

Ethnopharmacological study of the Selected Tribal Medicinal Plants of the Tboli People in Lake Sebu, South Cotabato

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DOI: [10.29303/jossed.v3i1.1367](https://doi.org/10.29303/jossed.v3i1.1367)

Article Info

Received: January 29, 2022

Revised: February 28, 2022

Accepted: March 5, 2022

Published: April 30, 2022

Abstrak: Around 80% of people around the world have practiced the use of ethnopharmacological medicine. Tboli tribe of Lake Sebu, South Cotabato, Philippines recognized medicinal plants for healing. There were two identified plants by the Tboli people, the Bulok bukay (*Stachytarpheta jamaicensis*) and Kluonmabu (*Ageratum conyzoides* L.) as a potential medicine for wound healing. The plants underwent antimicrobial analysis, phytochemical screening, and functional groups detection through FTIR. The zone of inhibition for Bulok bukay showed antimicrobial potential in all concentrations. Kluonmabu observed as not as effective as the other plant against *S. aureus*. With the $p = 0.0641$ at 0.05 level of significance, Bulok bukay showed to be effective against bacteria and comparable with a commercial antibiotic. Furthermore, phytochemical properties for both plants have been observed with more components of alkaloids and terpenoids in Bulok bukay. FTIR confirmed as well that two plants have the potential presence of functional groups against bacteria.

Keywords: Ethnopharmacological; Tboli Tribe; Tribal Medicinal Plants, Antimicrobial, Phytochemical; FTIR

Citation: Larida, A.Q., Soldevilla, J.B., Ursabia, C.J.B., & Blanco, D.R.C. (2022). Ethnopharmacological study of the Selected Tribal Medicinal Plants of the Tboli People in Lake Sebu, South Cotabato. *Journal of Science and Science Education*, 3(1), 1–8. <https://doi.org/10.29303/jossed.v3i1.1367>

INTRODUCTION

Various species of plants have been used to treat diseases all over the world since the prehistoric period. For instance, indigenous cultures in Rome, Egypt, Iran, Africa, and America made use of herbs as part of their healing rituals. Others such as the Unani, Ayurveda, and Chinese began establishing their own traditional medical systems. Many of these traditional healing practices continue to be performed today. According to the World Health Organization (WHO), around 80% of people around the world today still depend on herbal medicines in some aspects of their lifestyle (Khan, 2016; Hipol et al., 2012). There has been a renewed interest in the study of medicinal plants simply because they are considered to be "green medicine." Treatments such as this are more easily available, cheaper, and have little to no side effects on the patient; synthetic medicines, on the other hand, are known to possibly have harmful effects to humans and the environment (Chanda, 2013; Yang et al., 2014). Khan (2016) stressed that factors such as population growth, scarce drug supply, high-priced treatment costs, among others have led to the rising need to understand the nature of traditional medicinal plants using more advanced scientific processes. Currently, in the Philippines there are only ten medicinal plants approved by the Department of Health (DOH). Many of these accepted natural medicines are already being used in the production of topical

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ointments, creams, and liniments.

These may be used to treat wounds, skin diseases, and muscle pains as well as aid in massages and aromatherapies (Penecilla & Magno, 2011). According to Kala et al. (2006), as said by Tantiado (2012), indigenous knowledge on therapeutic plants is slowly beginning to decline and become obsolete as these traditions are being passed on primarily by word of mouth. Apparently, there is also a lack of recognition among younger generations due to shift in attitude and ongoing socio-economic changes. With the use of modern technological instruments, then, interest towards herbal medicine must be renewed as soon as possible through detailed documentation so as to preserve this indigenous knowledge. Taking advantage of both qualitative and quantitative tools, the present study is bent towards distinguishing and evaluating the healing properties of two of the most commonly-used medicinal plants of the Tboli ethnic community in Lake Sebu, South Cotabato by means of microbial assay procedures and phytochemical analysis, as well as providing sufficient documentation of these plants for the reference of future researchers.

This study aims to determine the true healing properties of the most common medicinal plants of the Tboli ethnic group in Lake Sebu, South Cotabato. Specifically, it aims to: (1) Perform a pharmacological bioassay on two plants recognized by the Tboli people as an effective treatment for wounds; (2) Determine the presence of the phytochemical such as alkaloids, flavonoids, phenols, quinones, tannins, and terpenoids on both plants, and; (3) Identify the functional groups present in both selected plants.

