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#### **Research Article**

### Environmental Conditions, Phytochemical Constituents, and Antibacterial Activities of Two Philippine Medicinal Vitaceae Species

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#### ABSTRACT

Plants are utilized for medicinal, therapeutic, nutritional, and industrial importance. This study investigated the phytochemical constituents and antibacterial activities of two medicinal Vitaceae species, namely: Cayratia trifolia and Tetrastiqma harmandii. The growing environment (location, elevation, soil properties, and associated plants) of the species in northeastern Cagayan, Luzon, Philippines, were assessed and leaf samples were collected for phytochemical screening and antibacterial analysis. T. harmandii dominated the population found growing in various habitats such as hills, residential areas, near coastal areas and island shorelines, nipa plantations, agricultural and grasslands, near bodies of water, caves, and secondary growth forests while C. trifolia were only found growing in swamps, hills, and secondary growth forests. Both plants were found climbing in plant species like ipil-ipil (Leucaena leucocephala) and kakawate (Gliciridia sepium). T. harmandii populations were growing in soils with lower soil pH and higher nutrient content as compared to C. trifolia populations which grew in soils with higher pH and lower nutrients. Antioxidant properties were exhibited by the presence of secondary metabolites. Alkaloids, flavonoids, phenols, terpenoids, anthocyanins, tannins, and saponins were detected in C. trifolia ethanolic leaf extracts while phenols, terpenoids, tannins, and saponins were detected in T. harmandii. Both species showed inhibitory activity against Staphylococcus aureus. In addition, a slight activity against *Klebsiella aerogenes* was observed for *T. harmandii*. Thus, *C. trifolia* and *T. harmandii* also have antibacterial properties.

Keywords: Antibacterial property, Cayratia trifolia (Linn.) Domin, Habitats and environment, Plant secondary metabolites, Staphylococcus aureus, Tetrastigma harmandii Planch

#### Introduction

Vitaceae species in the northern part of Luzon, Philippines, are diverse and some of these species are believed to be medicinal. Some of the Vitaceae species with medicinal properties are *Cayratia trifolia* (Linn.) Domin. and the common *Tetrastigma* species, *Tetrastigma harmandii* Planch (Figure 1). *C. trifolia*, locally known as *kalit-kalit* in the Philippines, is a herbaceous climber with trifoliolate leaves, ovate to oblong-ovate leaflets, and dark purple or black nearly spherical fruits [1] while *T. harmandii*, locally known as *ayo or ariwat*, is a woody vine with pedately compound leaves with 3(5)6 glossy dark green leaflets, simple tendrils, and rusty brown globose fruits [2]. Between the two Vitaceae species, only the *Tetrastigma* is parasitized by Rafflesia, which grows in the roots and stem of the said species [3]. The distribution of *C*. *trifolia* [4] and *T. harmandii* [2] in the Philippines and nearby countries was reported, however, their growing environments were the least explored.

*C. trifolia* and *T. harmandii* have been reported to possess various medicinal properties. *C. trifolia* was found to contain various phytochemicals such as steroids, flavonoids, tannins, carbohydrates [5], and stilbenes such as piceid, resveratrol, viviferin, ampelopsin [6]. Stilbenes in *Cayratia* 

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Figure 1. Medicinal Vitaceae species: *Cayratia trifolia* (A) and *Tetrastigma harmandii* (B)

were widely studied and were found to have various medical benefits. In terms of its pharmacological uses, the species was reported to have antioxidant activity [7], anti-ulcer property [8], hepatoprotective activity [9], anti-inflammatory activity [10], cardioprotective activities [11], antidiabetic properties [12, 13], antiviral effects [14], reduce vaginal and diseases, diarrhea, spleen disorders [15] and can treat scurvy [16]. On the other hand, *T. harmandii* was found to have anti-scabies and diuretic properties [17] and treat urinary diseases [16].

