ETHNOMEDICINAL VALUE, PHYTOCHEMICAL EVALUATION, AND PHARMACO-TOXICOLOGICAL PROFILING OF *Cipadessa baccifera* (Roth) Miq. AMONG COMMUNITIES OF BOKOD, BENGUET

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ABSTRACT

Cipadessa baccifera (Roth) Miq was studied for its traditional use, phytochemical properties, and pharmaco-toxicological properties. The descriptive qualitative method was used in this study to describe the traditional use of the medicinal plant, and the experimental quantitative method was used to determine the plant's components and capabilities. Assays such as Thin Layer Chromatography (TLC), Chorioallantoic Membrane Assay (CAM), Brine Shrimp Assay, and Zebrafish Embryotoxicity Assay were done to profile the C. baccifera. Based on the results, traditionally, the plant parts were used as a treatment for diarrhea, toothache, cough, colds, indigestion, flu symptoms, dysmenorrhea, urinary tract infection, bleeding and swelling of gums, skin rashes, lowering blood sugar, dandruff, natural family planning, animal farm deworming, and skin diseases. Its phytochemical contents include anthraquinones, anthrones, alkaloids, flavonoids, fatty acids, triterpenes, steroids, phenols, and essential oils. Furthermore, the CAM vascularity inhibition of Cipadessa baccifera concentrations increased with concentration, indicating that the inhibition of vascularity increased with concentration. Similarly, the brine shrimp cytotoxicity test revealed that the number of shrimp deaths increased as the concentration of plant extract increased. Furthermore, the plant extract's calculated LC50 was 434ppm. Microscopic analysis of the zebrafish embryo exposed to the ethanolic extract of the plant sample, on the other hand, revealed malformation in the larvae exhibiting scoliosis at 100ppm extract concentration. Additionally, at plant extract concentrations ranging from 250ppm to 1000ppm, retarded and coagulated embryos were observed. Given the findings of the research study, it is highly recommended that future studies include the ecology and biology of C. baccifera in the Philippines to establish a further taxonomic classification of C. baccifera endemic to the country.

Keywords: Pharmaco-toxicological properties, phytochemical analysis, CAM Assay, Embryotoxicity, Philippines

INTRODUCTION

Plant-derived medicines are frequently used to treat diseases in rural areas. This is due to the remoteness of health centers and hospitals, the constant availability of herbal medicines, and the fact that it is free. Users understand the specific part of the plant to be used, how to use it, the dosage, and the potency of various herbal plants.

Therefore, they are not at risk of being poisoned. Furthermore, most medicinal plant users use estimated dosages and follow the principle that "too much of anything is dreadful." So, once they felt relieved, they stopped taking the herbal extract. The utilization of herbal plants is also based on consumers' personal and collective experiences. They are thus unsure of all the illnesses that a herbal plant can cure. And these include *Cipadessa baccifera* (Roth) Miq., commonly called “shael or dael” in the community of Bokod, Benguet.

On the other hand, academically speaking, students frequently receive broad education on the value of biodiversity and the repercussions of resource degradation. However, it is frequently ignored to mention the significance of a particular plant or animal species to other species (Millstein, 2013). Educators frequently emphasize the importance of trees, plants, and other living things, but they rarely discuss each one's specific significance. Thus, this study may provide additional pieces of evidence. In the meantime, the Traditional and Alternative Medicine Act (TAMA) of 1997 recognizes the use of herbal medicine and encourages scientific research on alternative medicines.

The *Cipadessa plant* is said to be able to treat a variety of diseases. In contrast to other herbal plants, it was used not only to treat a variety of illnesses but also as a safe and effective natural birth control method. However, excessive use can result in infertility. Furthermore, the community claims that most bitter plants help treat illnesses, and this plant has a bitter, astringent flavor.

