Biological and environmental profiling of three Philippine *Tetrastigma* species (Vitaceae) in northeastern Cagayan, Luzon, Philippines

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**Abstract.** Opeña JM, Maramag CL, Alicay CB, Baloloy MV, Gaspar CA, Jr Pacris FA, Torres JS, Sumer LB, Bayani GU, Sotto RC. 2021. Biological and environmental profiling of three Philippine *Tetrastigma* species (Vitaceae) in northeastern Cagayan, Luzon, Philippines. *Biodiversitas* 22: 2956-2970. *Tetrastigma* species have considerable attention because of their medicinal importance, but little is known about these Philippine species. This study provides information on the biological (morphological, phytochemicals, nutritional, antimicrobial characterization) and environmental (diversity, distribution, growing environment, soil characteristics) profiles of the three Philippine *Tetrastigma* species namely, *Tetrastigma ellipticum* Merr., *Tetrastigma harmandii* Planch., and *Tetrastigma loheri* Gagnep. *Tetrastigma harmandii* growing in various habitats were widely distributed throughout northeastern Cagayan, while *T. ellipticum* and *T. loheri* were found only in the mountain forests of the northernmost part of northeastern Cagayan. *Tetrastigma ellipticum* was found only in Mt. Cagua forests and volcano, while *T. loheri* was found growing in the forests of Palau Island, Buwacag mountain, and Mt. Cagua. Most of the plant species growing within the *Tetrastigma* environments were native and endemic trees. Mountain species of *Tetrastigma* (*T. ellipticum* and *T. loheri*) grew in extremely acidic soils with high organic matter content. More secondary metabolites were detected in *T. ellipticum*, which was the only species among the three *Tetrastigma* species to contain alkaloids, flavonoids, and anthocyanin. Saponins were detected only in *T. harmandii*. Steroids were not detected in the three species. Moreover, *T. harmandii* was the only species to have an antibacterial property against *Staphylococcus aureus* and a slight inhibiting activity against *Klebsiella aerogenes*. Between the two edible *Tetrastigma* species, *T. harmandii* contains more macronutrients and micronutrients, while *T. loheri* contains higher crude protein, crude fat, and crude fiber.

**Keywords:** Chemical, antimicrobial, distribution, habitats, *Tetrastigma ellipticum*, *Tetrastigma harmandii*, *Tetrastigma loheri*

**INTRODUCTION**

*Tetrastigma* (Miq.) Planch is one of the most species-rich genera of the economically and agronomically important Vitaceae family (Habib et al. 2017). The genus is characterized by its unbranched to digitately branched tendrils, dioecious sexual system, and 4-lobed stigmas in female flowers (Chen et al. 2011). *Tetrastigma* is also famous in Southeast Asia for being the host plant of the *Rafflesia* (Wen 2007; Lianah et al. 2015) where *Tetrastigma harmandii* was reported as the single host plant of *Rafflesia lagascae* (as *R. manillana*) on Mt. Makiling, Laguna, Philippines (Yahya et al. 2010). *Tetrastigma* species grow suitably in hillside and valleys of shady and moist primary rainforests (Lianah et al. 2015). In the Philippines, there are about 24 species of *Tetrastigma*, of which 15 species are endemic. Species of the genera exhibited divergences with other Asian species at various times (Wen et al. 2013). Diversity, morphological characteristics, and geographical distribution of *Tetrastigma* species in the Philippines were reported by Wen et al. (2013) and Pelser et al. (2016). Recently, other *Tetrastigma* species have been subjected to pharmacological and phytochemical studies. Modern pharmacological studies showed that *T. hemsleyanum* had a wide range of pharmacological functions such as antiproliferative, antitumor, antivirus, anti-inflammatory, antivirus, liver protective, and immunoregulation activities (Zhong, et al. 2006; Xu et al. 2008; Yang and Wu, 2009; Ma et al. 2012; Wang et al. 2018; Ding et al. 2019; Ru et al. 2019). *Tetrastigma glabratum* plant extract was found to contain steroids, terpenoids, flavonoids, saponins, and tannins (Lianah et al. 2015), while leaves of *T. leucostaphylum* contain alkaloids, phytosteroids, saponin, diterpenes, cardiac glycosides, carbohydrate, fixed oils, and fats (Adarsh et al. 2013). Common Philippine *Tetrastigma* species specifically the *T. harmandii* is used as a souring ingredient for fish (Maghirang et al. 2018) and was found to have antimicrobial activities (Brown, 1920) and could treat urinary diseases (Quisumbing, 1951; Carag and Buot, 2017).