Despite the richness of knowledge on medicinal plants worldwide, a small number of scientific reports on *C. trifolia* and *T. harmandii* in the Philippines were reported. With the aforementioned health and medical benefits, this study compared the profiles of the two medicinal Vitaceae species commonly found growing in various habitats of the northeastern Cagayan, Luzon, Philippines in terms of their growing environment and their phytochemical and antibacterial properties.

# Material and Methods *Sampling methods*

Study permits were secured from the Department of Environment and Natural Resources (DENR) Region 2 Office and various Local Government Units (LGUs) of northeastern Cagayan namely Lal-lo, Camalaniugan, Aparri, Buguey, Santa Teresita, Gonzaga, and Santa Ana. The municipalities were visited for the presence of *C. trifolia* and *T. harmandii*. The study encountered 2 accessions of *C. trifolia* and 36 accessions of *T. harmandii*. The accessions were planted in the Cagayan State University (CSU) -Gonzaga field gene bank and nursery. A combination of walk/vehicular ride, visual encounter, and photo documentation was employed from June to October 2020.

### Geographical distribution and habitat conditions

The climatic condition during the experimental period is shown in Table 1. A geographical map for the medicinal Vitaceae species distribution in northeastern Cagayan was generated using the Google Earth Pro and the locations and elevations were determined using the Geocam Pro version 5.34 (Wazar-Apps, 6 Place Jacques Bonsergent, Paris, France). The various habitats where the two Vitaceae species grew and the plants growing within their environments were identified. The scientific, English and common/local names were provided.

Topsoil samples from various experimental sites where the species grew were gathered, pulverized, and subjected to air-drying at room temperature until total dryness. The soil pH, organic carbon (OM), phosphorus, potassium, and trace elements (copper, zinc, iron, manganese) were tested through the Potentiometric method, Walkey and Black Spectrophotometric, Olsen's method, Cold Sulfuric Extraction, and diethylenetriaminepentaacetic acid (DTPA) extraction, respectively [18]. The rating scales guided the interpretation of soil test results for total N, total P, and total K [19], pH, and micronutrients [18].

#### Plant sample collection

Fresh young and mature leaves of *C. trifolia* and *T. harmandii* were collected in Gonzaga, Lallo, and Santa Teresita, Cagayan. Their identities were verified through published photos of Vitaceae in Co's Digital Flora of the Philippines website [20].

#### Analysis of phytochemical constituents and antimicrobial activity of the medicinal Vitaceae species

#### Preparation of the ethanolic leaf extracts

The collected leaves were thoroughly washed with running tap water and rinsed with distilled water thrice. The leaves were air-dried at room temperature for 3-4 days, chopped into smaller pieces, oven-dried at 50°C for several hours, ground and pulverized using mortar and pestle then sieved to obtain a fine powder. The powder (200 g.L<sup>-1</sup>) was extracted using 90% ethanol at room temperature, filtered using a filter paper, and collected the crude extracts.

#### **Phytochemical screening**

The leaf crude extracts were subjected to phy-

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Table 1. Climatic condition of northeastern Cagayan, Euzon, Finippines during the study							
Month (2020)	Mean Temperature (°C)			Mean Precipitation	Mean Relative	Socon	
	Minimum	Average	Maximum	(mm)	humidity (%)	Season	
June	26	30	32	273.9	79	dry	
July	26	29	31	224	80	dry	
August	25	29	30	237.5	81	dry	
September	26	29	31	241.1	81	dry	
October	25	27	28	1,014.70	86	wet	

Table 1. Climatic condition of northeastern Cagayan, Luzon, Philippines during the study



Figure 2. Geographical distribution of *Cayratia trifolia* (Linn.) Domin. and *Tetrastigma harmandii* Planch. in the northeastern part of Cagayan, Philippines.

tochemical screening to identify the presence of eight secondary metabolites. The tests used for the determination of alkaloids, anthocyanin, flavonoids, phenols, saponins, steroids, tannins, and terpenoids were Meyer's test, NaOH test, Shinoda test, Ferric Chloride test, Froth test, Liebermann-Burchard reaction, Lead Acetate test, and Salkowski test, respectively [21].

