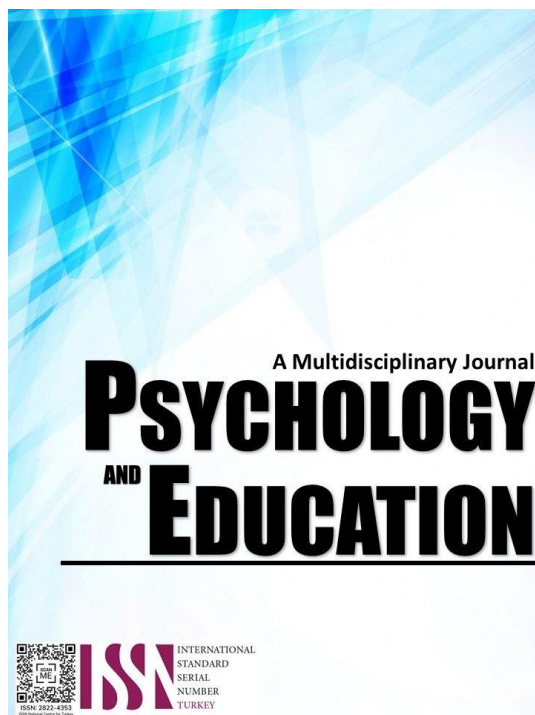


PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ANALYSIS OF PSEUDELEPHANTOPUS SPICATUS (JUSS.) ROHR. LEAF EXTRACTS



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Phytochemical Screening and Antimicrobial Analysis of *Pseudelephantopus Spicatus* (Juss.) Rohr. Leaf Extracts

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Abstract

The study determine the phytochemical components and antimicrobial activities of *Pseudelephantopus spicatus* (Juss.) Rohr. young and mature leaf. The collected leaves were air-dried for 1 week, pulverized and macerated with 95% of ethanol for 72 hours. Results of the phytochemical screening, alkaloid, saponin, tannin, phenol, protein, and carbohydrates are present in both young and mature leaf extracts. However, the diterpene test gave positive result in young leaf extract but not in mature leaf extract. Antimicrobial analysis of young and mature ethanol leaf extracts using disc diffusion method and with test microbes *E. coli*, *S. aureus*, *C. albicans*, and *A. niger* shows potent antimicrobial activities compared with the positive standards Cotrimoxazole, Ampicillin, Itraconazole and Fungisol. Generally, *Pseudelephantopus spicatus* leaf extracts contain both antibacterial activities (mean = 21.95 mm and 20.79 mm, respectively for *E. coli* and *S. aureus*, in the young leaf; mean = 23.66 mm and 21.23 mm, respectively for the same bacteria in the mature leaf) and antifungal activities (mean = 27.12 mm and 35.66 mm, respectively for *C. albicans* and *A. niger*, in the young leaf; mean = 34.96 mm and 33.43 mm, respectively for the same fungi, in the mature leaf).

Keywords: *phytochemical, screening, antimicrobial, analysis, extracts*

Introduction

Traditional and alternative medicine depended upon by many people in many developing countries including the Philippines (Elamin, Abdmageed, Shyoub and Mohammed, 2013). Botanical medicine is used by about four billion people (80%) of the world's population (World Health Organization, 2002). Herbal medicines represent a considerable proportion of the global drug market and have often maintained popularity (D' Britto, Dev, Prasad, Dwahan, Mantri and Prabhune, 2012).

Medicinal plants are useful for curing of many human diseases because of their phytochemical components that gives a defense mechanism and protection from various diseases. These phytochemicals are found in the leaves, roots, barks and stem of many plants (Nostro, Germanò, D'angelo, Marino and Cannatelli, 2000).

Pseudelephantopus spicatus (Juss.) Rohr. is medicinal plant listed in the book Medicinal Plants of the Philippines. It belongs to the Family Compositae and is commonly called Dog's Tongue. The bark of the plant is used as an antidote for snake bites in India (Gomes, Das and Sarkhel, 2010), used to cure fever, eye problems, and sprains in Jamaica (Picking, Youngerb, Mitchellc and Delgoda, 2011) and it is also used to treat dampness, nephritis, edema, scabies, and pneumonia (Lin, Yen and Chiu, 1991). It is also used to induce acute hepatic damages in rats and exhibits antifungal activity against *Candida albicans* and *Aspergillus niger* in Taiwan (Lin, et al., 1991) and exhibits antileishmanial activity (Odonne, 2011).

Research Questions

Pseudelephantopus spicatus has been widely used as herbal plants in many countries because of its medicinal properties. It is abundantly found in many waste areas in the Philippines and especially in the province of Maguindanao. The present study is conducted to determine this plant's phytochemical components and antimicrobial activities. The study determines the phytochemical components and antimicrobial activities of leaf extracts of *Pseudelephantopus spicatus*. Specifically, it answered the following queries:

1. What are the phytochemical components of young and mature leaf ethanol extracts of *Pseudelephantopus spicatus*?
2. What are the antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Escherichia coli* and *Staphylococcus aureus*?
3. Are there significant differences in antimicrobial activities between and among young and mature leaf extracts of *Pseudelephantopus spicatus* against *Escherichia coli* and *Staphylococcus aureus*?
4. What are the antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* and *Candida albicans*?
5. Are there significant differences in antimicrobial activities between and among young and mature leaf extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* and *Candida albicans*?

Methodology

Research Design

The experiment used ethanol in extracting the plant metabolites present in the leaves of the *Pseudelephantopus spicatus*. The procedure

of Tiwari et al. (2011) is used to analyze phytochemical components. For the antimicrobial analysis of the crude extract, the Kirby-Bauer test (also called disc diffusion test) was followed using two bacterial species (*Escherichia coli* and *Staphylococcus aureus*) and two fungal species (*Candida albicans* and *Aspergillus niger*).