Cipadessa baccifera (Roth) Miq. is a member of Meliaceae, locally known as "shael or dael" by the Ibaloi, Kalanguya, and Karao communities of Bokod, Benguet. It is a shrub that may be found in forest reserves and lands close to agricultural areas in Benguet Philippines. The medium-sized tree has distinctive brown outer and scarlet interior bark. The leaflets are oppositely organized, oval-elliptic, with an inequilateral base and acuminate tip, and measure 2.7-9.8 cm in length and 0.8-4.9 cm in breadth. The petiole is about 0.3-0.7 cm long. The leaves have a bitter flavor and are odd-pinnately, spirally oriented. When young, the flowers are white and the fruits are green; when fully ripe, they turn violet (Figure 1). Compared to pine trees, this plant receives less attention from conservationists. Additionally, *Cipadessa baccifera* was discovered in Thailand, China, India, Vietnam, and Indonesia (JSTOR Global Plants, 2021).

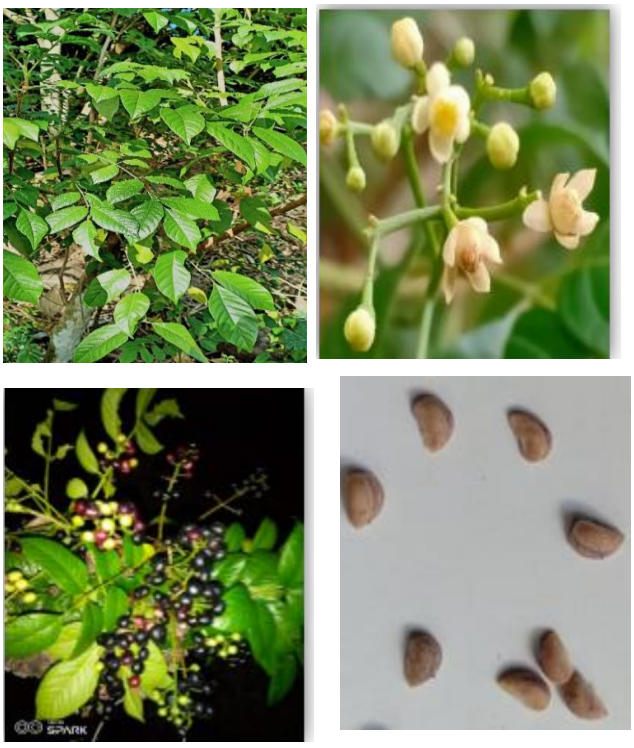

Figure 1. Leaves, flowers, fruit, and seeds of Cipadessa baccifera

OBJECTIVES OF THE STUDY

This study sought to document the traditional medicinal importance of Cipadessa baccifera with its pharmaco-toxicological profiles, specifically the following:

1. To describe the traditional use of *Cipadessa baccifera found in Bokod, Benguet;*

2. To determine the pharmaco-toxicological profiles of *Cipadessa baccifera* in terms of

    2.1. Phytochemical analysis,
    2.2. CAM vascularity Bioassay,
    2.3. Cytotoxicity Bioassay, and
    2.4. Teratogenic Assay;

3. To identify the LC_{50} of the ethanolic extract of *C. baccifera*; and
4. To find the significant differences between the concentrations used in

    4.1. Cytotoxicity test (1000ppm, 750ppm, 500ppm, 250ppm, 100ppm),
    4.2. CAM vascularity Assay (1000ppm, 750ppm, 500ppm, 250ppm, 100ppm).

METHODOLOGY

Research Design. The study utilized descriptive qualitative and quantitative experimental design. A descriptive study can respond to inquiries about what, where, when, and how, but not why (McCombes, 2020). While an experiment is a type of research method that involves the manipulation of one or more independent variables and measuring their effect on one or more dependent variables (McCombes, 2020).

A survey interview on the plant's traditional uses using a researcher-made questionnaire was the first stage of the investigation. Based on the results of the interview, an experimental study was conducted.

Research Respondents. The respondents for the survey conducted were 72 male and female Senior Citizens, 60 years old and above coming from the ten barangays of Bokod, Benguet. They were randomly selected based on their age, their knowledge of herbal medicines, and their willingness to participate in the study.

Data Gathering Procedure. Collection of Plant Material. Cipadessa baccifera leaves, both young and old, were collected and then thoroughly washed and air-dried.

Ethanolic Extraction of C. baccifera A 50 g sample of air-dried Cipadessa baccifera leaves were mixed with 250 ml of pure ethanol (90%). The filtrates were collected and filtered through filter paper into sterile 500ml Erlenmeyer flasks. These were labeled properly, wrapped in aluminum foil, and refrigerated for further testing.