#### Determination of antibacterial activity

The antibacterial properties of the plant crude extracts against gram-negative bacterial strains *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *K. aerogenes*, and *A. baumannii*; and gram-positive bacteria *S. aureus* and *E. faecium* were tested using the paperdisc diffusion method [21]. Bacterial strains were from the Central Analytical Laboratory of CSU-Tuguegarao City, Cagayan, Philippines. The inoculum size of each test organism was standardized. The standardized inocula were then plated on Mueller-Hinton agar through cotton swabbing and the plates were incubated for 12 hours. Six sterilized paper discs (6-mm diameter) from Whatman No.1 filter paper were immersed in ethanolic leaf extracts and placed on inoculated Mueller-Hinton agar plates with the bacterial strains. The sample plates containing six paper discs were replicated thrice. The sample plates were then incubated at 37°C for 24 hours. Zones of inhibition were measured using a ruler and their corresponding interferences were described [21].

#### **Results and Discussions**

# Geographical distribution of medicinal Vitaceae species and their habitats

*C. trifolia* populations in the northeastern Cagayan were few as compared to the populations of T. harmandii. C. trifolia were only found growing in Sta. Maria, Lal-lo and Luga, Santa Teresita, Cagayan while T. harmandii were distributed in all the municipalities of northeastern Cagayan (Figure 2). Both *C. trifolia* and *T. harmandii* grew in northeastern Cagavan environments with 25-32°C temperature, 224-1,014 mm rainfall, and 79-86% relative humidity (Table 1). It was observed that T. harmandii grew in lower elevations (34.14 meters above sea level (masl) lower) to higher altitudes (103.94 masl higher) as compared to C. trifolia. Both species grew in the lowland, near a swap, in hilly areas, and in secondary growth forests, however C. trifolia was found growing in flooded areas where the roots are fully submerged. Meanwhile, T. harmandii was found growing in other habitats such as residential areas, near coastal areas of Aparri, Buguey, Gonzaga, and Sta. Ana; in nipa plantations; various agricultural lands planted with vegetables, rice, and corn; grasslands/grazing areas; near bodies of water such as riverbanks, creeks, irrigation dam, and canals, and freshwater spring; outside the caves of Sta. Teresita and Lal-lo; and also near the seashores of Aparri, Buguey, Gonzaga, and Palaui Island Protected Landscape and Seascape in Sta. Ana, Cagayan (Figure 3, Table 2).

Both *Cayratia* and *Tetrastigma* species were

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Figure 3. Tetrastigma harmandii Planch. and Cayratia trifolia (Linn.) Domin. growing in various habitats in northeastern Cagayan, Luzon, Philippines. *T. harmandii* growing in A. nipa plantation, B. agricultural land, C. residential area, D. grassland, E. near coastal area, F. dam, G. creek, H. river, I. spring, J. outside the cave, and K. secondary growth forest; *C. trifolia* growing in L. secondary growth forest and M. swampy area.

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## Table 2. Growing environment of *Cayratia trifolia* and *Tetrastigma harmandii* in northeastern Cagayan, Luzon. Philippines