METHOD

Data Gathering Procedure

1. Preliminary Phase

Identification of the Plants Specimens of the two selected medicinal plants will be brought to taxonomist Miss Fe Sangalang, MSc. for taxonomic identification. Upon confirmation of their species, additional information including the plants' English and local names, biogeographical, and ecological descriptions will also be documented. Two kilograms of each of the two selected plants (not including those already approved by the DOH) within the ethnic group were collected. These plants were collected around Lake Sebu, South Cotabato.

2. Experimental Phase

a. Extraction Process

Each plant was extracted using the ethanol extraction method. Both plant samples were air-dried for 2-3 days with their roots cut off. The dry samples were crushed and weighed in grams. Afterward, each plant sample was stored in a glass jar and soaked in pure ethanol for 24 hours. After soaking, the plant-infused ethanol was filtered, separating it from the solid plant components. This liquid was poured into a round-bottom flask which will then be attached to a rotary evaporator, where the solvent was left to evaporate at 50°C with a speed of 90 revolutions per minute (rpm). The compound left in the flask when all of the solvents have evaporated will be the plant extract used in the microbial assay, phytochemical screening, and FTIR spectroscopy.

b. Microbial Assay

The present study made use of the modified Kirby-Bauer disc diffusion method for antimicrobial susceptibility testing: The inoculum was first prepared from the primary culture plate by using an inoculation loop to touch the top parts of *Staphylococcus aureus* (ATCC 6538) colonies to be tested and transferring these growths to a tube with saline solution. This tube was compared to the 0.5 McFarland turbidity standard and the test suspension's density was adjusted to the standard. Mueller Hinton agar (MHA) discs were inoculated by dipping a sterile swab into the inoculum. These swabs were streaked evenly all over the surface of each medium three times. The swabs were passed around the edge of the agar surface. Afterward, the inoculum was left to dry in an incubator at 35°C for 24 hours. The extract-impregnated discs with concentrations of 1.00 gram per milliliter, 0.5, 0.25, 0.125, and 0.0625 g/ml were attached to the surface of the agar plate using sterile forceps, each disc was gently pressed down to make sure they were in complete contact with the agar surface. This setup was triplicated for each plant. Within 30 minutes of preparation, the plates were transferred to an incubator at 35°C. After 18-24 hours of incubation, the zones of inhibition were measured and recorded in millimeters (Acharya, 2013). In addition to performing the assay on the plants with varying concentrations, the same procedure was used to determine the zones of

inhibition of the commercial antibiotic's gentamicin and oxacillin. These results were compared to the performance of the two plant extracts.

c. Phytochemical Screening

The following phytochemical procedures were performed on both plants:

Alkaloid detection by Wagner's Test. The extracts were dissolved individually in dilute hydrochloric acid (HCl), and then filtered. These filtrates were treated with Wagner's reagent (iodine in potassium iodide). Alkaloids are present if there is a formation of brownish to reddish precipitate (Tiwari et al., 2011).

Flavonoid detection by Alkaline Reagent Test. Extracts were treated with drops of sodium hydroxide (NaOH) solution. Flavonoids are present if there is a formation of strong yellow color which becomes colorless in the addition of a dilute acid (Tiwari et al., 2011).

Phenol detection by Ferric Chloride Test. Three to four drops of ferric chloride (FeCl_3) solution will be treated to the extract. Phenols are present if there is a formation of a bluish-black color (Tiwari et al., 2011).

Quinone detection. Around 0.5 g of plant extract was utilized and added to 1 ml extract and 1 ml concentrated sulfuric acid (H_2SO_4). The formation of red color indicates the presence of quinones (Kala et al., 2012).

Tannin detection by Gelatin Test. One percent (1%) gelatin solution containing sodium chloride (NaCl) was added to the extract. Tannins are present if there is a formation of a white precipitate (Tiwari et al., 2011).

Terpenoid detection by Salkowski Test. The extract was mixed with 2 ml chloroform. Three milliliters (3 ml) of sulfuric acid (H_2SO_4) concentrate will be added cautiously to form a layer. Terpenoids are present if there is a reddish-brown discoloration of the interface (Singh et al., 2019).