However, underutilized Philippine plant species such as the *Tetrastigma* have been given low priority in most research and development programs. Little is known about...
their utilization, geographical extent, morphological variation, and potential in terms of medicine and food. Also, *Tetrastigma* plants are not commonly known by the local people due to limited information. Thus it is important to document the distribution and diversity of *Tetrastigma* as well as the morphological, phytochemical, medicinal, and nutritional properties of *Tetrastigma* species found in the country.

To contribute to the database about the *Tetrastigma* species in the Philippines, exploratory trips had to be conducted to determine the existence and distribution of *Tetrastigma* species in various municipalities of northeastern Cagayan, Philippines. Additionally, it hopes to look into the *Tetrastigma* species growing in protected areas of northeastern Cagayan such as the Paluai Island Protected Landscape and Seascape (an island in the northeastern extremity of Luzon) in Santa Ana, Cagayan and Mt. Cagua (an active volcano lying in the eastern border of the municipality of Gonzaga, Cagayan which is part of the Sierra Madre range in the eastern coast of Luzon). Documentation of the morphology and growing environment to include the soil characteristics and the plants that grow within the *Tetrastigma* environment need to be carried out. This study also aimed to investigate the phytochemical and antimicrobial properties of the three Philippine *Tetrastigma* species. Furthermore, it sought to determine the nutritional content of the two common edible species, *T. harmandii*, and *T. loheri*. Hence, this study will be a great help to prepare strategies for conservation, propagation, and eventually in the commercialization of these underutilized *Tetrastigma* species.

**MATERIALS AND METHODS**

**Prior Informed Consent (PIC) and selection of local researchers**

To satisfy the legal requirements of EO 247 (Bioprospecting) and RA 9147 (Wildlife Resources Conservation and Protection Act), prior informed consent from the Local Government Units (LGUs) through the Office of the Municipal Mayor, Municipal Environment and Natural Resources Office (MENRO) and the community/barangay was obtained through letters asking for permission to conduct the research and collect plant and soil samples in the identified areas. Moreover, a gratuitous permit was secured from the Department of Environment and Natural Resources Region 2 Office for conducting the study in Mt. Cagua and Paluai Island Protected Landscape and Seascape. Selection of local researchers (forest guides, MENRO and Barangay officials, Coast Guards) was made with the stakeholders in different municipalities of northeastern Cagayan based on their indigenous knowledge of the *Tetrastigma* species resources in the study sites. The local researchers per municipality were involved during the fieldwork in their respective areas.

**Sampling site description**

The municipalities in northeastern Cagayan, Philippines namely Lal-lo, Camalaniugan, Aparri, Buguey, Santa Teresita, Gonzaga, and Santa Ana were visited for the presence of *Tetrastigma* species. Northeastern Cagayan experienced a dry season from March to July, while the wet season occurs from September to January. We assessed habitats and environments where the *Tetrastigma* species grew. The presence of *Tetrastigma* species was investigated in various habitats (sole or combination). The latitude, longitude, and elevations were recorded using the Geocam Pro version 5.34 (Wazar-Apps, 6 Place Jacques Bonsergent 75010 Paris, France) and a geographical map was generated using Google Earth Pro.

**Sampling methods**

The study encountered 49 accessions (in population) of *Tetrastigma* species in 7 municipalities, namely Lal-lo, Aparri, Camalaniugan, Buguey, Santa Teresita, Gonzaga, and Santa Ana. About 5 accessions of *T. ellipticum*, 36 accessions of *T. harmandii*, and 8 accessions of *T. loheri* were collected. Accession number coding follows the year of collection and collection number. The accessions were planted in the Cagayan State University-Gonzaga *Tetrastigma* field gene bank and nursery. The species were located in different barangays of the municipalities (Table 1). A combination of walk/vehicular ride, visual encounter, and photo documentation was carried out from June to October 2020.

**Identification and morphological description of the *Tetrastigma* species**

Morphological identification of the *Tetrastigma* species by referring to the species description was further performed according to Pelser et al. (2016). During the sampling, the following morphological characters were recorded: old stem shape and appearance, leaf characteristics such as leaf organization, young leaf color, number of leaflets, leaflet shape, leaf margin, type of venation on the adaxial surface, presence of leaf and petiole indumentum, and tendril morphology (Table 2).