The extracts were placed in a water bath for 72 hours at 50°C, to remove excess alcohol. The researchers used a serial dilution procedure to generate concentrations of 1000 parts per million (ppm), 750 ppm, 500 ppm, 250 ppm, and 100 ppm from one milligram of the plant's crude extract in 1L of distilled water.

Phytochemical Analysis. The presence of any secondary metabolites was determined through phytochemical screening of each plant extract. Each plant extract was spotted on a 7 x 4 cm TLC that had been marked and labeled before being developed in an acetate-methanol (7:3) solution in the developing chamber. The spots on the TLC plates for a specific metabolite were observed and then exposed to UV light and a hot plate to evaluate the separation of the individual components (Claustra et al., 2005).

Brine Shrimp Assay. In a petri dish, 38 grams of sea salt and one liter of distilled water were combined to make a saltwater solution that was used to hatch brine shrimp. 15 g of brine eggs were combined with water. Five inches away from the Petri plate was a 60-100 watt light bulb. The eggs and nauplii (larva) were collected after another 24 hours of observation. They were separated from the empty eggs, and 10 nauplii were pipetted into each of the six-well plates. Plant ethanolic extracts in concentrations of 1000 ppm, 750 ppm, 500 ppm, 250 ppm, and 100 ppm were applied to nauplii. The number of survivors was counted every three hours, and the death percentage was computed. The absence of controlled forward motion during 30 seconds of observation was the bioassay's mortality endpoint. For each concentration and control, the nauplii's percent lethality was calculated using the formula below. The percentage of death was calculated by counting the number of dead and alive nauplii on

each plate. (Sarah, et.al, 2017). The following concentrations were used: 1000 ppm, 750 ppm, 500 ppm, 250 ppm, and 100 ppm.

$$\% \text{ Death} = \left(\frac{Number\ of\ Dead\ Nauplii}{Number\ of\ Dead\ Nauplii + Number\ of\ Live\ Nauplii}\right) 100$$

Chorioallantoic Membrane Assay. Thirty-five fertilized duck embryos aged 8 days were incubated for two days at 37.5°C, and 70% humidity. Before windowing, a cotton gauss was soaked in 70% isopropyl alcohol and used to wipe the ducks' shells. A one-by-one cm window was cut in the shell to expose the CAM for experimental manipulation. The sterile filter paper discs absorbed the test plant extract. The treated filter paper discs were then placed onto the CAM after 24 hours of soaking. The treated eggs were incubated for two days after being sealed with sterile plastic tape. The CAMs were harvested on the tenth day of incubation by counting the proangiogenic and antiangiogenic vasculature. Concentrations of 1000 ppm, 750 ppm, 500 ppm, 250 ppm, and 100 ppm were used. The CAM vascularity inhibition was expressed as a percentage of the control using the formula below:

$$\% \text{ CAM vascularity inhibition} = \left(\frac{No.of\ branch\ points\ (treated) - No.of\ branch\ points\ (negative\ control)}{No\ of\ Branch\ points\ (negative\ control)}\right) x\ 100$$

Zebrafish Embryotoxicity and Teratogenicity. Zebrafish embryos were exposed for 96 hours in 96-well plates to 1000ppm, 750ppm, 500ppm, 250ppm, and 100ppm plant extract with eight replicates for each. Aquarium water was used as a negative control. Then, the zebrafish embryos were examined under the microscope after 24 hours.

Treatment of Data. The result of the survey and interview was descriptively interpreted. Analysis of variance (ANOVA), using robust variants of the F-statistic, and effect size omega squared (ω2) was used to determine the difference in the antiangiogenic and vascularity inhibition properties of *C. baccifera* across treatment groups.Fisher's exact test (G) and Cramer's V effect size estimate were used to find out the difference in cytotoxicity across treatment groups. Probit analyses were performed to determine the LC_{50} of *C. baccifera* extract. All data were imported and analyzed in SPSS Statistics version 26, and inferential statistics assumed an alpha level of 0.05.

