents used by Sudheer K. (2016), which used Mayer's reagent, Wagner's reagent, Bouchardat's reagent, and Valser's reagent to determine if alkaloids are present. The screening for flavonoids used the Cyanidin and Beta-Smith Metcalf test to determine if flavonoids are present, which is similar to the test that Manuel et al. (2014) used. The screening for tannins was based on the study of Torres et al. (2012), which also used the gelatin test, the gelatin block test, and the ferric chloride test to determine if tannins are present.

Table 1. Phytochemical screening of alkaloids

Screening for alkaloids			
Qualitative Test	Positive Results	Actual Results	Remark
Mayer's reagent	Production of ppt.	X	X
Wagner's reagent	Production of ppt.	X	X
Bouchardat's Reagent	Production of ppt.	X	X
Valser's Reagent	Production of ppt.	X	X
INTERPRETATION		X	

Legend: Where x is the actual results, Remark and Interpretation

Table 2. Phytochemical screening of flavonoids

Screening for flavonoids			
Qualitative Test	Positive Results	Actual Results	Remark
Bate-Smith Metcalf Test	Red violet color	X	X
Cyanidin Test	Color change: green / red	X	X
INTERPRETATION		X	

Legend: Where x is the actual results, Remark and Interpretation

Table 3. Phytochemical screening of tannins

Screening for tannins			
Qualitative Test	Positive Results	Actual Results	Remark
Gelatin Test	Production of ppt.	X	X
Gelatin Block Test	Production of ppt.	X	X
Ferric Chloride Test	Greenish Blue/ Greenish Black Blue	X	X
INTERPRETATION		X	

Legend: Where x is the actual results, Remark and Interpretation

Production of Syrup

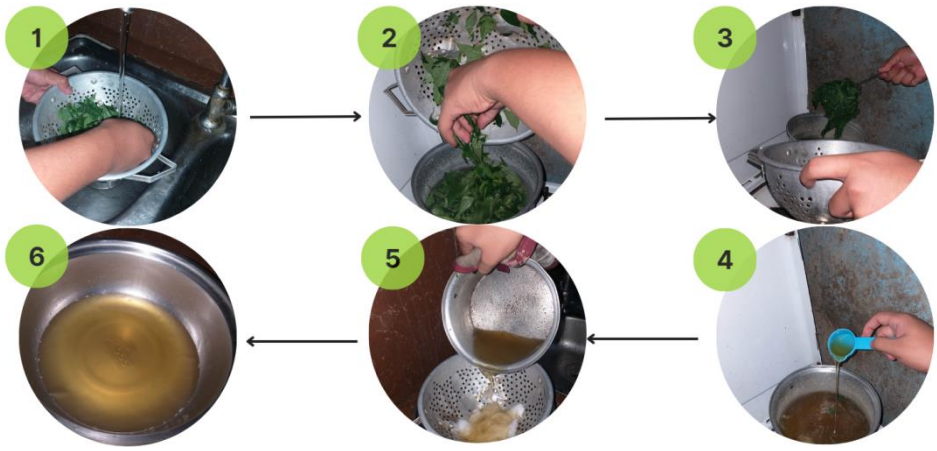

Figure 1. Production of syrup

Ten grams of serpentina leaves were added to the beaker with 750 milliliters of water and waited until it boiled. When the water with serpentina leaves boils, the serpentina leaves are removed from the beaker, and 7 spoons of honey are added and waited until it cooled down (Based on the established method by CUTM Courseware, 2020).

Disk diffusion test with 4 different concentrations, 25%, 50%, 75%, and 100%.

This study used the disk diffusion method with 4 different concentrations, namely 25%, 50%, 75%, and 100% concentration, to determine the growth of inhibition of serpentina leaves as syrup on *Escherichia coli* causing diarrhea. The microbial inhibition activity was tested at a certified laboratory testing site.

According to Kocsis et al. (2018). Disk diffusion is a standardized technique for testing the rapid growth of pathogens; this method is also classified as agar diffusion.