**Multivariate analysis of the *Tetrastigma* species**

Visualization of the morphological similarities/differences among the *Tetrastigma* species and their populations in northeastern Cagayan was done through Principal Coordinate Analysis (PCoA) based on Euclidean distance and Bray-Curtis similarity index. Principal Coordinate Analysis (PCoA) was done using the Paleontological Statistics Software (PAST 4.03) (Hammer et al. 2001).

**Environmental analysis**

The plant species growing within the *Tetrastigma* species-environment were identified. The scientific name, English name, and common/local name were provided. Characteristics of soils where the *Tetrastigma* species grew were also determined. Soil samples where the *Tetrastigma* species grew were collected in various experimental sites. Topsoils were gathered, pulverized, subjected to air-drying at room temperature until total dryness, and were analyzed according to the method outlined by Motsara and Roy (2008). The soil pH, organic carbon (OM), available phosphorus,
available potassium, and trace elements such as copper, zinc, iron, manganese were tested through the Potentiometric method, Walkey and Black Spectrophotometric, Olsen’s method, Cold Sulfuric Extraction, and DTPA extraction, respectively. Interpretation of soil test results was guided by the rating scales of Horneck et al. (2011) for total N, total P, and total K, while the rating scales of Motsara and Roy (2008) were used for the pH and micronutrients.

Table 1. List of characterized *Tetrastigma* accessions and their place of collection