Identification of Functional Groups This study made use of Fourier-transform infrared spectroscopy (FTIR) to identify the functional groups available in both plants. By interpreting the infrared absorption spectrum, the chemical bonds contained within the plants are detected. A small amount of each plant extract was pounded with potassium bromide (KBr) to create a fine powder with no visible crystallites. This powdered sample was pressed into the sample pellet and positioned in the FTIR spectrometer for infrared spectrum measurement.

d. Determination of Functional Groups through FTIR

Chemical characterization of the oil extract was conducted using Fourier Transform Infrared Spectroscopy (FTIR) using horizontal attenuated total reflectance accessory. It utilized IRAffinity-1S FTIR Spectrophotometer (Shimadzu) with 12,000 spectra with a high stable dynamic alignment mechanism and robust S/N ratio of 30,000:1. The extract was placed on an attenuated total reflection crystal plate and the IR spectra were then collected. The FTIR spectra of samples were determined in the mid IR range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} . Each spectrum was collected in transmittance mode (Vidyadharani, and Dhandapani, 2013).

Data Analysis

The present study determined the antimicrobial properties of two selected medicinal plants of the Tboli ethnic group situated in Lake Sebu, South Cotabato. It first used a descriptive method for analyzing the obtained data based on what was observed during the microbial bioassay procedures. The Kruskal-Wallis H Test was then utilized to compare the differences between the zones of inhibition of each concentration per plant as obtained from the microbial bioassay, with an α -value of 0.05. According to McDonald (2014), the said statistical test is used when there is one nominal variable and one measurement variable in the study. It is considered to be the nonparametric version of the ANOVA test wherein its measurement variable does not meet the normality assumption of the standard one-way ANOVA. This test will be used to determine whether there is a significant difference among the zones of inhibition produced by each plant extract in varying concentrations. This will also conclude whether the plant contains antimicrobial properties based on the results. The Levene test followed, which determined whether the variances are equal among three sample groups, which included: the concentration with the highest antimicrobial

effects, oxacillin, and gentamicin. According to Brown and Forsythe, as said in Levene Test for Equality of Variances, this step is crucial in the study especially because the data which was obtained comes from a non-normal distribution. Lastly, a t-test was conducted to determine whether there is a significant difference between the results of the plant extract and oxacillin and gentamicin combined. The method of computation highly depended on the results of Levene's test, which is the equality of variance among the sample groups. The aforementioned statistical procedures were conducted electronically, using XLSTAT, an add-on in Microsoft Excel which allows users to perform parametric and nonparametric statistical tests without having to compute the values manually.