	Luzon, Pin	nppmes			
Species	Collec- tion Site	Elevation (m asl)	Habitat	Soil chemical properties	Native/endemic plants growing within the environ- ment
C. trifo- lia	Lal-lo, Sta. Tere- sita	53.04 – 78.03	lowland, near a swamp, hills, secondary growth forests/ open for- ests	pH: $6.78-7.27$ $\pm 0.61$ , OM (%): 2.79- $3.51 \pm 0.22$ , P (ppm): 12.70- $14.90 \pm 0.64$ , K (ppm): 117.0- $258.0 \pm 40.78$ , Cu (ppm): 0.88- $1.62 \pm 0.22$ , Zn (ppm): 0.98- $1.68 \pm 0.20$ , Fe (ppm): 3.20- $9.60 \pm 1.86$ , Mn (ppm): 3.80- $18.0 \pm 4.10$	Leucaena leucocephala (Lam.) de Wit [Lead tree/ Ipil- ipil] Gliciridia sepium (Jacq.) Kunth ex Walp. [St. Vincent Plum/Kakawate] Swietenia mahogani (L.) Jacq. [Mahogany]
manan	Aparri, Camala- niugan Buguey,	101.97	residential areas, near coastal ar- eas (ap- prox. 5- 200 meters	± 0.43, OM (%): 0.41-3.99 ± 1.06, P (ppm): 20.80- 100.0 ± 25.67, K (ppm): 296.0- 707.0 ± 128.69, Cu (ppm): 0.28-	Melanolepis multiglandulosa (Reinw. Ex Blume) Reichb.&Zoll. [Alim/Alem] Gnetum gnemon Linn. [Bago] Macaranga tanarius (Linn.) MuellArg [Elephant's ear/Binunga,Samak] Antidesma bunius (L.) Spreng [Currant tree/Bignay] Antidesma ghaesembilla Gaertn [Black Currant
	Sta. Teresita, Gonzaga, Sta. Ana, Palaui Is-		from the sea), nipa plan- tations, agricul- tural lands, grasslands, near rivers	2.28 $\pm$ 0.28, Zn (ppm): 0.48- 1.38 $\pm$ 0.62, Fe (ppm): 7.60- 26.20 $\pm$ 9.57, Mn (ppm): 5.20- 37.60 $\pm$ 5.37	tree/Arosep,Binayuyo] Phragmites vulgaris (Lam.) Trin. [Common Reed/Tambo,Tanobong] Pterocarpus indicus Willd. [Rosewood/Narra] Kleinhovia hospita Linn. [Timanga tree/Biknong] Hibiscus tiliaceus Linn. [Sea Rosemallow/Malabago] Donax canniformis (G. Forst.) K. Schum. [Common donax/Darumaka]
	land		creeks, dam, swamp, spring, and irrigation canals, outside the caves, secondary growth forests / open for- ests Palaui Is- land coast- line		Harpullia arborea (Blanco) Radik. [Tulip wood tree/Uas]Spathodea campanulata P.Beauv. [African tulip tree/Sirit-sirit]Ficus nota (Blanco) Merr. [Sacking tree/Tibig]Ficus septica Blanco [Hauili fig tree/Hauili, Ria-ria]Ficus septica Blanco [Hauili fig tree/Hauili, Ria-ria]Ficus ulmifolia Lam. [Isis, Oplas]Ficus benjamina Linn. [Weeping fig/Balete]Gmelina arborea Robx. [White teak/Gmelina]Swietenia mahogani (L.) Jacq. [Mahogany]Ceiba pentandra (L.) Gaertn [White silk cotton tree/Kapok]Eucalyptus globulus Labill [Blue gum tree/Eucalyptos]Areca catechu L. [Areca nut palm/Buñga, Boa] Syzigium cumini (L.) Skeels [Java plum/Duhat] Saribus rotundifolius (Lam.) Blune [Fan palm/Anahaw] Jatropha curcas Linn. [Physic nut tree/Tubang-bakod] Pandanus tectorius Parkinson ex Du Roi [Screw pine/Pandan] Nypa fruticans Wurmb. [Sasa/Nipa] Morus alba Linn. [Mulberry/Morera] Gliciridia sepium (Jacq.) Kunth ex Walp. [St. Vincent Plum/Kakawate] Leucaena leucocephala (Lam.) de Wit [Lead tree/ Ipil- ipil]

Remarks: \*pH: Potentiometric; N: Walkey & Black Spectrophotometric; P: Olsen; K: Cold Sulfuric Extraction; Cu, Zn, Fe, Mn: diethylenetriaminepentaacetic acid (DTPA)

n = 5, mean  $\pm$  Std. Error.

Observations were done on June-October 2020.