| Accession number | Species          | Collection site  | Latitude  | Longitude  | Elevation (m asl.) | Population-specific characters                                      |
|------------------|------------------|------------------|-----------|------------|--------------------|---------------------------------------------------------------------|
| 20-001           | *T. harmandii* Planch. | Aparri, Cagayan  | 18.3797°N | 121.575°E  | 42.06              |                                                                      |
| 20-002           |                  |                  | 18.3803°N | 121.58102°E | 45.11              |                                                                      |
| 20-003           |                  |                  | 18.3801°N | 121.579°E  | 18.90              |                                                                      |
| 20-004           |                  |                  | 18.3807°N | 121.581°E  | 28.04              |                                                                      |
| 20-006           | *T. harmandii* Planch. | Camalaniugan, Cagayan | 18.2646°N | 121.6613°E  | 35.97              |                                                                      |
| 20-007           |                  |                  | 18.2644°N | 121.6611°E  | 41.15              |                                                                      |
| 20-008           | *T. harmandii* Planch. | Buguay, Cagayan  | 18.19613°N | 121.83661°E | 53.95              |                                                                      |
| 20-009           |                  |                  | 18.19648°N | 121.83664°E | 49.07              |                                                                      |
| 20-010           |                  |                  | 18.18765°N | 121.84537°E | 50.90              |                                                                      |
| 20-011           |                  |                  | 18.27522°N | 121.86894°E | 46.94              |                                                                      |
| 20-012           | *T. harmandii* Planch. | Sta. Teresita, Cagayan | 18.2299°N | 121.88542°E | 43.89              | Light green young and mature leaves (*T. harmandii*)                  |
| 20-014           |                  |                  | 18.22839°N | 121.8835°E  | 52.12              |                                                                      |
| 20-015           |                  |                  | 18.21513°N | 121.87775°E | 52.12              |                                                                      |
| 20-016           |                  |                  | 18.21561°N | 121.879°E   | 49.99              |                                                                      |
| 20-017           |                  |                  | 18.20853°N | 121.90755°E | 56.08              |                                                                      |
| 20-018           |                  |                  | 18.23117°N | 121.9505°E  | 74.07              |                                                                      |
| 20-019           | *T. harmandii* Planch. | Gonzaga, Cagayan  | 18.29163°N | 122.1074°E  | 181.97             | Light green mature leaves with conspicuous yellow midrib (20-020)     |
| 20-020           |                  |                  | 18.28507°N | 122.1114°E  | 152.10             |                                                                      |
| 20-021           |                  |                  | 18.28415°N | 122.11287°E | 146.91             |                                                                      |
| 20-022           |                  |                  | 18.28517°N | 122.11428°E | 134.11             |                                                                      |
| 20-023           | *T. ellipticum* Merr. | Mt. Cagua,        | 18.21905°N | 122.11102°E | 828.14             | Dark green mature leaves with visible yellow spots (20-027 *T. ellipticum*) |
| 20-024           |                  |                  | 18.21688°N | 122.11425°E | 862.89             |                                                                      |
| 20-025           | *T. ellipticum* Merr. | Gonzaga, Cagayan  | 18.21934°N | 122.111172°E | 818.98             |                                                                      |
| 20-026           | *T. loheri* Gagnep. |                  | 18.2254°N  | 122.10846°E | 937.87             |                                                                      |
| 20-027           | *T. ellipticum* Merr. | Gonzaga, Cagayan  | 18.22379°N | 122.1033°E  | 861.97             | Red newly emerged leaves; light green young fully opened leaves (*T. loheri*) |
| 20-028           | *T. ellipticum* Merr. | Gonzaga, Cagayan  | 18.22634°N | 122.09602°E  | 698.91             |                                                                      |
| 20-029           | *T. loheri* Gagnep. |                  | 18.39578°N | 122.23983°E | 152.10             | Light green newly emerged leaves; light green fully opened leaves (*T. loheri*) |
| 20-030           | *T. loheri* Gagnep. | Buwacac mountains, Sta. Ana, Cagayan | 18.39592°N | 122.23999°E | 123.14             |                                                                      |
| 20-031           |                  |                  | 18.39608°N | 122.24022°E | 156.06             |                                                                      |
| 20-032           |                  |                  | 18.39538°N | 122.23931°E | 127.10             |                                                                      |
| 20-033           | *T. harmandii* Planch. | Palau Island, Cagayan  | 18.54369°N | 122.15144°E | 45.11              | Red to reddish-yellow newly emerged leaves; red to reddish light green young fully opened leaves (*T. loheri*) |
| 20-034           | *T. loheri* Gagnep. | Sta. Ana, Cagayan  | 18.55114°N | 122.1505°E  | 86.87              |                                                                      |
| 20-035           |                  |                  | 18.55207°N | 122.14882°E | 56.08              |                                                                      |
| 20-036           | *T. harmandii* Planch. | Lal-Lo, Cagayan  | 18.13551°N | 121.6983°E  | 100.89             | Undulate, thick, large leaves; prominent leaf venation and lenticels in the old stem (20-042 *T. harmandii*) |
| 20-037           |                  |                  | 18.13625°N | 121.70089°E | 107.59             |                                                                      |
| 20-038           | *T. harmandii* Planch. | Gonzaga, Cagayan  | 18.24956°N | 121.99788°E | 64.92              | Large rounded old stems (20-043, 20-049 *T. harmandii*)             |
| 20-039           |                  |                  | 18.25316°N | 121.99811°E | 66.14              |                                                                      |
| 20-040           |                  |                  | 18.25275°N | 122.00239°E | 70.10              |                                                                      |
| 20-041           |                  |                  | 18.24962°N | 122.01225°E | 109.12             |                                                                      |
| 20-042           |                  |                  | 18.24288°N | 122.04192°E | 187.154             |                                                                      |
| 20-043           | *T. harmandii* Planch. | Gonzaga, Cagayan  | 18.28612°N | 122.01881°E | 46.94              |                                                                      |
| 20-044           |                  |                  | 18.34764°N | 122.08643°E | 39.93              |                                                                      |
| 20-045           |                  |                  | 18.35026°N | 122.08727°E | 63.09              |                                                                      |
| 20-046           |                  |                  | 18.37334°N | 122.12352°E | 35.05              |                                                                      |
| 20-047           |                  |                  | 18.41915°N | 122.12968°E | 32.92              |                                                                      |
| 20-048           |                  |                  | 18.42862°N | 122.12618°E | 52.12              |                                                                      |
Table 2. Morphological characters of the Tetrastigma species that were measured qualitatively

| Character                                      | State                                      | Reference                  |
|------------------------------------------------|--------------------------------------------|----------------------------|
| Old stem shape and characteristics            | (1) rounded woody/semi-woody               | Habib et al. 2018          |
|                                                | (2) flat woody/semi-woody                 |                            |
|                                                | (3) rounded herbaceous                     |                            |
|                                                | (4) flat herbaceous                        |                            |
| Leaf organization                             | (1) pinnately trifoliolate                 | Pelser et al. 2016; Habib et al. 2018 |
|                                                | (2) pedately compound                      |                            |
|                                                | (3) palmately compound                     |                            |
| Leaflet number of mature leaves               | (1) 3 leaflets                             |                            |
|                                                | (2) 4 leaflets                             |                            |
|                                                | (3) 5 leaflets                             |                            |
|                                                | (4) 6 leaflets                             |                            |
|                                                | (5) 7 leaflets                             |                            |
| Newly emerged leaf color                      | (1) light green                            |                            |
|                                                | (2) red                                    |                            |
|                                                | (3) reddish yellow                         |                            |
| Leaflet shape                                 | (1) elliptical                             |                            |
|                                                | (2) elliptic-oblong                        |                            |
|                                                | (3) lanceolate                             |                            |
| Leaf margin                                   | (1) serrate                                | Habib et al. 2018          |
|                                                | (2) entire                                 |                            |
|                                                | (3) crenate                                |                            |
| Type of venation                              | (1) cross-venulate                         |                            |
|                                                | (2) pinnate                                |                            |
| Leaf and petiole indumentum                   | (1) absent                                 | Habib et al. 2018          |
|                                                | (2) present in the leaf, absent in the petiole |                        |
|                                                | (3) absent in the leaf, present in the petiole |                      |
|                                                | (4) present in both leaf and petiole       |                            |
| Tendril morphology                            | (1) forked/branched                        | Pelser et al. 2016; Habib et al. 2018 |
|                                                | (2) unforked/unbranched                    |                            |