Distilled water was used as a negative control, Cotrimoxazole and Ampicillin as positive control for antibacterial test, and Itraconazole and Fungisol as positive control in the antifungal analysis. The treatments for both antibacterial and antifungal analysis were: Antibacterial Analysis – T1 (Negative control - distilled water), T2 (Cotrimoxazole), T3 (Ampicillin), T4 (Young Leaf), and T5 (Mature Leaf); and Antifungal Analysis - T1 (Negative control - distilled water), T2 (Itraconazole), T3 (Fungisol), T4 (Young Leaf), and T5 (Mature Leaf).

Collection of Samples

Healthy and disease free leaves of *Pseudelephantopus spicatus* (Juss.) Rohr. were collected from Madia, Datu Saudi Ampatuan, Maguindanao where this plant is available and accessible for the researcher. The researcher collected young leaves from upper part of the plant and mature leaves from the middle portion. The plant samples were collected from January 17 - 30, 2019.

Materials

Apparatus

Alcohol Lamp	Graduated cylinder
Beaker	Liquid Dropper
Cheese cloth/filter paper	Test tube
Centrifuge Tube	Magnetic stirrer
Electric blender	Petri dish
Erlyenmeyer flask	Stirring rod
Florence Flask	Test tube
Funnel	Test tube rack
Forcep	Watch glass
Hot Plate	Wire Gauze

Equipment

Rotary evaporator
Laboratory oven
Analytical balance
Triple Beam Balance

Chemicals

α -naphthol solution	Hydrochloric acid
Benedict's solution	Sodium chloride
Chloroform	Sulphuric acid
Nitric acid	Ninhydrin reagent
Copper acetate solution	Lead Acetate
Distilled water	Sodium hydroxide solution
Diluted HCl	Ethanol
Nitric acid	Mueller Hinton Agar
Ferric chloride solution	Potato dextrose agar

Potassium mercuric/bismuth iodine

Positive Controls

Bacteria: Cotrimoxazole and Ampicillin

Fungi: Itraconazole and Fungisol

Microbes Used in the Study

Bacteria: *Escherichia coli* and *Staphylococcus aureus*

Fungi: *Candida albicans* and *Aspergillus niger*

Procedure

Preparation of the Plant Sample

The leaves of the plants were removed from their stem, washed thoroughly with running tap water, and air-dried in a shady area for five (7) days or until the leaves become crispy. The air-dried leaves of the plant were weighed and pulverized using electric blender.

Preparation of Plant Crude Extracts

The researcher weighed three hundred fifty grams (350 g) of the pulverized leaves in separate containers and labeled as Young leaf and mature leaf. Each bottle was filled with one and half liter of ethanol. The mixtures were allowed to stand for seventy-two (72)

hours to allow the solvent to extract the bioactive components of the plant. The two mixtures were then filtered with cheese cloth and concentrated in vacuo maintained at 40 oC. Each extract was placed in a plastic vial and labeled as young leaf extract and mature leaf extract. The bottle was covered tightly to prevent evaporation of the solvent and placed in a secured place.

Preparation of Culture Media

The antimicrobial activities of the plant extracts was determined using the standard of Kirby-Bauer test (or disc diffusion test) method which was standardized by the World Health Organization in 1961. The Mueller Hinton Agar (MHA) was used as media for *Escherichia coli* and *Staphylococcus aureus* bacteria and Potato Dextrose Agar for *Candida albicans* and *Aspergillus niger* fungi.

The Mueller Hinton Agar was prepared by dissolving 38 g of the agar in one liter (1 L) liter of distilled water in an Erlenmeyer flask. The mixture was heated with occasional stirring in a hot plate until the agar was completely dissolved. It was then sterilized and autoclaved at 121 oC for 15 minutes. The sterilized mixture was allowed to cool but not solidify in a sterile environment. Approximately 20 ml of the agar solution was poured in a sterile petri dish and covered to prevent contamination. The agar solution was then allowed to harden. The same procedure was done for Potato Dextrose Agar.

Preparation of Plant Extracts

Approximately five grams (5 g) of each of the young and mature ethanol crude extracts of *Pseudelephantopus spicatus* were placed in a sterile petri dishes. Each extract was diluted with 5 ml distilled water and was mixed with sterile glass rod. Approximately fifty (50) pieces of sterile filter disc (with diameter of 6 mm) were immersed and coated with each of the two crude extract extracts. The petri dishes were covered and set aside while preparing for the positive controls.

Preparation of Positive Controls

The positive controls for antibacterial analysis were Cotrimoxazole and Ampicillin, and Itraconazole and Fungisol for antifungal analysis. Fifty milligrams (50 mg) of each of the positive controls were dissolved in 10 mL distilled water in a sterile petri dish. Approximately fifty pieces of sterile filter disc were immersed in each of the mixture.

Inoculation of the Microbes

Two bacteria (*Escherichia coli* and *Staphylococcus aureus*) and two fungi (*Candida albicans* and *Aspergillus niger*) were used as test organisms in the antimicrobial analysis of the young and mature leaf ethanol extracts of the plants. The microbes were obtained from the Microbiology Laboratory of Notre Dame of Dadiangas University in General Santos City. Using sterile cotton swabs, each of the microbes were streaked in the prepared media.