RESULTS AND DISCUSSION

1. Traditional Uses of *Cipadessa baccifera* ("Shael")

The source of information on traditional applications of *C. baccifera* were 72 Senior Citizens, 60 years and older, from the ten barangays of Bokod, Benguet Municipality, with 22 (31%) men and 50 (69.44%) women. Table one summarizes each plant's specific uses, including where and how to use it as herbal medicine. Ninety-seven percent of respondents mentioned the utilization of plant leaves, 8% explained the usability of stems, and 1% mentioned roots. Eighty-five percent of the participants mentioned that plant extracts of "shael" could be used as a treatment for diarrhea or loose bowel movement (LBM), 36% for stomachache, 11% for dysmenorrhea, 19% for flu and flu-like symptoms, 4% for birth control, 4% for skin diseases and wounds, 3% for toothache, 2% for birth control, and 1% for others.

Cipadessa baccifera has been traditionally used as an alternative medicine for different ailments such as diarrhea, toothache, stomachache, cough, colds, bleeding and swelling gums, and indigestion for children and adult ages. The community of Bokod Benguet refers *C. baccifera* as "*empai*t" or "*mankel-it*" because of its bitter taste. Despite this, *C. baccifera* in certain parts of Bokod, Benguet has been widely considered as herbal traditional medicine for an array of threatening diseases. The elderlies of the Bokod community also claimed that *C. baccifera* has been also effective as an alternative contraceptive formula for women. The ethnomedical use of this plant as an herbal

medicine in the communities of Bokod has not been scientifically proven, however, the traditional use of this plant has made rounds across the globe.

Table 1

Traditional Uses of "Shael" Plant (Cipadessa baccifera)

Part of Plant	Where to Use	How to Utilize
Leaves	For Diarrhea/LBM	Boil 1 *tangkay* (stem) of leaves plus 1 cup water. Do not overboil. Then drink the concoction or boil water, then soak the leaves for 10 minutes. Then drink the concoction for 1 day only, 1-3 cups, depending on severity. Or Squeeze the leaves, crash, and then immediately sip the solution. It can be used for human and animal diarrhea. The stem could also be used.
	Toothache	Boil the leaves in water and then gargle with it. Or Blend some leaves into a paste and apply it to the sore tooth.
	Stomachache	Boil the leaves, then consume one cup or more. Or Ground the dried leaves into a powder then use it as tea.
	For Natural Family Planning	Boil the leaves for a few minutes then drink I cup before and after intercourse.
	As antidiabetic	Boil the leaves in water and drink the decoction.
	For treatment of Flu and flu-like symptoms: cough/colds/fever/body pains	Bathe in water that has been boiled with the plant leaves.
	Relief of dysmenorrhea	Boil water, remove from heat, add 8–10 leaves, and then after 3 minutes, sip the resulting fluid.
	Decrease in blood sugar/diabetes	Boil the leaves, then consume 1 cup daily.
	Urinary tract infection (UTI)/ urine with blood	Boil the leaves, then take 1-2 cups of the resulting concoction to feel better.
	Skin rashes	Boil the leaves then use them as bathwater.
	Removal of dandruff	
	Animal deworming	Boil the leaves, then place them in a bottle and allow the animal (for example, a cow) to drink the concoction.
	Animal (ex. cow, carabao) cures for skin diseases, boils, and wounds.	Use powdered dried leaves or crush and grind fresh leaves to apply the plant extract directly to the animals' skin twice daily until it is visibly healed.
Roots	For treatment of indigestion	Boil the roots, then drink the decoction.
	Flu	
Bark Skin	Bleeding and swelling gums	Slice off a little piece of bark skin, then apply it like a plaster to the gums that are swollen or bleeding. Or Utilize the paste-like material made from plant bark skin, and then apply it directly to the affected area.
Other Use		
Leaves	As "condiment" for hard drinks (liquor)	Add a few leaves to 1 bottle of liquor. Accordingly, it reduces the effect of hangovers and reduces the bitter taste of the liquor.

According to the report (Flowers of India, ND) the leaves of *C. bacciferra* have been used in significant parts of India as a powerful anti-venom property, especially for the treatment of cobra poison. It is also used in treating cough, cold, and diabetes. Phytoaccumulation of mineral elements in *C. baccifera* leaf has been also proven in the study conducted by (Jebarubi et al, 2018). Notably, men also tend to add leaves of the plant to their drinking alcohol, which according to them it reduces the effect of hangovers and the bitter taste of liquor. Plant leaves were the most widely utilized component. It can be used by boiling and then drinking the resulting decoction.