Names inside brackets [ ] are common names of plant species.

found creeping on the ground but most of the vines were climbing on plant species such as Ipil-ipil [Leucaena leucocephala (Lam.) de Wit] and Kakawate [Gliciridia sepium (Jacq.) Kunth ex Walp.]. Native/endemic trees such as Alem [Melanolepis multiglandulosa (Reinw. Ex Blume) Reichb. & Zoll.], Bago [Gnetum gnemon Linn.], Samak [Macaranga tanarius (Linn.) Muell.-Arg], Bignay [Antidesma bunius (L.) Spreng], Binayuyo [Antidesma ghaesembilla Gaertn], Tanobong [Phragmites vulgaris (Lam.) Trin.], Narra [Pterocarpus indicus Willd.], Biknong [Kleinhovia hospita Linn.], Malabago [Hibiscus tiliaceus Linn.], Darumaka [Donax canniformis (G. Forst.) K. Schum.], Uas [Harpullia arborea (Blanco) Radik.], Tibig [Ficus nota (Blanco) Merr.], Ria-ria [Ficus septica Blanco], Oplas [Ficus ulmifolia Lam.], and other species also grew within the *T*. harmandii environment (Table 2).

Concerning soil growing conditions, C. trifo*lia* grew in soils with slightly acidic to moderately alkaline pH, medium to high organic matter content, medium amount of phosphorus, and low to high potassium content. Furthermore, the soils contained a high amount of copper, low to medium zinc, low to high iron, and a high to very high amount of manganese. On the other hand, T. harmandii grew in soils with strongly acidic to slightly acidic pH, low to medium organic matter content, high to excessive phosphorus content, and high potassium content. In addition, the soils also had low to high copper, very low to medium zinc, and high to very high iron and manganese contents (Table 2). Both species can grow in marginal soils with acidic conditions and low nutrient content. C. trifolia grew in soils with low potassium, low zinc, and iron contents while T. harmandii grew in soils with low organic matter and low micronutrients such as copper and zinc.

Comparing the soil properties where *C. trifolia* and *T. harmandii* thrived, the former species grew in soils with higher pH (0.56-1.53 higher) as compared to the latter species which grew in soils with a lower pH (0.56-1.53 lower). *T. harmandii* grew in soils with lower organic matter (0.48-2.38% lower), higher phosphorus (8.10-85.10 ppm higher), higher potassium (179.0-449.0 ppm higher), lower zinc, (0.30-0.50 ppm lower), and higher copper (0.66 ppm higher), iron (4.40-16.60 ppm higher), and manganese (1.40-19.60 ppm higher) contents as compared to *C. trifolia* which

grew in soils with lower macronutrients and micronutrient levels.

#### Phytochemical properties of the medicinal Vitaceae species

Phytochemical screening of plants posts an important role in identifying new sources of therapeutically, nutritionally, and industrially important compounds or chemicals. To compare the two medicinal Vitaceae species, their phytochemical profiles were examined (Table 3). C. trifolia leaves contained almost all the secondary metabolites tested namely alkaloids, flavonoids, phenols, terpenoids, anthocyanins, tannins, and saponins except steroids while the *T. harmandii* leaves only contained phenols, terpenoids, tannins, and saponins. Steroid was not present in the two medicinal Vitaceae species. The presence of saponins, alkaloids, flavonoids, and tannins in C. trifolia [22] and saponins in T. harmandii [23] plant extracts were also reported in other studies. Medicinal plants were reported to contain some natural products or bioactive substances such as tannins, alkaloids, terpenoids, steroids, flavonoids, [24], phenolic compounds [25], and saponins [26].