To ensure even distribution of the microbes in the media, during the streaking process, the petri dish was rotated by 60o and the rubbing procedure was repeated twice until all the surface was approximately covered with the microbes. The media was allowed to dry for 3-5 minutes but not longer than 15 minutes for absorption of excess moisture. Thirty (30) sets of petri dishes with MHA were prepared for *E.coli* and *S. aureus* bacteria and 30 pieces with potato dextrose agar for *C. albicans* and *A. niger*. A total of sixty (60) pieces petri dishes with cultured media were prepared.

Thirty (30) pieces of petri dishes with MHA media were inoculated with *Escherichia coli* and *Staphylococcus aureus*. Another Thirty (30) pieces of petri dishes with PDA media were inoculated with *Candida albicans* and *Aspergillus niger*. Each petri dish was streaked with microbes using sterile cotton swab (also called cotton applicator) and set aside for 10 minutes to allow absorption of the moisture. The culture media then were inoculated with the extracts coated in a filter disc in three replicates. Three pieces filter discs were place in one petri dish in a triangular position as shown below. The procedure was repeated for all the remaining two plant extracts, four positive controls and one negative control. After inoculation, the petri dishes were place in an incubator maintained at 40 oC for 48 hours for bacteria *E. coli* and *S. aureus* and 72 hours for fungi *C. albicans* and *A. niger*.

Measuring the Zone of Inhibition

The zone of inhibition (measure in millimeter) is the area where the microbes were eliminated because of the inhibitory effect of the treatments. It was measured using a Vernier caliper at the widest diameter from one edge of the zone to the other edge. For *Escherichia coli* and *Staphylococcus aureus* bacteria, the zone of inhibition were measured after 48 hours and seventy-two hours (72 hrs) for the fungi *Candida albicans* and *Aspergillus niger* to allow maximum effect of the treatments. The data were recorded and tabulated in a table.

Data Analysis

The gathered data were tabulated and analyzed for their mean zone of inhibition expressed in millimeter (mm). Analysis of Variance (NOVA) at .05 degrees of freedom to determine the significance of the extracts. Statistical analysis was carried out using post hoc test for the differences in antimicrobial activities of leaf extracts of *Pseudelephantopus spicatus* against microbes and Scheffe test for pair-wise comparison of treatments. The statistical comparisons between the different treatments and the interaction between microbes as affected by different extracts were done using two-way factorial analysis of variance (ANOVA).

Results and Discussion

Phytochemical Components of *Pseudelephantopus spicatus* Extracts

The phytochemical components are found in the young and mature leaf extracts of *Pseudelephantopus spicatus* (Table 1).

Table 1. *Phytochemical Screening of Pseudelephantopus spicatus Leaf Extracts*

Phytochemical	Young Leaf Extract	Mature Leaf Extract
Alkaloids	+	+
Flavonoids	-	-
Saponins	+	+
Tannins	+	+
Diterpenes	+	-
Phenols	+	+
Protein	+	+
Carbohydrates	+	+
Phytosterols	-	-
Anthraquinone Glycosides	-	-

Legend: (+) means presence of the phytochemical component in plant crude extract
(-) means absence of the phytochemical component in plant crude extract

Alkaloids, saponins, tannins, phenols, protein, and carbohydrates are present in both young and mature leaf extracts but absent in flavonoid, phytosterol, and Anthraquinone glycosides for both young and mature leaf extracts. However, diterpene is present in young leaf extracts but absent in mature leaf extracts. The result of this study is differ to the study of Khatun (2014) on the aerial parts of *Pseudelephantopus spicatus* using methanol as solvent of the plant which contain Flavonoids, alkaloids, steroids, and tannins.

Plants having alkaloids are used for treating tumors, psychiatric and palpitation, nocturnal leg cramps and diarrhea. It was also found out that phenols act as antioxidants and reactive atoms that contribute to tissue damage in the body (Onike, 2010). The presence of saponins exhibits anti-inflammatory, anti-hepatotonic, hypoglycemic, anti-microbial and antiviral; used in detergents and molluscicides. Tannins are also used as anti-fungal, anti-biotic, anti-inflammatory, analgesic, astringent and wound healing (Visweswari, 2013).

Antimicrobial Activity of Leaf Extracts of *Pseudelephantopus spicatus* Against *Escherichia coli*

Antimicrobial activity of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Escherichia coli* is as follows (Table 2).

Table 2. *Antimicrobial Activity of Leaf Extracts of Pseudelephantopus spicatus Against Escherichia coli*

Treatment	Zone of Inhibition			Mean s.d.		* Susceptibility
	R ₁	R ₂	R ₃	(mm)		
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00	0.00	Resistant
T ₂ – Cotrimoxazole (Positive Control)	17.10	14.93	18.83	16.95	1.22	Susceptible
T ₃ – Ampicillin (Positive Control)	15.60	14.47	17.63	15.90	1.44	Intermediate
T ₄ – Young Leaf Extract	22.00	21.73	22.13	21.95	0.09	Susceptible
T ₅ – Mature Leaf Extract	24.00	24.47	22.50	23.66	1.06	Susceptible

*Susceptibility means the sensitivity of the bacteria to antibiotic expressed as:

R – Resistant, if the Mean value is ≤ 10 mm

I – Intermediate, if the Mean value is 11 – 15 mm, and

S – Susceptible, if the Mean value is ≥ 16

Among the treatments used against *Escherichia coli*, mature leaf extracts (T₅) has the highest mean value of 23.66 mm, followed by young leaf extracts (T₄) with the mean value of 21.95 mm. Cotrimoxazole (T₂) has 16.95 mm mean value while Ampicillin (T₃) has 15.90 mm mean value with intermediate susceptibility. *Escherichia coli* is susceptible to both mature and young leaf extracts. However, there is no clear zone around the petri discs of distilled water indicated by 0.00 mean value for three replicates.