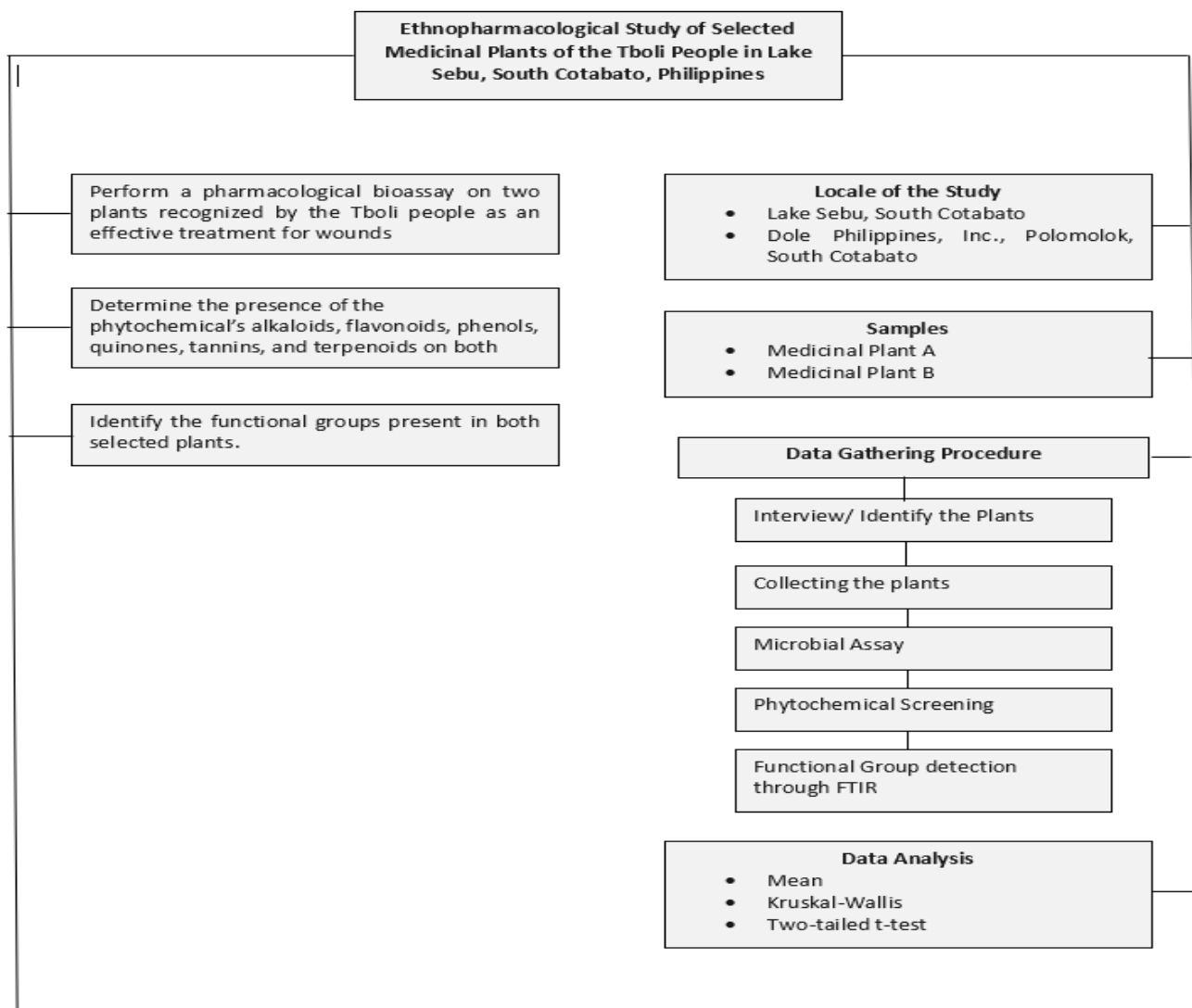


Figure 1. Research Design

RESULT AND DISCUSSION

Measurement of Zone of Inhibition of *S. jamaicensis* and *A. conyzoides* under varying concentrations

Initial observations show that *S. jamaicensis* has antimicrobial properties due to the direct relationship between its zone of inhibition and concentration, such that as concentration (g/mL) increases, the zone of inhibition increases as well. On the other hand, with *A. conyzoides* there is only one plate among all 18 trials which showed a zone of inhibition, while all other plates revealed that the said plant is not effective against *S. aureus* under the given concentrations due to the absence of a zone of inhibition. The zones of inhibition of gentamycin and oxacillin were also measured after being incubated under the same conditions. Table 3 shows the names of the antibiotics and their average zone of inhibition in millimeters.

Table 1. Zone of inhibition of two identified medicinal plants.

Name of Plant	Concentration (g/mL)	Zone of Inhibition (mm)		
		A	B	C
<i>S. jamaicensis</i>	1.00	14.50	15.50	17.00
	0.50	12.00	13.50	13.50
	0.25	10.00	10.00	11.00
	0.12	5.50	7.50	9.50
	0.06	4.50	7.50	7.50
<i>A. conyzoides</i>	1.00	0.00	0.00	9.00
	0.50	0.00	0.00	0.00
	0.25	0.00	0.00	0.00
	0.12	0.00	0.00	0.00
	0.06	0.00	0.00	0.00

The Zones of Inhibition measured on two commercially-available antibiotics

Table 2. Zone of inhibition of the Commercialized antibiotics

Antibiotic Name	Average Zone of Inhibition (mm)
Gentamycin	12.25
Oxacillin	14.75

Due to the general lack of presence of zones of inhibition in *A. conyzoides* extract, only the data from *S. jamaicensis* extract underwent statistical treatment. Instead of manually computing, Microsoft Excel's add-in XLSTAT was used for both Kruskal-Wallis and t-test analyses. The first test conducted was the Kruskal-Wallis Test, which determined whether there is a significant difference among the zones of inhibitions produced by the five different concentrations of *S. jamaicensis* extract.

Kruskal-Wallis test results of the different concentrations

Table 3. Kruskal-Wallis of the different concentrations of the two plants

H (observed value)	13.04
Hc (critical value)	9.49
Degrees of Freedom	4.00
One-tailed p-value	0.01
Alpha value	0.05

The rejection region for this test is $R = \{H \geq 9.488\}$. Since it is observed that $H = 13.040 \geq 9.488$, at the level of significance $\alpha=0.050$ the decision is to reject the null hypothesis of absence of difference between the 5 samples. In other words, the difference between the varying concentrations of *S. jamaicensis* is significant. With this, it can be concluded that the plant contains antimicrobial properties and the best concentration among the five in which *S. aureus* is most susceptible is at 1 g/mL.