In the preparation of inoculums, pick around 3-5 well-isolated colonies of the microorganism and use them to inoculate it in the brain heart infusion broth. For the incubation, it should have a degrees Celsius of 37 and should last for 2-8 hours until the researchers observe a light to moderate turbidity growth. Following those, the researcher will now compare it to the McFarland Standard. After that, using a sterile cotton swab dipped in the inoculums, streak the Mueller Hinton Agar (Squeeze the swab against the tube wall to remove the excess). Using sterile forceps, pick the paper disk soaked with the product and distribute it to the inoculated agar, pressing them into firm contact. For the next 24 hours, incubate the plate at 37°C. After 24 hours of incubation, observe the zone of inhibition, measuring it by a caliper or ruler. Moreover, finally, record the result. This was based on the procedures of Jan Hudzicki (2009).

Table 4. Experimental setup for the microbial inhibition against *Escherichia coli.*

Experimental Group: Serpentina leaves-based syrup			
Trial 1	Trial 2	Trial 3	Trial 4
X/X/X	X/X/X	X/X/X	X/X/X

Legend: Where X is the percentage result of the Laboratory tests.

The experimental setup was tested with 4 different concentrations (25%, 50%, 75%, and 100%) for the antimicrobial test of the serpentina leaves syrup and was replicated 3 times at each concentration. The disk diffusion test was conducted at a certified laboratory testing site.

Table 5. Positive control set up for the microbial inhibition against *Escherichia coli.*

Experimental Group: Serpentina leaves-based syrup			
Trial 1	Trial 2	Trial 3	X
X	X	X	

Legend: Where X is the microbial inhibition and effectiveness of the commercial product

The positive control group used a commercially used drug as the treatment for the control group. The Positive control group undergoes 3 trials. It was conducted at a certified laboratory testing site.

Shelf-life determination

The shelf life can be influenced by many factors, including exposure to light, heat, and moisture; gas transmission; mechanical stresses; and contamination by microorganisms, according to Manzocco et al. (2016). The serpentina leaves-based syrup was kept at room temperature (Average room temperature in the Philippines is 25℃ to 32℃) and was observed daily. Its appearance and odor were observed, coinciding with Randive et al. (2017).

Table 6. Indicators for the deterioration of the serpentina leaves-based syrup.

Characteristics	Normal	Sign of deterioration
Appearance	Mould-free Yellow Green Color	discoloration and/or Formation of molds
Odor	Bittersweet Smell	Foul smell and/ or loss of Bittersweet Smell

Table 7. Observation of Serpentina leaves syrup at room temperature on a daily basis.

Room temperature (25℃ to 32℃)		
Characteristics	Normal	Sign of deterioration
Day 1	X	X
Day 2	X	X
Day 3	X	X
Day 4	X	X
Day 5	X	X
Day 6	X	X
Day 7	X	X
Day 8	X	X
Day 9	X	X
Day 10	X	X
Day 11	X	X
Day 12	X	X
Day 13	X	X
Day 14	X	X

Legend: Where X is the observation.

Disposal of product

After all treatments and laboratory analyses, the materials for the actual experimentation (treated and untreated) were properly disposed of through an autoclave method.

One-way ANOVA

The statistical tool used in this study is the One-way ANOVA, which aims to determine the significant difference between the positive control group, which uses the commercially used product, and the experimental group with 4 different concentrations, which uses the serpentina leaves-based syrup, coinciding with Mehta et al., 2020, in which uses the One-way ANOVA in the antifungal and antibacterial activities of extracts from 4 indigenous plants.

This is the formula that was used to determine the significant difference in the microbial inhibition of both the experimental and positive groups:

$$F = \frac{MST}{MSE}$$

$$MST = \frac{\sum_{i=1}^{k}(T_i^2/n_i) - G^2/n}{k-1}$$

$$MSE = \frac{\sum_{i=1}^{k}\sum_{j=1}^{n_i} Y_{ij}^2 - \sum_{i=1}^{k}(T_i^2/n_i)}{n-k}$$

Figure 2. Formula of One-way ANOVA

F stands for variance ratio of the overall test;
MST stands for mean square of between groups; and
MSE stands for mean square of within groups

Decision rule:

Accept Ho if the computed f- value is less than (<) f- critical value at df and level of significance; and
Reject Ho If the computed f-value is greater than (>) f- critical value at df and level of significance.

As said by Rebecca B. (2022), One-way ANOVA (Analysis of Variance) is a standard statistical tool that employs a single independent variable and examines the differences between 3 or more groups.