**Biochemical and microbial screening in the Tetrastigma species**

*Preparation of the ethanolic leaf extracts*

Freshly collected young and mature leaves of the Tetrastigma species were washed thoroughly with running tap water and rinsed thrice with distilled water. The leaves were air-dried at room temperature for 3-4 days and then chopped into smaller pieces. The air-dried leaves were oven-dried at 50°C for several hours, ground and pulverized using mortar and pestle, and sieved. The fine powder was then mixed with 90% ethanol (200 g l⁻¹) at room temperature and filtered. The crude extracts were collected for phytochemical and microbial analyses.

*Phytochemical screening*

The leaf crude extracts were subjected to screening for the presence of the eight secondary metabolites, namely alkaloids, flavonoids, phenols, terpenoids, anthocyanin, tannins, steroids, and saponins. These were determined following the procedures of Guevarra (2005). Phytochemical screening of alkaloids, anthocyanin, flavonoids, phenols, saponins, steroids, tannins, and terpenoids was done through Meyer’s test, NaOH test, Shinoda test, Ferric Chloride test, Froth test, Liebermann-Burchard reaction, Lead Acetate test, and Salkowski test, respectively.

*Microbial sensitivity test*

The ethanolic leaf extracts were subjected to antimicrobial susceptibility test against gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella aerogenes, and Acinetobacter baumannii; and gram-positive bacteria, namely Staphylococcus aureus and Enterococcus faecium using the paper disc diffusion method (Guevarra, 2005). Bacterial strains were obtained from the Central Analytical Laboratory of the Cagayan State University, Tuguegarao City, Cagayan, Philippines. The inoculum size of each tested organism was standardized. The standardized inocula were then plated on Mueller-Hinton agar through cotton swabbing and the plates were incubated for 12 h. Six sterilized Whatman No.1 filter paper discs, 6 mm diameter, were immersed in the ethanolic leaf extracts and the discs were then 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 h. Zones of inhibition were measured and their corresponding interferences were described following the rating scale of Guevarra (2005).
Nutritional and mineral analysis

Young and mature leaves of the known edible Tetrastigma species (T. harmandii and T. loheri) were analyzed following the procedures of Motsara and Roy (2008). The total nitrogen of the Tetrastigma species was determined following the Kjeldahl Jaudber-Gunning method, while the total phosphorus, total potassium, and trace elements (copper, zinc, iron, and manganese) were analyzed using the Vanadomolybdate, Flame Atomic Emission, and Microwave Plasma Atomic Emission Spectroscopy tests, respectively. Moreover, 100 grams of dried leaf tissues were subjected to proximate analysis where crude protein content was determined through the Kjeldahl method, while crude fat and crude fiber content were measured through Filter Bag Technique (ANKOM), and calcium content by Titrimetry test. Interpretation of plant tissue analysis results follows the rating scale of Jones (1967).

RESULTS AND DISCUSSION

Morphological characteristics of the three Tetrastigma species

This study supplements the information provided by Pelser et al. (2016) on the key morphological features of the three Philippine Tetrastigma species identified in this study. The old stem shape and appearance, leaf appearance and leaflet shapes, leaf margin, type of venation on the adaxial leaf surface, petiole color, and presence of indumentum in the leaf and petiole were reported in this study. On the other hand, characteristics on tendril structure, number of leaflets, and leaf morphology were the same as those observed by Pelser et al. (2016). Key morphological features are presented in Figure 1 and Table 3.