Two-Tailed t-test results of Antimicrobial effect of *S. jamaicensis* extract on *S. aureus*

Table 4. T-test results of Antimicrobial Effect

t (observed value)	1.73
t (critical value)	7.26
Degrees of Freedom	6.00
Two-tailed p-value	0.06
Alpha value	0.05

Since it is observed that $p = 0.06 \geq 0.05$, at the level of significance $\alpha=0.050$ the decision is to reject the null hypothesis of equality of the means. In other words, the difference between the means is significant. This signifies that the antimicrobial effects of *S. jamaicensis* extract on *S. aureus* is comparable to that of gentamicin or oxacillin on the same bacteria.

Phytochemical Screening Results of *S. jamaicensis* and *A. conyzoides*

The crude ethanolic extract of both plants were each subjected to phytochemical screening to test for the presence of six phytochemicals known for their antimicrobial properties, namely: alkaloids, flavonoids, phenols, quinones, tannins, and terpenoids. The analysis revealed the presence of all six mentioned phytochemicals on both plant extracts.

Table 5. Results of the screening for antimicrobial phytochemicals

Phytochemicals	Plant Extracts	
	<i>S. jamaicensis</i>	<i>A. conyzoides</i>
Alkaloids	++	+
Flavonoids	+	+
Phenols	+	+
Quinones	+	+
Tannins	+	+
Terpenoids	++	+

Note: A “++” sign on the table denotes that the plant was more reactive towards the given phytochemical test compared to the other

The different phytochemicals were observed to both plants. However, terpenoids and alkaloids are much reactive for *S. jamaicensis* only. This might be the reason of strong antimicrobial property of *S. jamaicensis*.

FTIR Results of the S. jamaicensis and A. conyzoides

To identify the plants' dominant functional groups and confirm the presence of phytochemicals, the crude extracts were further subjected to FTIR spectroscopy.