Escherichia coli is susceptible to Cotrimoxazole (T₂), Young leaf extract (T₄) and Mature leaf extract (T₅). It is further supported by the study conducted by Ragasa (2001) which reported that *Pseudelephantopus spicatus* exhibits antibacterial activities.

Antimicrobial Activity of Leaf Extracts of *Pseudelephantopus spicatus* Against *Staphylococcus aureus*

Antimicrobial activity of young and mature leaf extracts of *Pseudelephantopus spicatus* against *S. aureus* is shown as follows (Table

3).

Among the treatments used against *Staphylococcus aureus*, Cotrimoxazole (T2) has the highest mean value of 21.66 mm, followed by mature leaf extracts (T5) with mean value of 21.23, while young leaf extracts (T4) has a total mean of 20.79 mm and 11.25 mm on the Ampicillin (T3). Both young and mature leaf extracts are having susceptible effect on the growth of *Staphylococcus aureus*. It is further supported by the study conducted by Ragasa (2001) which reported that the plant *Pseudelephantopus spicatus* exhibits antibacterial activities.

Table 3. *Antimicrobial Activity of Leaf Extracts of Pseudelephantopus spicatus Against Staphylococcus aureus*

Treatment	Zone of Inhibition			Mean s.d.		* Susceptibility
	R ₁	R ₂	R ₃	(mm)		
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00	0.00	Resistant
T ₂ – Cotrimoxazole (Positive Control)	19.60	21.80	23.43	21.61	2.71	Susceptible
T ₃ – Ampicillin (Positive Control)	9.33	10.00	14.43	11.25	0.45	Intermediate
T ₄ – Young Leaf Extract	20.00	20.93	21.37	20.79	0.92	Susceptible
T ₅ – Mature Leaf Extract	22.70	20.27	20.73	21.23	1.39	Susceptible

*Susceptibility means the sensitivity of the bacteria to antibiotic expressed as:

R – Resistant, if the Mean value is ≤ 10 mm

I – Intermediate, if the Mean value is 11 – 15 mm, and

S – Susceptible, if the Mean value is ≥ 16

Antimicrobial Activity of Leaf Extracts of *Pseudelephantopus spicatus* Against *Candida albicans*

Antimicrobial activity of young and mature leaf extracts of *Pseudelephantopus spicatus* against *C. albicans* is shown as follows (Table 4).

Table 4. *Antimicrobial Activity of Leaf Extracts of Pseudelephantopus spicatus Against Candida albicans*

Treatment	Zone of Inhibition			Mean (mm)	s.d.
	R ₁	R ₂	R ₃		
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00	0.00
T ₂ – Itraconazole (Positive Control)	10.63	10.80	11.40	10.94	0.54
T ₃ – Fungisol (Positive Control)	33.60	34.63	35.07	34.43	1.04
T ₄ – Young Leaf Extract	22.97	27.73	30.37	27.12	5.45
T ₅ – Mature Leaf Extract	31.03	36.70	37.17	34.97	4.34

*Susceptibility means the sensitivity of the bacteria to antibiotic expressed as:

R – Resistant, if the Mean value is ≤ 10 mm

I – Intermediate, if the Mean value is 11 – 15 mm, and

S – Susceptible, if the Mean value is ≥ 16

Among the five treatments used against *Candida albicans*, mature leaf extracts (T5) has the highest mean value of 34.97 mm followed by Fungisol (T3) with 34.43 mm mean value, while young leaf extract (T4) has 27.12 mm mean value and 10.94 mm on the Itraconazole. Mature leaf extract has better effect than young leaf extract. The antifungal activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* showing closely similar to zone of inhibition with the standard used Fungisol. It can be noticed that mature leaf extract is more effective in inhibiting *C. albicans* than the young leaf extract.

The result of this study is similar to an earliest study (Lin, Yen and Chiu, 1991) in Taiwan which exhibited antifungal activities of the leaf extracts against *Candida albicans*.

Antimicrobial Activity of Leaf Extracts of *Pseudelephantopus spicatus* Against *Aspergillus niger*

Antimicrobial activity of young and mature leaf extracts of *Pseudelephantopus spicatus* against *A. niger* is shown as follows (Table 5).

Among all the treatments, Fungisol (T3) has the highest mean value of 35.83mm followed by young leaf extract (T4) with a mean value of 35.66 mm, mature leaf extract (T5) 33.43 mm and 11.09 mm on Itraconazole (T2). The antifungal activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* showing closely similar to zone of inhibition with the standard used Fungisol.

Table 5. *Antimicrobial Activity of Leaf Extracts of Pseudelephantopus spicatus Against Aspergillus niger*

Treatment	Zone of Inhibition			Mean (mm)	s.d.
	R ₁	R ₂	R ₃		
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00	0.00
T ₂ – Itraconazole (Positive Control)	10.10	11.37	11.80	11.09	1.20
T ₃ – Fungisol (Positive Control)	35.10	37.33	35.07	35.83	0.02
T ₄ – Young Leaf Extract	37.83	33.77	35.37	35.66	1.74
T ₅ – Mature Leaf Extract	34.63	31.20	34.47	33.43	0.12

The result of this study is similar to an earliest study (Lin, Yen and Chiu, 1991) in Taiwan which exhibited antifungal activities of the leaf extracts against *Aspergillus niger*.

Summary of Antimicrobial Activities of Different Leaf Extracts of *Pseudelephantopus spicatus* Against *Escherichia coli* and *Staphylococcus aureus*

The summary of antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Escherichia coli* and *Staphylococcus aureus* are shown as follows (Table 6).

The summary of antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* expressed as zone of inhibition of the two bacterial species.