Data gathering and analyses

Data were collected following the experiment. The results of the laboratory analyses, such as the alkaloids, flavonoids, and tannins determination and disk diffusion test with 4 different concentrations, the result of the shelf-life determination, and the statistical analysis, specifically the one-way ANOVA of the 5 groups, were gathered. The data collected was analyzed, and conclusions in the study were drawn.

RESULTS AND DISCUSSION

Laboratory analyses

Alkaloids, flavonoids, and tannins determination

Screening for alkaloids, tannins, and flavonoids was conducted at a certified laboratory testing site with the help of a qualified scientist. The screening for alkaloids was based on the standard reagents used by Sudheer K. (2016), which used Mayer's reagent, Wagner's reagent, Bouchardat's reagent, and Valser's reagent to determine if alkaloids are present. The screening for flavonoids used the Cyanidin and Beta-Smith Metcalf test to determine if flavonoids are present, which is similar to the test that Manuel et al. (2014) used. The screening for tannins was based on the study of Torres et al. (2012), which also used the gelatin test, the gelatin block test, and the ferric chloride test to determine if tannins are present.

Table 8. Phytochemical screening of alkaloids

Screening for alkaloids			
Qualitative Test	Positive Results	Actual Results	Remark
Mayer's reagent	Production of ppt.	W/ ppt.	+
Wagner's reagent	Production of ppt.	W/ ppt.	+
Bouchardat's Reagent	Production of ppt.	W/ ppt.	+
Valser's Reagent	Production of ppt.	W/ ppt.	+
INTERPRETATION		Presence of alkaloids	

Table 8 showed that serpentina leaves have a 1 remark on Mayer's Reagent and Valser's Reagent, while the table also showed that Serpentina leaves had 2 remarks on Wagner's Radiant and Bouchardat's Reagent. Interpreting that alkaloids are present on Serpentina leaves. With the presence of Alkaloids in the leaves of Serpentina shown in the results, serpentina can inhibit the growth of *Escherichia coli* that causes diarrhea, coinciding with Yuemi et al. (2021) which, based on their study, alkaloids exhibited selective antibacterial activity against *E. coli.*

Table 9. Phytochemical screening of flavonoids

Screening for flavonoids			
Qualitative Test	Positive Results	Actual Results	Remark
Bate-Smith Metcalf Test	Red violet color	Light Yellow color sol'n	-
Cyanidin Test	Color change: green / red	W/ ppt.	+
INTERPRETATION		Presence of flavonols	

Table 9 showed that serpentina leaves have a 1 remark on the Cyanidin test and no remarks on the Bate-Smith Metcalf Test. Interpreting that flavonols, a subclass of flavonoids is present on Serpentina leaves. With flavonols still present, even though flavonoids are not, serpentina can still inhibit the growth of *E. coli* because they are a subclass of flavonoids. According to Mengying et al. (2021), every flavonoids that was tested had inhibitory effects on *E. coli*. Moreover, hydroxyl-group flavonoids (Which flavonols have in position 3 of the C ring) exhibited greater inhibitory properties than methoxyl-group flavonoids.

Table 10. Phytochemical screening of tannins

Screening for tannins			
Qualitative Test	Positive Results	Actual Results	Remark
Gelatin Test	Production of ppt.	W/ ppt.	+
Gelatin Block Test	Production of ppt.	W/ ppt.	+
Ferric Chloride Test	Greenish Blue/ Greenish Black Blue	Greenish Black sol'n	+
INTERPRETATION		Presence of tannins	

Table 10 shows that serpentina leaves have a 1 mark on all the tests used to determine the presence of tannins (The tests are the Gelatin Test, Gelatin Block Test, and Ferric Chloride Test), interpreting that tannins are present on Serpentina leaves. With the results showing the presence of tannins, it gives Serpentina leaves the ability to inhibit the growth of *Escherichia coli.*, coinciding with Gregor et al. (2020) in which tannic acid has the strongest antibacterial qualities of all the studied samples due to it inhibiting *E. coli* with the lowest MIC value.

Based on the study of Sohaib et al. (2020) the leaves, seeds, and roots of serpentina contain many phytochemical contents, where tannins, alkaloids, and flavonoids are mentioned, which can inhibit the growth of *E. coli*.

These findings indicate that serpentina leaves are a very potent aid against *Escherichia coli* causing diarrhea. Due to the presence of these chemicals, namely the alkaloids, flavonols, a subclass of flavonoids, and tannins, serpentina leaves can have a lot of implications, most notably its pharmacological and microbial inhibition implications, which are a very big help for some aspects of the community, to be more specific, aspects of the community that is experiencing the harmful effects of Diarrhea.