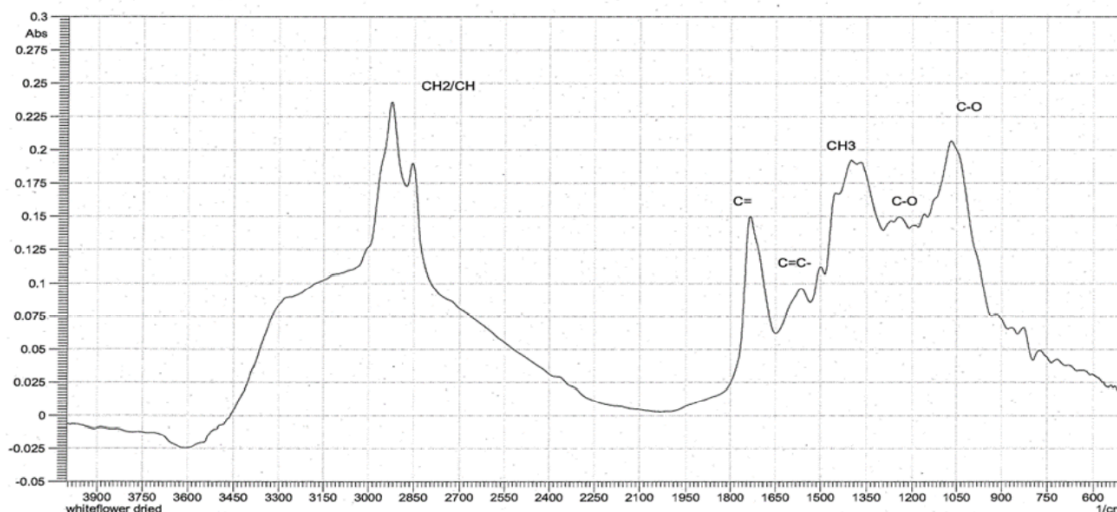


Figure 2. *S. Jamaicensis*

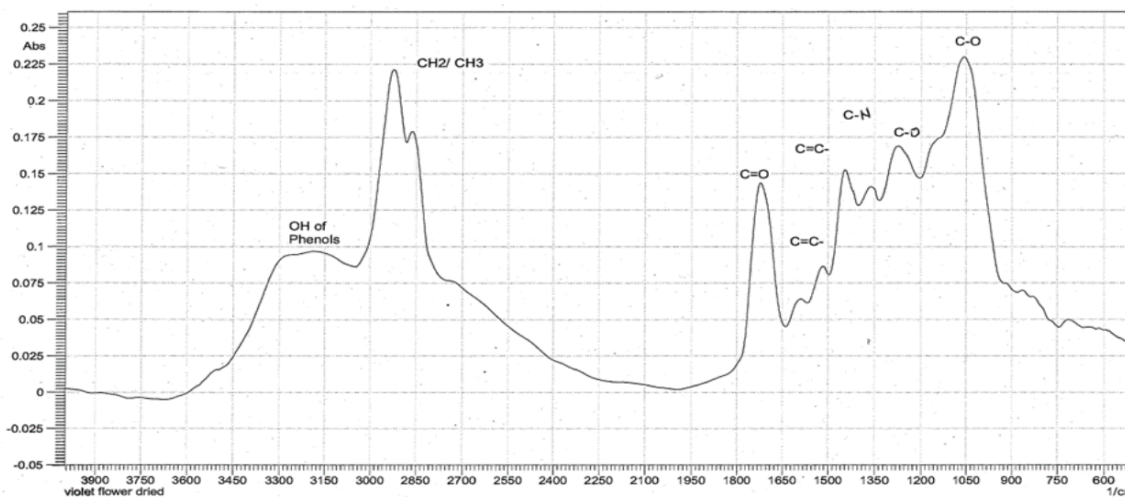


Figure 3. *A. conyzoides*

Every functional group has its own assigned wave number (cm^{-1}), which is read by the FTIR spectrometer. The occurrence of a peak at specific wavelengths indicates the presence of the assigned functional group. Infrared (IR) spectra of *S. jamaicensis* (kluonmabu) and *A. conyzoides* (bulok bukay) extract showed carbonyl -C-O peak at $1310\text{-}1400\text{ cm}^{-1}$ and at 1050 cm^{-1} and carbonyl C=O peak of esters at $1750\text{-}1725\text{ cm}^{-1}$. Peaks found at $1450\text{-}1510\text{ cm}^{-1}$ and at $1615\text{-}1580\text{ cm}^{-1}$ were assigned to aromatic ring stretch C=C-C of aromatic ring. On the contrary, a hydroxyl OH peak of phenol was observed at $3100\text{-}3400\text{ cm}^{-1}$, although this peak can be more prominently observed in *S. jamaicensis*. Peak at $2800\text{-}3000\text{ cm}^{-1}$ was assigned to methyl CH_3 group, which can be observed in both plant extracts. These peaks indicated the presence of flavonoids, phenols, quinones, tannins, and terpenoids. On the other hand, presence of tertiary amine peak at $1360\text{-}1310\text{ cm}^{-1}$ particularly in *S. jamaicensis* extract indicated the presence of alkaloids. The antimicrobial properties of both *S. jamaicensis* (kluonmabu) and *A. conyzoides* (bulok bukay) are explained with the presence of such phytochemicals with antimicrobial properties. Although little to no zone of inhibition was observed in *A. conyzoides* given the study's microbial assay setup, this does not completely invalidate its possible antimicrobial characteristics when used in higher concentrations

CONCLUSION

The Tboli people recognize various plants from around Lake Sebu which they claim to be of antimicrobial nature. Many of these are freshly harvested, pounded, and applied directly on open wounds. In this study, *S. jamaicensis* and *A. conyzoides* were chosen by the tribal community to be subjected to laboratory analysis. While this study recognizes the medicinal use of both plants in the Tboli culture, *S. jamaicensis* is shown to be the more effective plant compared to *A. conyzoides* in terms of its antimicrobial effects such that its zones of inhibition are already comparable with those of oxacillin and gentamicin, which are two commercially-recognized antibiotics sold in the market to treat various kinds of bacterial infections. Additionally, the presence of the six antimicrobial phytochemicals may explain the antimicrobial nature of *S. jamaicensis*, and at the same time provide an idea that *A. conyzoides* may also exhibit antimicrobial properties in a different environment, such as with higher concentrations. With this, the results of this study do not invalidate the possible antimicrobial effects of *A. conyzoides* as it may still display the property on concentrations much higher than what the present study settled for, and on a different controlled environment.

ACKNOWLEDGMENTS

Thank you to the whole team of Dole Philippines, Inc. Philippines – Laboratory Services for allowing the researchers to perform the laboratory, particularly to Dr. Artemio Tulio, Jr., Lalaine S. Balane, RCh, Christine Anne Jomocan-Apura, RCh, and Anna June S. Cañete, RCh. The same intensity of gratitude as well to the LGU-Lake Sebu, South Cotabato, Philippines for the algal samples

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