2. Phytochemical Contents of *Cipadessa baccifera*

Table 2
Phytochemistry of "Shael" Plant (C. bacciferra)

Phytochemical Components	
Anthraquinones	Present
Anthrones	Present
Alkaloids	Present
Flavonoids	Present
Fatty acids	Present
Triterpenes	Present
Steroids	Present
Phenols	Present
Essential Oils	Present

It can be gleaned from table 2 that *C. baccifera* contains amounts of phytochemicals that have pharmaceutical value. These include anthraquinones, anthrones, alkaloids, flavonoids, fatty acids, triterpenes, steroids, phenols, and essential oils. A similar result was found by Jeevitha et al (2017) in the phytochemistry of *C. baccifera*. In addition to these compounds, they found glycosides, amino acids, carbohydrates, saponins, and phytosterol (Jeevitha et al., 2017; Bakshu et al., 2016; Archaya, 2014), coumarin (Murugammal & Ilavarasan, 2016), however, Markute and Shinde (2019) did not detect glycoside in their analysis; and flavonoid and terpenoid were negative for the test conducted by Venkata et al. (2010) but positive for a steroid. Accordingly, the presence of flavonoids, phenols, and triterpenes acts as antioxidants, protecting the cells from free radical damage (Roghini & Vijayalakshmi, 2018). Hence, the presence of alkaloids indicates that *C. baccifera* might have a biologically active component and is partially responsible for its antimicrobial activity. Moreover, the presence of disease-preventive antioxidants and antimicrobial molecules suggests that *Cipadessa baccifera* has pharmaceutical value. However, the traditional use of *Cipadessa baccifera* in the Bokod Benguet communities to treat diarrhea, toothache, stomachache, and diabetes warrants further scientific investigation.

3. CAM Vascularity of *Cipadessa baccifera*

3.1. The CAM profile of C. baccifera

Table 3
CAM Vascularity Inhibition of C. baccifera Ethanolic Extract

Replicates	Concentration of Plant Extract					Ibuprofen (+ control)	Water (- control)
	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm		
1	27	28	38	38	40	35	25
2	26	28	37	37	37	36	26
3	29	29	35	36	35	32	30
4	29	30	35	36	38	38	27
5	32	28	35	32	36	40	28
6	30	30	30	32	32	44	28
7	30	32	36	40	37	35	28
Mean	29	29.29	35.14	35.85	36.43	37.15	27.43
CAM Vascularity Inhibition	5.72%	6.78%	28.11%	30.69%	32.77%		

The CAM vascularity inhibition of *Cipadessa baccifera* at various concentrations is shown in Table 3. As the concentration rises, the amount of biochemicals in the plant sample becomes more concentrated, which explains why vascularity inhibition rises with concentration. The flavonoids and phenols found in the ethanolic extract of the plant *Cipadessa baccifera* have been linked to anti-tumor and anti-metastatic properties. This model and research, however, are not being used to determine the exact and most effective concentrations or to make a direct translation to clinical and medical application, but rather to determine general trends and interactions of

models and drugs to determine if these therapies are viable treatment options that should be investigated further (Ho and Kuo, 2007).

4. Statistical difference of C. baccifera extracts in terms of anti-angiogenic and vascularity inhibition

Table 4

Descriptive and Inferential Statistics on the Difference in the Antiangiogenic and Vascularity Inhibition Properties of C. baccifera Across Treatment Groups

	Descriptives		Test of difference		
Concentration	***M***	***SD***	***F***	***p***	**ω^2**
Number of branch points with antiangiogenic vasculature					
100 ppm	29.00	2.00	18.15[a,b]	**.000***	.647[L]
250 ppm	29.29	1.50			
500 ppm	35.14	2.54			
750 ppm	35.86	2.97			
1000 ppm	36.43	2.51			
Vascularity inhibition (%)					
100 ppm	5.81	5.77	8.37[c,d]	**.000***	.457[L]
250 ppm	6.99	6.47			
500 ppm	28.76	15.01			
750 ppm	31.30	15.24			
1000 ppm	33.53	15.87			