Disk diffusion test with 4 different concentrations, 25%, 50%, 75%, and 100%

In the preparation of inoculums, pick around 3-5 well-isolated colonies of the microorganism and use them to inoculate it in the brain heart infusion broth. For the incubation, it should have a degrees Celsius of 37 and should last for 2-8 hours until the researchers observe a light to moderate turbidity growth. Following those, the researcher will now compare it to the McFarland Standard. After that, using a sterile cotton swab dipped in the inoculums, streak the Mueller Hinton Agar (Squeeze the swab against the tube wall to remove the excess). Using sterile forceps, pick the paper disk soaked with the product and distribute it to the inoculated agar, pressing them into firm contact. For the next 24 hours, incubate the plate at 37°C. After 24 hours of incubation, observe the zone of inhibition, measuring it by a caliper or ruler. Moreover, finally, record the result. This was based on the procedures of Jan Hudzicki (2009)

Table 11. Result of the Antimicrobial Test (25% concentration)

25% Concentration of Serpentina Leaves Syrup			
Replicate 1	Replicate 2	Replicate 3	Intermediate
11mm	13mm	13mm	65% Effective

Table 11 shows that the Serpentina leaves syrup has a 65% effectiveness against *E. coli* at 25% concentration making it intermediate. 11mm, 13mm, and 13 mm are the growth inhibition of serpentina leaves syrup against *E. coli*, which makes it 65% effective or intermediate; 50% to 70% are considered Intermediate according to the certified laboratory testing site, in which the disk diffusion test occurred. A ruler or a caliper, by mm, was used to measure the zone of inhibition.

Table 12. Result of the Antimicrobial Test (50% concentration)

50% Concentration of Serpentina Leaves Syrup			
Replicate 1	Replicate 2	Replicate 3	Intermediate
13mm	13mm	12mm	65% effective

Table 12 shows that the Serpentina leaves syrup has a 65% effectiveness against *E. coli* at 50% concentration, making it intermediate. 13mm, 13mm, and 12 mm are the growth inhibition of serpentina leaves syrup against *E. coli*, which makes it 65% effective or intermediate; 50% to 70% are considered Intermediate according to the certified laboratory testing site, in which the disk diffusion test occurred. A ruler or a caliper, by mm, was used to measure the zone of inhibition.

Table 13. Result of the Antimicrobial Test (75% concentration)

75% Concentration of Serpentina Leaves Syrup			
Replicate 1	Replicate 2	Replicate 3	Intermediate
15mm	15mm	15mm	70% effective

Table 13 shows that the Serpentina leaves syrup has a 70% effectiveness against *E. coli* at 75% concentration, making it intermediate. 15mm, 15mm, and 15 mm are the growth inhibition of serpentina leaves syrup against *E. coli*, making it 70% effective or intermediate; 50% to 70% are considered Intermediate according to the certified laboratory testing site, where the disk diffusion test occurred. A ruler or a caliper, by mm, was used to measure the zone of inhibition.

Table 14. Result of the Antimicrobial Test (100% concentration)

100% Concentration of Serpentina Leaves Syrup			
Replicate 1	Replicate 2	Replicate 3	Susceptible
18mm	20mm	17mm	80% effective

Table 14 shows that the Serpentina leaves syrup has an 80% effectiveness against *E. coli* at 100% concentration, making it Susceptible. 18mm, 20mm, and 17mm are the growth inhibition of serpentina leaves syrup against *E. coli* which makes it 80% effective or Susceptible; 75% and above are considered Susceptible according to the certified laboratory testing site, in which the disk diffusion test occurred. A ruler or a caliper, by mm, was used to measure the zone of inhibition.

Table 15. Result of the Positive Control Group.

Positive control group: commercial product			
Replicate 1	Replicate 2	Replicate 3	Susceptible
24mm	24mm	26mm	95% effective

Table 15 shows that the positive control group has a 95% effectiveness against E. coli, which makes it susceptible to infection. 24mm, 24mm, and 26mm are the growth inhibition of the commercial product against *E.*

coli, making it 95%effective or susceptible; 75% and above are considered Susceptible according to the certified laboratory testing site where the disk diffusion test occurred. A ruler or a caliper, by mm, was used to measure the zone of inhibition.