### Antibacterial properties of the medicinal Vitaceae species

To compare the two medicinal Vitaceae species, their antibacterial activities were examined (Table 4). When tested against Staphylococcus aureus, a gram-positive bacterial strain, C. trifolia and T. harmandii exhibited antibacterial activity with 12 mm and 10 mm mean zones of inhibition, respectively. The two medicinal Vitaceae species showed a partially active reaction against the said pathogenic bacterial strain. The antibacterial activity of *C. trifolia* n-hexane plant extract against S. *aureus* was also reported [27]. Furthermore, *T*. harmandii leaf extract demonstrated a least active reaction against Klebsiella aerogenes with a 9 mm mean zone of inhibition. Based on the phytochemical screening, the species contain secondary metabolites such as flavonoids and terpenoids which are known to have antibacterial activities [28, 29]. Moreover, both species contain tannins and saponins which were reported to demonstrate an antibacterial action against S. aureus [30, 31, 32]. S. aureus infection is a major cause of diseases such as bacteremia, skin and soft tissue infections, endocarditis, metastatic infections, sepsis, and toxic

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Secondary Metabolites	Test Method	C. trifolia	T. harmandii			
Alkaloids	Mayer's Test	Present	Absent			
Anthocyanin	NaOH Test	Present	Absent			
Flavonoids	Shinoda Test	Present	Absent			
Phenols	Ferric Chloride Test	Present	Present			
Saponins	Froth Test	Present	Present			
Steroids	Liebermann-Buchard Reaction	Absent	Absent			
Tannins	Lead Acetate Test	Present	Present			
Terpenoids	Salkowski Test	Present	Present			

Table 3. Phytochemical screening of Cayratia trifolia and Tetrastigma harmandii ethanolic leaf extracts

Table 4. Antibacterial activity of Cayratia trifolia and Tetrastigma harmandii ethanolic leaf extracts

Pactoria	C. trifolia		T. harmandii		
DdCleIId	Zone of Inhibition (mm)*	Activity	Zone of Inhibition (mm)*	Activity	
Staphylococcus aureus	12.0	+	10.0	+	
Escherichia coli	6.0	-	6.0	-	
Pseudomonas aeruginosa	6.0	-	6.0	-	
Enterococcus faecium	6.0	-	6.0	-	
Klebsiella pneumonia	6.0	-	6.0	-	
Klebsiella aerogenes	6.0	-	9.0	+ / -	
Acinetobacter baumannii	6.0	-	6.0	-	

Remarks: Control (distilled water) - 6.0 mm

n = 3, (+) inhibited, (-) uninhibited

\*(< 10 mm) maybe expressed as inactive, (10-13 mm) partially active, (14-19 mm) active, (> 19 mm) very active

shock syndrome [33]. Thus, the two medicinal Vitaceae species have potential in the treatment of these diseases.

#### Conclusion

*C. trifolia* and *T. harmandii* were both found growing in northeastern Cagayan. *T. harmandii* grew in a wide range of habitats, from lower to higher elevations, and soils with higher nutrients while *C. trifolia* only grew in some habitats with mid-elevations and soils with higher pH. Both species grew in environments where ipil-ipil (*Leucaena leucocephala*) and kakawate (*Gliciridia sepium*) trees were found. Furthermore, *C. trifolia* ethanolic leaf extract contains more secondary metabolites as compared to *T. harmandii*. Both species can inhibit the activity of *S. aureus* while a slight inhibiting activity against *K. aerogenes* was observed in the ethanolic leaf extracts of *T. harmandii*.

With our findings, the potential of utilizing the species in product development for medicinal, therapeutic, and industrial purposes is an opportunity. The present findings on the habitat conditions of the two medicinal Vitaceae species will guide future researchers in investigating their growing environments in other places, localities, or ecosystems. Moreover, the concentrations in various plant parts and the specific identities of the phytochemicals present in the two Vitaceae species should be further explored. Initial findings on the antibacterial potential of *C. trifolia* and *T. harmandii* against *S. aureus* will guide future researchers in evaluating the efficacy of medicinal Vitaceae plant extracts in treating various diseases caused by the pathogenic bacteria.

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Regional Soils Laboratory of the Cagayan Valley Integrated Agricultural Laboratory, Department of Agriculture Region 2, Tuguegarao City, Cagayan.

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