Note: a: $df_1 = 4$, $df_2 = 14.74$ b: Based on Welch's method
c: $df_1 = 4$, $df_2 = 21.60$ d: Based on Brown-Forsythe method
*: Significant L: Large effect size

There was a significant difference in the number of branch points with antiangiogenic vasculature, $F(4, 14.74) = 18.15$, $p < .0005$, $\omega^2 = .647$, and the CAM vascularity inhibition, $F(4, 21.60) = 8.37$, $p < .0005$, $\omega^2 = .457$, across treatments with varying level of concentrations. The large effect size estimate also indicates that the difference of at least one group is very large.

5. Multiple pairwise comparisons of treatment groups

Table 5

Multiple Pairwise Comparison of Treatment Groups for the Difference in the Antiangiogenic and Vascularity Inhibition of C. baccifera

Comparisons	M_{diff}	*SE*	*p*	*d*
Number of branch points with antiangiogenic vasculature				
100 ppm vs. 250 ppm	−0.29	0.94	.998[ns]	0.16
100 ppm vs. 500 ppm	−6.14	1.22	.003*	2.68[L]
100 ppm vs. 750 ppm	−6.86	1.35	.003*	2.71[L]
100 ppm vs. 1000 ppm	−7.43	1.21	.000*	3.28[L]
250 ppm vs. 500 ppm	−5.86	1.12	.003*	2.81[L]
250 ppm vs. 750 ppm	−6.57	1.26	.004*	2.80[L]
250 ppm vs. 1000 ppm	−7.14	1.10	.001*	3.46[L]
500 ppm vs. 750 ppm	−0.71	1.48	.987[ns]	0.26[S]
500 ppm vs. 1000 ppm	−1.29	1.35	.871[ns]	0.51[M]
750 ppm vs. 1000 ppm	−0.57	1.47	.994[ns]	0.21[S]
Vascularity inhibition (%)				
100 ppm vs. 250 ppm	−1.18	3.28	.996[ns]	0.19
100 ppm vs. 500 ppm	−22.95	6.08	.034*	2.02[L]
100 ppm vs. 750 ppm	−25.49	6.16	.022*	2.21[L]
100 ppm vs. 1000 ppm	−27.72	6.38	.017*	2.32[L]
250 ppm vs. 500 ppm	−21.77	6.18	.044*	1.88[L]
250 ppm vs. 750 ppm	−24.31	6.26	.028*	2.08[L]
250 ppm vs. 1000 ppm	−26.55	6.48	.021*	2.19[L]
500 ppm vs. 750 ppm	−2.54	8.09	.998[ns]	0.17
500 ppm vs. 1000 ppm	−4.77	8.26	.976[ns]	0.31[S]
750 ppm vs. 1000 ppm	−2.23	8.32	.999[ns]	0.14

Note: * : Significant *ns:* Not significant
S: Small effect size M: Medium effect size
L: Large effect size

Based on the pairwise comparisons, the data is divided into two subsets. The antiangiogenic and vascularity inhibition properties of *C. baccifera* extract at 100- and 250-ppm concentrations are statistically equal. The same is true for treatments with 500-, 750-, and 1000-ppm concentrations. However, such properties were significantly stronger in those treated with 500-, 750-, or 1000-ppm concentrations than in those treated with 100- or 250-ppm concentrations.

6. Cytotoxicity of *Cipadessa baccifera*

6.1. The Cytotoxicity profile of *C. baccifera*

The aforementioned table showed that as concentration increased, the total number of shrimps alive decreased. In contrast, as the level of concentration increases, so does the total number of deaths, as does the percentage of deaths. Specifically, it could be gleaned from table

4 at 100ppm, out of 3 trials, the percent death is 36.67%; at 250ppm is 46.67%; at 500ppm is 53.33%; at 750ppm 60%; and at 1000ppm is 60.67%.