In connection Rafi et al. (2021), that also used the same technique, the disk diffusion method, for their antibacterial test. They found that minimum inhibitory concentration (MIC) of aqueous leaf extract fo serpentina (RSALE) and encapsulated gold nanoparticles (R-AuNPs) stops or inhibits bacterial growth, while MIC50 of RSALE and R-AuNPs prevents or inhibits half of the bacteria, indicating that the results of this study are more effective. Even the lowest percentage of bacterial inhibition (65%) still demonstrated a higher inhibitory effect.

Based on the inhibitory efficacy of the serpentina leaves-based syrup that showed intermediate to susceptible effectiveness against *E. coli* causing diarrhea, the Serpentina leaves-based syrup can now be used as a potent aid against diarrhea, due to the pharmacological and microbial inhibition efficiency implications. Other than that, it can also be immediately used as the formulation of the serpentina leaves-based syrup can be easily replicated and is already been proven effective against *E. coli* causing diarrhea.

Shelf-life determination

The shelf life can be influenced by many factors, including exposure to light, heat, and moisture; gas transmission; mechanical stresses; and contamination by microorganisms, according to Manzocco et al. (2016). The serpentina leaves-based syrup was kept at room temperature (Average room temperature in the Philippines is 25℃ to 32℃) and was observed daily. Its appearance and odor were observed, coinciding with Randive et al. (2017).

Table 16. Result of the shelf life determination

Room temperature (25℃ to 32℃)		
Characteristics	Normal	Sign of deterioration
Day 1 (October 8, 2022)	No Change (Mold-free Yellow Green Color)	No change (Bittersweet smell)
Day 2 (October 9, 2022)	No Change (Mold-free Yellow Green Color)	No change (Bittersweet smell)
Day 3 (October 10, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 4 (October 10, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 5 (October 11, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 6 (October 12, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 7 (October 13, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 8 (October 14, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 9 (October 15, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 10 (October 16, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 11 (October 17, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)
Day 12 (October 18, 2022)	No Change (Mold-free Yellow Green Color)	No Change (Bittersweet smell)

Day 13 (October 19, 2022)	Slight Change (Mold-free losing Yellow Green Color)	No Change (Bittersweet smell)
Day 14 (October 20, 2022)	Slight Change (Mold-free losing Yellow Green Color)	Slight Change (Losing bittersweet smell)

Table 16 shows that the serpentina leaves-based syrup doesn't change from the 1st day until the 12th day at room temperature (The average room temperature in the Philippines is 25℃ to 32℃), while on the 13th day, the syrup finally changed in terms of color, starting to lose the yellow-green color; In contrast, on the 14th day, it finally changes appearance (losing its yellow-green color) and odor (losing the bittersweet smell). In conclusion, the serpentina leaves-based syrup's shelf life can last up to 2 weeks or 14 days at room temperature.

In support of this result, a study conducted by Manitoba, 2016, states that products with an 87% moisture content, which serpentina leaves have, can last up to 10 days. Still, due to the addition of honey, the shelf life can now be prolonged for a counted number of days; in this scenario, the shelf life of the serpentina leaves was extended for another 4 days, as microorganisms can't grow fast on a high amount of sugar (G. Hyslop, 2021), in which honey has.

One-way ANOVA

The statistical tool used in this study is the One-way ANOVA, which aims to determine the significant difference between the positive control group, which uses the commercially used product, and the experimental group with 4 different concentrations, which uses the serpentina leaves-based syrup, coinciding with Mehta et al., 2020, in which uses the One-way ANOVA in the antifungal and antibacterial activities of extracts from 4 indigenous plants.

This is the formula that was used to determine the significant difference in the microbial inhibition of both the experimental and positive groups:

$$F = \frac{MST}{MSE}$$

$$MST = \frac{\sum_{i=1}^{k}(T_i^2/n_i) - G^2/n}{k-1}$$

$$MSE = \frac{\sum_{i=1}^{k}\sum_{j=1}^{n_i} Y_{ij}^2 - \sum_{i=1}^{k}(T_i^2/n_i)}{n-k}$$

Figure 3. Formula of One-way ANOVA

F stands for variance ratio of the overall test;
MST stands for mean square of between groups; and
MSE stands for mean square of within groups

As said by Rebecca B. (2022), One-way ANOVA (Analysis of Variance) is a standard statistical tool that employs a single independent variable and examines the differences between 3 or more groups.