Comparing the highest mean value of the two microbes in young and mature leaf extracts, *E. coli* has the highest mean of 23.66 mm from mature leaf extracts and 21.95 mm mean value from young leaf extracts. However, on the lowest concentration, *S. aureus* with 20.79 mm under young leaf extracts and 20.79 mm mean value under mature leaf extracts.

Table 6. *Summary of Antimicrobial Activities of Different Leaf Extracts of Pseudelephantopus spicatus Against Escherichia coli and Staphylococcus aureus*

Treatment	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Mean	s.d.	Mean	s.d.
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00
T ₂ – Cotrimoxazole (Positive Control)	16.95	1.22	21.61	2.71
T ₃ – Ampicillin (Positive Control)	15.90	1.44	11.25	0.45
T ₄ – Young Leaf Extract	21.95	0.09	20.79	0.92
T ₅ – Mature Leaf Extract	23.66	1.06	21.23	1.39

Moreover, result shows that young and mature leaf extracts are better in inhibiting the growth of microbes and it is consistent to all treatments.

Summary of Antimicrobial Activities of Different Leaf Extracts of *Pseudelephantopus spicatus* Against *Candida albicans* and *Aspergillus niger*

The summary of antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Candida albicans* and *Aspergillus niger* are shown as follows (Table 7).

Comparing the highest mean value of the extracts in the microbes, young leaf extract has the highest mean value of 35.66 mm in *A. niger*, followed by mature leaf extract in *C. albicans* with a mean value of 34.97mm. Mature leaf extract in *A. niger* has 33.43 mm mean value. However, on the lowest mean value among the two extract is the young leaf extract in *C. albicans* with 27.12 mm mean value.

Moreover, result shows that young and mature leaf extracts has antifungal activities in inhibiting the growth of two bacteria. Comparing the mean value of young and mature leaves extract, mature leaf extract is consistent among two bacteria. Furthermore, among the two fungi inoculated, *A. niger* is more susceptible than *C. albicans*.

Table 7. Summary of Antimicrobial Activities of Different Leaf Extracts of *Pseudelephantopus spicatus* Against *Candida albicans* and *Aspergillus niger*

Treatment	<i>Candida albicans</i>		<i>Aspergillus niger</i>	
	Mean	s.d.	Mean	s.d.
T ₁ – Distilled H ₂ O (Negative Control)	0.00	0.00	0.00	0.00
T ₂ – Itraconazole (Positive Control)	10.94	0.54	11.09	1.20
T ₃ – Fungisol (Positive Control)	34.43	1.04	35.83	0.02
T ₄ – Young Leaf Extract	27.12	5.45	35.66	1.74
T ₅ – Mature Leaf Extract	34.97	4.34	33.43	0.12

Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Escherichia coli*

The differences in antimicrobial activities between young and mature leaf extracts of *Pseudelephantopus spicatus* against *Escherichia coli* are as follows (Table 8).

Table 8. ANOVA Results for the Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Escherichia coli*

Sources of Variation	df	SS	MS	F (ratio)	F (Prob.)	Interpretation
Between Groups	3	127.399	42.47	22.775	.000*	Significant
Within Groups	8	14.917	1.87			
Total	11	142.316				

*p < .05 is Significant at .01 level (2-tailed)

The differences in the antimicrobial activities between young and mature leaf and within the various treatments (T₁ – T₅) based on ANOVA are significant (Table 8). The measures of significant differences have the values F (3, 11) = 22.775, p = .000).

The post hoc test for differences in antimicrobial activities of different extracts of *Pseudelephantopus spicatus* against *Escherichia coli* (Table 9).

Table 9. Post Hoc Test for the Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Escherichia coli*

<i>Escherichia coli</i>		Mean	Deviation	p value	Interpretation
Cotrimoxazole	Ampicillin	1.023	.838		NS
	Young Leaf Extract	5.000	.014*		S
	Mature Leaf Extract	1.703	.538		NS
Ampicillin	Young Leaf Extract	6.023	.005*		S
	Mature Leaf Extract	7.727	.001*		S
Young Leaf Extract	Mature Leaf Extract	1.703	.538		NS

*p < .05 is Significant (S) at .01 level (2-tailed)

Pair-wise comparison using Scheffe test in Table 9 shows that Cotrimoxazole (T₂) in comparison with Ampicillin (T₃) don't exhibit significant difference. However, when Cotrimoxazole (T₂) compared with each of Young leaf extract and mature leaf extract shows statistically significant differences. This indicates that the *E. coli* bacteria are sensitive to the young leaf extract and mature leaf extract than to the Cotrimoxazole.

Moreover, when Ampicillin (T₃) compared to young leaf extract and mature leaf extract, they reveal significant differences. Still another Positive Control (Ampicillin) is resistant to *E. coli* bacteria than the two extracts coming out of *Pseudelephantopus spicatus*. But, when Young Leaf Extract and Mature Leaf Extract underwent test of difference, it found out to have no significant difference. Hence, they are mostly like the same effect on the *E. coli* bacteria. Thus, the young leaf and mature leaf extracts have better antimicrobial activities against *E. coli* compared to the positive controls such as: Cotrimoxazole and Ampicillin.

Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Staphylococcus aureus*

The differences in antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Staphylococcus aureus* are as follows (Table 10).

Table 10. ANOVA Results for the Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Staphylococcus aureus*

Sources of Variation	df	SS	MS	F (ratio)	F (Prob.)	Interpretation
Between Groups	3	221.068	73.689	20.708	.000*	Significant
Within Groups	8	28.468	3.558			
Total	11	249.536				

*p < .05 is Significant (S) at .01 level (2-tailed)

The differences in the antimicrobial activities between young and mature leaf and within the various treatments (T1 – T5) based on ANOVA are significant (Table 10). The measures of significant differences have the values $F(3, 11) = 20.708$, $p = .000$.