Table 6
Cytotoxicity of C. baccifera ethanolic extract on Brine shrimp (Artemia salina)

Concentration of Extract	Number of *Artemia salina* surviving after 24 hours			Total Number of Shrimp Alive	Total Number of Shrimp Death	Percent Death
	T1	T2	T3			
100ppm	6	6	7	19	11	36.67%
250ppm	6	5	5	16	14	46.67%
500ppm	5	5	4	14	16	53.33%
750ppm	4	4	4	12	18	60.00%
1000ppm	3	3	4	10	20	66.67%

The aforementioned table showed that as concentration increased, the total number of shrimps alive decreased. In contrast, as the level of concentration increases, so does the total number of deaths, as does the percentage of deaths. Specifically, it could be gleaned from table 4 at 100ppm, out of 3 trials, the percent death is 36.67%; at 250ppm is 46.67%; at 500ppm is 53.33%; at 750ppm 60%; and at 1000ppm is 60.67%. This might be an indication that the increase in concentration is associated with the increase in the amount of biochemical present in the sample. Moreover, the increased mortality rate of the brine shrimp as the concentration increases after exposure is also an indication of the toxic potential of the ethanolic extract of *C. baccifera* in living cells, this might due to the reason that *C. baccifera* contains phenols and alkaloids that can kill cells in certain amount or concentration.

7. Statistical difference of C. baccifera extracts in terms of cytotoxicity

It can be gleaned above that there was no significant difference in the mortality of *A. salina* across treatment groups, G = 6.46, p = .174, V = .209. This means that the cytotoxicity is statistically the same across treatment groups. There may be observable differences, but these are rather small.

Table 7
Descriptive and Inferential Statistics on the Difference of Cytotoxicity of C. baccifera Extract Across Treatment Groups

	Descriptives		Test of difference		
Concentration	***f***	**%**	***G***	***p***	***V***
100 ppm	11	36.67	6.46	$.174^{ns}$	$.209^{S}$
250 ppm	14	47.67			
500 ppm	16	53.33			
750 ppm	18	60.00			
1000 ppm	20	66.67			

Note: ns: Not significant S: Small effect size

8. Multiple pairwise comparisons of treatment groups

Table 8
Multiple Pairwise Comparison of Treatment Groups for the Difference in the Cytotoxicity of C. baccifera.

Comparisons	*OR*	OR^{-1}
100 ppm vs. 250 ppm	0.66	1.51
100 ppm vs. 500 ppm	0.51	1.97
100 ppm vs. 750 ppm	0.39	2.59
100 ppm vs. 1000 ppm	0.29	3.45
250 ppm vs. 500 ppm	0.77	1.31
250 ppm vs. 750 ppm	0.58	1.71
250 ppm vs. 1000 ppm	0.44	2.29
500 ppm vs. 750 ppm	0.76	1.31
500 ppm vs. 1000 ppm	0.57	1.75
750 ppm vs. 1000 ppm	0.75	1.33

For the multiple comparisons (table 4.2), 100- and 250-ppm concentrations of *C. baccifera* extract have roughly the same level of cytotoxicity. The same holds for the concentration pairs 250- and 500-ppm, 500- and 750-ppm, and 750- and 1000-ppm. Nonetheless, *C. baccifera* extract at 500 to 1000 ppm is two to three times more cytotoxic than 100 ppm, 750 to 1000 ppm is twice as cytotoxic as 250 ppm, and 1000 ppm is also twice as cytotoxic as 500 ppm.

9. LC_{50} of *C. baccifera* Extract

This work aimed to develop a statistical model that can forecast Artemia salina mortality based on Cipadessa baccifera extract concentrations. The null hypothesis for the analysis objective was: "The mortality observed in the data was statistically the same as the mortality predicted from the model". Using Probit regression/analysis, the result is shown in Tables 8 and 9.

Table 8
Parameter Estimates of the Probit Model in Predicting Mortality from the Concentration of C. baccifera Extract

Parameter	*b*	SE_b	*z*	*p*
Constant	−0.349	0.195	−1.790	.074
	[−0.544, −0.154]			
Concentration (ppm)	0.001	0.000	2.510	.012
	[0.000, 0.001]			

Note: **Overall fit: $c^2(3) = 0.202$, $p = .977$ (ns).** 95% CI was reported in square brackets.

*: Significant ns: Not significant

So based on the computed p-value=0.977 (overall fit), the model fits the data, hence the null hypothesis could be accepted.