Table 17. ANOVA table

Source	SS	DF	MS	F
Between treatments	312.933	4	78.233	73.344
Within Treatments	10.667	10	1.067	
Total	323.600	14		

Legend: Where SS is the sum of squares, df is the degrees of freedom, MS is the mean square, and F is the F value.

Decision rule:

If computed f-value is less than f- critical value at df and level of significance; accept Ho; and
If computed f-value is greater than f- critical value at df and level of significance; reject Ho.

Results:

To assess whether there is a significant difference between the groups, the researcher applied the decision rule:
- if F is greater than 3.48, reject the null hypothesis
- F > 3.48
- F = 73.344

The One-way ANOVA was utilized in this study to determine the significant difference between the 5 groups of this study, namely the experimental group that are 25% concentration, 50% concentration, 75% concentration, and 100% concentration, which all uses the serpentina leaves-based syrup, and the positive control group which uses a commercially used product. The result of the statistical test using One-way ANOVA showed a significant difference in the microbial inhibition efficacy across the 5 groups as the F value, which is 73.344, is greater than (>) the F critical value, which is 3.48, that's why the null hypothesis is rejected which means there is a significant difference; $F(4,10) = 73.344$, $p < 0.05$.

CONCLUSION AND RECOMMENDATION

Conclusions

Based on the results that were obtained and analyzed, the conclusions have been drawn:

1. The phytochemical contents of serpentina leaves that can inhibit the growth of *E. coli* are alkaloids, flavonols, and tannins. Due to the presence of these chemicals, it can give a lot of implications, most notably its pharmacological and microbial inhibition implications.
2. Serpentina leaves syrup is 65% effective with 25% and 50% concentration, 70% effective with 75% concentration, and 80% effective with 100%. Serpentina leaves syrup effectiveness ranges from Intermediate to susceptible effectiveness against *Escherichia coli*. Due to this inhibitory efficacy, the syrup can now be used as a potent aid against diarrhea.
3. Serpentina leaves syrup can last for up to 2 weeks at room temperature. With this time span, individual will now have enough time to heal before the spoliation of the syrup.
4. There is a significant difference between the 5 groups, namely 25% concentration, 50% concentration, 75% concentration, and 100% concentration, which uses the serpentina leaves-based syrup, and the positive control group, which uses a commercially used product, F (73.344) > 3.48.

Recommendation

For this paper's improvement, the following recommendations were enumerated:

1. The researcher suggests that the phytochemical contents of serpentina leaves should be quantitatively analyzed.
2. The researcher suggests that another bacteria should be added alongside *Escherichia coli.*

3. The researcher suggests that the microbial analysis of the product should be tested.
4. The research suggests that after the microbial analysis is conducted, the product should be distributed to communities.
5. The researcher suggests lengthening the shelf-life of the product.

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APPENDICES

A. Shelf life determination

Figure. 4 *1st Observing day*

Figure. 5 *2nd Observing day*

Figure. 6 *3rd Observing day*

Figure. 7 *4th Observing day*

Figure. 8 *5th Observing day*

Figure. 9 *6th Observing day*

Figure. 10 *7th Observing day*

Figure. 11 *8th Observing day*

Figure. 12 *9th Observing day*

Figure. 13 *10th Observing day*

Figure. 14 *11th Observing day*

Figure. 15 *12th Observing day*

Figure. 16 *13th Observing day*

Figure. 17 *14th Observing day*

B. Phytochemical Analyses

Figure. 18 *Screening of alkaloids*

Figure. 19 *Screening of Flavonoids*

Figure. 20 *Screening of Tannins*

C. Disk Diffusion Test with 4 different concentrations

Figure. 21 *Disk Diffusion Test*

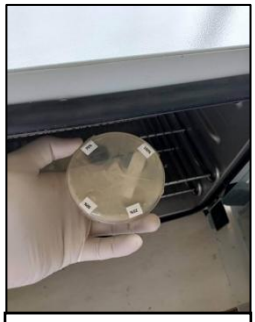

Figure. 22 *Disk Diffusion Test*

Figure. 23 *Disk Diffusion Test*

Figure. 24 *Disk Diffusion Test*