The post hoc test for differences in antimicrobial activities of different extracts of *Pseudelephantopus spicatus* against *Staphylococcus aureus* (Table 11).

Table 11. Post Hoc Test for the Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Staphylococcus aureus*

<i>Staphylococcus aureus</i>		Mean Deviation	p value	Interpretation
Cotrimoxazole	Ampicillin	10.177	.001*	S
	Young Leaf Extract	0.640	.981	NS
	Mature Leaf Extract	0.197	.999	NS
Ampicillin	Young Leaf Extract	9.537	.002*	S
	Mature Leaf Extract	9.980	.002*	S
Young Leaf Extract	Mature Leaf Extract	0.443	.993	NS

*p < .05 is Significant (S) at .01 level (2-tailed)

Pair-wise comparison using Scheffe test in Table 11 shows that Cotrimoxazole (T2) in comparison with Ampicillin (T3) exhibit significant difference. However, when Cotrimoxazole (T2) compared with each of Young Leaf Extract (T4) and Mature Leaf Extract (T5) showed no significant differences. This indicates that the Cotrimoxazole when compared to the Young Leaf Extract and Mature Leaf Extract have the same effect to *E. coli* bacteria susceptibility.

Moreover, when Ampicillin (T3) compared to young leaf extract and mature leaf extract extracts of *Pseudelephantopus spicatus*, they reveal significant differences. Meaning, Young Leaf Extract and Mature Leaf Extract is susceptible to *S. aureus* bacteria than the Ampicillin. But, when Young Leaf Extract and Mature Leaf Extract underwent test of difference, it found out to have no significant difference. Hence, they are most likely the same effect on the *S. aureus* bacteria.

Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus spicatus* Against *Candida albicans*

The differences in antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Candida albicans* are as follows (Table 12).

Table 12. ANOVA Results for the Differences in Antimicrobial Activities of Different Extracts of *Pseudelephantopus*

Sources of Variation	df	SS	MS	F (ratio)	F (Prob.)	Interpretation
Between Groups	3	1129.449	376.483	54.742	.000*	Significant
Within Groups	8	55.019	6.877			
Total	11	1184.468				

*p < .05 is Significant (S) at .01 level (2-tailed)

The differences in the antimicrobial activities between young and mature leaf and within the various treatments (T1 – T5) based on

ANOVA are significant (Table 12). The measures of significant differences have the values $F(3, 11) = 54.742$, $p = .000$.

The post hoc test for differences in antimicrobial activities of different extracts of *Pseudelephantopus spicatus* against *Candida albicans* (Table 13).

Pair-wise comparison using Scheffe test in Table 13 showed that Itraconazole (T2) in comparison with each of Fungisol (T3), Young Leaf Extract (T4) and Mature leaf Extract (T5) revealed significant differences. This means that each of Treatments (T3,T4,T5) is better than the Positive Control Itraconazole in susceptibility of the fungi - *Candida albicans*.

Table 13. *Post Hoc Test for the Differences in Antimicrobial Activities of Different Extracts of Pseudelephantopus spicatus Against Candida albicans*

<i>Candida albicans</i>		Mean Deviation	p value	Interpretation
Itraconazole	Fungisol	24.490	.000*	S
	Young Leaf Extract	16.180	.001*	S
	Mature Leaf Extract	24.023	.000*	S
Fungisol	Young Leaf Extract	7.310	.055	NS
	Mature Leaf Extract	0.533	.996	NS
Young Leaf Extract	Mature Leaf Extract	7.843	.040*	S

* $p < .05$ is Significant (S) at .01 level (2-tailed)

On the other hand, when Fungisol (T3) is paired with compared Young Leaf Extract and Mature Leaf Extract, it was found out to have no significant differences. This indicates that Fungisol (T3) has the same anti-fungal effects with that of Young Leaf Extract and Mature Leaf Extract.

Finally, when Young Leaf Extract and Mature Leaf Extract underwent test of difference, it found out to have significant difference. Hence, Mature Leaf Extract is better in its anti-fungal effects that the Young Leaf Extract, particularly on *Candida albicans*.

Differences in Antimicrobial Activities of Different Leaf Extracts of *Pseudelephantopus spicatus* Against *Aspergillus niger*

The differences in antimicrobial activities of young and mature leaf extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* are as follows (Table 14).

Table 14. *ANOVA Results for the Differences in Antimicrobial Activities of Different Leaf Extracts of Pseudelephantopus spicatus Against Aspergillus niger*

Sources of Variation	df	SS	MS	F (ratio)	F (Prob.)	Interpretation
Between Groups	3	1294.284	431.428	168.073	.000*	Significant
Within Groups	8	20.783	2.598			
Total	11	1315.067				

* $p < .05$ is Significant (S) at .01 level (2-tailed)

The differences in the antimicrobial activities between young and mature leaf and within the various treatments (T1 – T5) based on ANOVA are significant (Table 14). The measures of significant differences have the values $F(3, 11) = 168.073$, $p = .000$.

The post hoc test for differences in antimicrobial activities of different extracts of *Pseudelephantopus spicatus* against *Aspergillus niger* (Table 15).

Pair-wise comparison using Scheffe test in Table 15 shows that Itraconazole (T2) in comparison with each of Fungisol (T3), Young Leaf Extract (T4) and Mature Leaf Extract (T5) revealed significant differences. This means that each of Treatments (T3,T4,T5) is better than the Positive Control Itraconazole in susceptibility of the fungi - *Aspergillus niger*.