Table 9
Predicted concentration for a given probability of mortality

Probability	Concentration (ppm)
0.36	0.00
0.40	118.74
0.50	**433.64**
0.60	748.54
0.70	1085.45
0.80	1479.74
0.90	2026.56
0.95	2478.13
0.99	3325.20

The above table illustrated that a lethal concentration of 434 ppm of *Cipadessa baccifera* extract is sufficient to cause 50% mortality in the sample, which may mean, this concentration of the chemical present in the plant extract kills 50% of the brine shrimp in a single exposure.

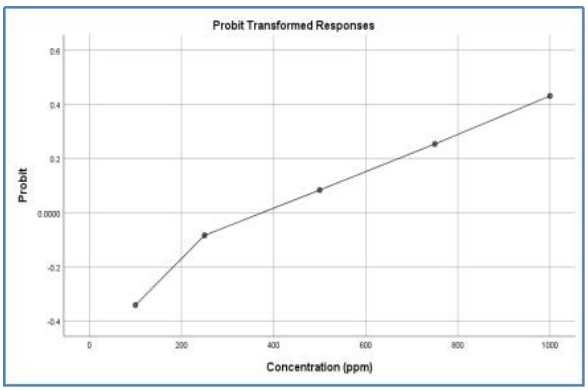

Figure 2. Probit graph of LC_{50} of C. baccifera Ethanolic Extract

10. Embryotoxicity of the Ethanolic Extract of *Cipadessa baccifera*

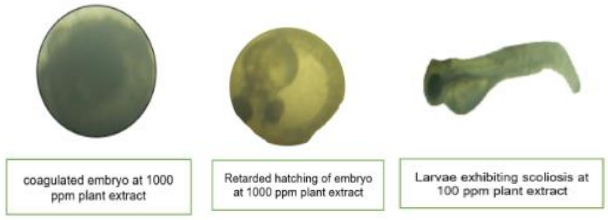

Figure 3. Embryotoxicity of the ethanolic extracts of C. baccifera

Based on the zebrafish embryo microscopic analysis shown above, there were malformations on the 3rd day. The larvae exhibit scoliosis at 100ppm extract concentration, this may imply that the plant extract disrupted the normal development of the embryo. Furthermore, retarded and coagulated embryo at 1000ppm plant extract concentration demonstrates that at higher concentrations, the plant extract may be toxic to the embryo during development. Similar results were observed at 250, 500, and 750ppm. Additionally, the retarded and coagulated embryos at concentrations ranging from 200 ppm to 1000 ppm demonstrate the plant extract's consistent cytotoxicity. Yumnamcha et al. (2015) discovered that the phytochemistry of *Millettia pachycarpa* contains alkaloids, saponins, phenolic compounds,

and triterpenoids with strong hemolytic activity, which caused cell membrane death in zebrafish. However, the toxicity of saponin in the zebrafish embryo has yet to be established. Prior research suggested that it could be caused by physicochemical properties and membranolytic effects on the chorion. *Cipadessa baccifera* phytochemistry revealed that the plant extract contains alkaloids, saponins, and triterpenoids, which could explain why zebrafish embryo hatching was delayed in different concentrations.

CONCLUSIONS

Based on the findings the following conclusions were drawn:

1. *C. baccifera* has been traditionally used as an alternative medicine for toothache, stomachache, diarrhea, cough, and colds in the Ibaloi, Kalanguya, and Karao communities of Bokod Benguet.
2. *C. baccifera* leaf ethanolic extract contains amounts of phytochemicals like alkaloids, phenols, triterpenes, anthrones, flavonoids, and essential oil which have pharmaceutical value.
3. *C. baccifera* leaf ethanolic extract showed cytotoxic effects wherein mortality rate increases as concentration increases. While test on zebrafish confirmed embryotoxicity and teratogenicity of the plant extract.
4. *C. baccifera* CAM concentrations had statistically significant differences while cytotoxicity concentrations had no significant differences.
5. *C. baccifera* LC50 ethanolic extract was recorded to be at 434ppm.

RECOMMENDATIONS

Given the study's findings, future research should concentrate on the ecology and biology of *C. baccifera* in the Philippines to create a new taxonomic classification of the endemic *Cipadessa baccifera*. And to address the safety issues concerning herbal use, it is recommended that the result of the study should be presented to the community.

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