On the other hand, when Fungisol (T3) is paired with compared Young Leaf Extract and Mature Leaf Extract, it was found out to have no significant differences.

This indicates that Fungisol (T3) has the same anti-fungal effects with that of Young Leaf Extract and Mature Leaf Extract against *Aspergillus niger*.

Finally, when Young Leaf Extract and Mature Leaf Extract underwent test of difference, it found out to have no significant difference. Hence, Mature Leaf Extract is most likely the same with that the Young Leaf Extract in its anti-fungal effects on *Aspergillus niger*.

Table 15. Post Hoc Test for the Differences in Antimicrobial Activities of Different Leaf Extracts of *Pseudelephantopus spicatus* Against *Aspergillus niger*

<i>Aspergillus niger</i>		Mean Deviation	p value	Interpretation
Itraconazole	Fungisol	24.743	.000*	S
	Young Leaf Extract	24.567	.000*	S
	Mature Leaf Extract	22.343	.000*	S
Fungisol	Young Leaf Extract	0.177	.999	NS
	Mature Leaf Extract	2.400	.401	NS
Young Leaf Extract	Mature Leaf Extract	2.223	.461	NS

Conclusions

Alkaloids, saponins, tannins, phenols, proteins, and carbohydrates are found in both young and mature leaf extracts. However, in diterpene test it gives positive result only in young leaf extract.

Young and mature leaf extracts contain antibacterial and antifungal activities. The mean value shows that the four microbes *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans* were inhibited.

Future researchers should include other solvent as a base for the plant extract; should add more microbes in the study, and should isolate pure compounds from *Pseudelephantopus spicatus*. It is recommended that future include dose-response relationship. Also use other method of Bioassay to test antimicrobial activity of the plant and more replicates.

References

- Arquion, R., Galanida, C., Villamor, B., and Aguilar, H. (2015). Ethnobotanical study of indigenous plants used by local people of Agusan del Sur, Philippines. Retrieved from: <https://po.pnureserachportal.org/ejournal/index.php/apherj/article/view/102>
- Asif HM, Akram M, Saeed TM, Khan I, Akhtar N, Ur Rehman R, Ali Shah SM, Ahmed K, Shaheen G. (2011). Review paper carbohydrates. International Research Journal of Biochemistry and Bioinformatics. 2011;1(1):001-005. Retrieved from: <https://www.interestjournals.org/IRJBB>
- Baguio, M. (2018). Antimicrobial Activity Of The Ethanol Extract Of *Caesalpinia Pulcherrima* (Linn.) Sw. Leaves. Master's thesis from Mindanao State University – Graduate School, Tamontaka, Datu Odin Sinsuat, Maguindanao.
- Bekalo, T. (2009). An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. Journal of Ethnobiology and Ethnomedicine. Retrieved from: <https://ethnobiomed.biomedcentral.com/ARTICLES/10.1186/1746-4269-5-26>
- Brazier, Yvette. (2018). "What are bacteria and what do they do?." Medical News Today. MediLexicon, Intl., 31 Oct. 2017. Web. 18 Dec. 2018. Retrieved from: <https://www.medicalnewstoday.com/articles/157973.php>
- Brook, G., Butel, J., and Ornston, N. (1997). Medical Biology. 20th Edition. San Francisco, C.A.
- Croteau R, Kutchan, TM, Lewis NG. (2000). Natural products (secondary metabolites). In Buchanan B, Gruissem W, Jones R (Eds.), Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists. 2000:12501318. Retrieved from: www.sciencedirect.com/reference/165048
- Dixon, R.A. (2001). Natural products and plant disease resistance. Nature 411:843-847. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/11459067>
- D' Britto V., Dev P.P., Prasad B.L.V., Dwahan A., Mantri V.G. and Prabhune A. (2012). Medicinal plant extracts used for blood sugar and obesity therapy shows excellent inhibition of invertase activity: synthesis of nanoparticles using this extract and its cytotoxic and genotoxic effects, International Journal of Life Science and Pharma Research, 2(3), 61-74. Retrieved from: <https://books.google.com.ph>
- Elamin A.E., Abdmageed M.A.M., Shyoub M.E. and Mohammed R. R. (2013). Evaluation of Toxicity of some plants having traditional uses in Sudan on Brine Shrimp, Global Journal of Traditional Medicinal Systems, 2(1), 19-23. Retrieved from: <https://www.researchgate.net>
- Gest, Howard. (2003). Microbes: An Invisible Universe. Revised Edition. Washington D.C. ASM Press. 1752 N St. N.W. Washington, DC, USA.

- Gomes A., Das R. and Sarkhel S. (2010). Herbs and herbal constituents active against snake bite, Indian Journal of Experimental Biology, 48, 865-878. Retrieved from: <https://www.researchgate.net>
- Hendrix, Mary Lynn. (2008) "Protein." Microsoft® Encarta® 2009 [DVD]. Redmond, WA: Microsoft Corporation.
- Jean Bouchaudon, Pierre Le Roy, and Mayer Naoum Messer. (1974). "Process for the preparation of ampicillin." U.S. Patent US3978078. Retrieved from: <https://www.drugbank.ca/drugs/DB00415>
- Jong-Soo Woo, Hong-Gi Yi. (1998). "Antifungal oral composition containing itraconazole and process for preparing same." U.S. Patent US6039981. Drug created on June 13, 2005 07:24 / Updated on April 27, 2019 05:44. <https://www.drugbank.ca/drugs/DB01167>
- Keeling CI, Bohlmann J. (2006). Genes, enzymes, and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytologist; 170:657-675. Retrieved from: <https://www.ncbi.nlm.nih.gov>
- Khatun, Rahman, Haque, Akter, and Jhumur. (2014). Cytotoxicity Potentials of Eleven Bangladeshi Medicinal Plants. Hindawi Publishing Corporation. The Scientific World Journal. Volume 2014, article ID 913127. Retrieved from: <http://dx.doi.org/10.1155/2014/913127>
- Lalisan, Jeda a., Nuñega, Olga M., Uy, Mylene M. (2014). Brine Shrimp (*Artemia salina*) Bioassay of the medicinal plant *Pseudelephantopus spicatus* from Iligan City, Philippines. Retrieved from: <https://pdfs.semanticscholar.org>
- Lin C.C., Yen M.H., and Chiu H.F. (1991). The pharmacological and pathological studies on Taiwan folk medicine (VI): The effects of *Elephantopus scaber* subsp. *oblanceolata*, *E. mollis* and *Pseudelephantopus spicatus*, American Journal of Chinese Medicine, 19(1), 41-50. Retrieved from: <https://www.ncbi.nlm.nih.gov>
- Microbiology Society. (2018). <https://microbiologyonline.org/about-microbiology/introducing-microbes/fungi>
- Middleton E. Jr. (1998). Effect of plant flavonoids on immune and inflammatory cell function. Advances in Experimental Medicine and Biology; 439:175-182. Retrieved from: <https://link.springer.com>
- Ncube NS, Afolayan AJ, Okoh AI. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806. Retrieved from: <https://www.ajol.info>
- Odonne, G., G. Herbet, V. Eparviera, G. Bourdye, R. Rojas, M. Sauvaine and D. Stien. (2011). Antileishmanial sesquiterpene lactones from *pseudelephantopus spicatus*, a traditional remedy from the chayahuira amerindians (peru). Part III Journal of Ethnopharmacology 137: 875– 879. Retrieved from: <https://www.ncbi.nlm.nih.gov>
- Onike, Rahaman. (2010). Phytochemical Screening Tests and Medicinal Values of Plants Active Properties. Phytochemical Screening Tests and Medicinal Values of Plants Active Properties. Retrieved from: <https://wrytestuff.com/swa599816-Phytochemical-Screening-Test-And-Medicinal-Values-of-Plants-Active-Properties.htm> on 20/11/2010
- Phoebe Hinton – Sheley. (2018). <https://www.news-medical.net/life-sciences/What-is-Aspergillus-niger.aspx>
- Picking D., Younger N., Mitchell S. and Delgoda R. (2011). The prevalence of herbal medicine home use and concomitant use with pharmaceutical medicines in Jamaica, Journal of Ethnopharmacology, 137(1), 305-311. Retrieved from: <https://www.ncbi.nlm.nih.gov>
- Quisumbing, Eduardo. (1978). Medicinal Plants of the Philippines. The Publishing Co., Inc., Quezon City, Philippines.
- Ragasa C. and Rideout J. (2001). An Antifungal Cadinanolide from *Pseudelephantopus spicatus*, Chemical and Pharmaceutical Bulletin, 49(10), 1359-1361. Retrieved from: <https://www.researchgate.net>
- Shahriar M, Bahar ANM, Hossain MI, Akhter S, Haque MA, Bhuiyan MA. (2012). Preliminary phytochemical screening, In vitro Anti-oxidant and cytotoxic activity of five different extracts of *Withania somnifera* root. International Journal of Pharmacy; 2:450-453 ISSN 2249-18848. Retrieved from: <https://www.researchgate.net>
- Sarr, C., Evers, C., and Sarr, L., (2012). Biology: Applications and Concepts. 8th Edition. Cengage Learning Asia Pte Ltd. Pasig City, Philippines.
- Tendencia, E. A. (2004). Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment (pp. 13-29). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. Retrieved from: www.seafdec.org.ph
- Tiwari, P., Kamar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical Screening and Extraction: A Review. International Pharmaceutica Scientia. Jan-Mar, 2011/Vol. I/Issue I. Retrieved from: <http://www.ipharmaceutica.com>
- Veena Sharma, Sadhana Sharma, Pracheta, and Ritu Paliwal. (2011). *Withania somnifera*: A rejuvenating ayurvedic medicinal herb for the treatment of various human ailments. International Journal of Pharmatech Research; 3:187-192. Retrieved from: <https://www.researchgate.net/>

publication/230852241_Withania_somnifera_A_rejuvenating_ayurvedic_medicinal_herb_for_the_treatment_of_various_humanaillments

Vincenzo, L., Lattanzio, V., Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects Phytochemistry. Advances in Research 2006: 23-67 ISBN: 81-308.0034-9. Retrieved from: <https://trnres.com>

Visweswari G, Christopher R and Rajendra W. (2013). Phytochemical screening of active secondary metabolites present in Withania somnifera root: Role in traditional medicine. Karnataka, India. Int J Pharm Sci Res 2013; 4(7); 2770-2776. doi: 10.13040/IJPSR.09758232.4(7).2770-76. Retrieved from: www.sciepub.com

Wagner H, Norr H, Winterhoff H. (1994). Plant adaptogens. Phytomedicine 1994; 1:63-76. Retrieved from: [https://doi.org/10.1016/S0944-113\(11\)80025-5](https://doi.org/10.1016/S0944-113(11)80025-5)

World Health Organization. (2002) Who launches the first global strategy on traditional medicine. Geneva: Press release WHO 2002/38, <http://www.who.int/mediacentre/news/releases/release38/en>.

Zwenger S, Basu C. (2008). Plant terpenoids: applications and potentials. Biotechnology and Molecular Biology Reviews; 3:001-007. Retrieved from: <https://www.sciencedirect.com/science/article/pii/S1074552114002415>

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