Figure 1. Tetrastigma species growing in the northeastern part of Cagayan, Luzon, Philippines, namely Tetrastigma ellipticum Merr. (A), Tetrastigma harmandii Planch. (B), and Tetrastigma loheri Gagnep (C) which were found in various habitats in Cagayan. Newly emerged leaves of Tetrastigma ellipticum Merr. (D), and Tetrastigma harmandii Planch. (E) were observed during asexual propagation. Newly emerged and young fully opened leaves of Tetrastigma loheri Gagnep found in Palau Island, Santa Ana (F, I) and Mt. Cagua, Sierra Madre Mountain range in Gonzaga (G, H, J, K) Cagayan have different colors as observed during asexual propagation. A unique accession of Tetrastigma ellipticum Merr. found in Mt. Cagua forest has dark green mature leaves with visible yellow spots (L). Tetrastigma harmandii Planch species in northeastern Cagayan bear young, green, globose (M), and mature, brown, globose fruits (N) during the month of June to October.
Table 3. Morphological characteristics of three *Tetrastigma* species growing in various habitats of the northeastern part of Cagayan, Philippines

| Scientific name | Old stem shape and characteristics | Leaf morphology and characteristics | Number of leaflets | Leaf margin | Type of venation on the adaxial leaf surface | Petiole color | Leaf and petiole indumentum | Tendril |
|-----------------|------------------------------------|------------------------------------|-------------------|------------|---------------------------------------------|--------------|-----------------------------|--------|
| *Tetrastigma ellipticum* Merr. | Rounded, semi-woody to woody stem | Palmitately compound leaves with glossy, light to dark green leaflets | 3, 4, 5 | Serrate | Pinnate | Young and mature leaves have both green petioles | Absent | Forked/branched tendrils |
| *Tetrastigma harmandii* Planch. | Flat, woody stem (others have rounded stem) | Pedatetally compound leaves with glossy, light green young leaflets | (3) 5 (7) | Serrate | Cross-venulate | Young and mature leaves have both green petioles | Absent | Unforked/unbranched tendrils |
| *Tetrastigma loheri* Gagnep. | Rounded, semi-woody to woody stem | Pinnately trifoliolate leaves with dark green leaflets | 3 | Serrate | Pinnate | Young leaves have green to reddish petioles while mature leaves have green petioles | Absent | Unforked/unbanced / simple tendrils |

*Tetrastigma ellipticum* Merr.

The plants were encountered in the forests and near the volcano crater of Mt. Cagua, Sierra Madre Mountain range in Gonzaga, Cagayan. Species found in the other areas of Mt. Cagua with an altitude of 861.97 m asl. had yellow spots in the leaflets. The newly emerged leaves were light green and trifoliolate which could be confused with those of *T. loheri* considering that both species were found in the forests of Mt. Cagua. The only difference in the newly emerged young leaves of *T. ellipticum* from *T. loheri* was the broader lamina of its trifoliolate leaves unlike the elongated lamina of the trifoliolate leaves of *T. loheri* (Figure 1, Table 3). The older stem of the *T. ellipticum* species varied from semi-woody to woody. The species encountered were in their vegetative stage and were not yet flowering or fruiting.

*Tetrastigma harmandii* Planch.

Most of the species found in the municipalities of northeastern Cagayan, except in the municipalities of Buguey, Gonzaga, and Lal-lo, had no flowers. In the three municipalities, some of the plants bore fruits (Figure 1) in June to October. The color of the leaves depended on the location. The plants found in the shaded area had darker green leaves compared to those in exposed areas. Some accessions found in Cabiraoan, Gonzaga have light green mature leaves with conspicuous yellow midribs. Also, an accession found in Santa Maria, Lal-lo had unique morphological characters in that the lenticels of the old stem as well as the leaf venation were prominent. It also had undulate, thick, and large leaflets resembling the leaves of Robusta coffee. *Tetrastigma harmandii* was observed to be the most common *Tetrastigma* species in Cagayan. The leaves were used as a souring agent in cooking and were known to remove the fishy smell of fish-based dishes.

*Tetrastigma loheri* Gagnep.

*Tetrastigma loheri* species in Mt. Cagua (Sierra Madre Mountain range) in Gonzaga, Palau Island (Protected Landscape and Seascape), and Buwacag mountains in Santa Ana were morphologically different in terms of young leaf characteristics. *Tetrastigma loheri* species encountered in the forests and near the volcano crater of Mt. Cagua Sierra Madre Mountain range in Gonzaga had red to light green newly emerged leaves, while species encountered in the mountain forest of Buwacag in Santa Clara, Santa Ana had light green newly emerged leaves (Figure 1, Table 3). However, *T. loheri* growing in mountain forests of Palau Island in Santa Ana had red to reddish-yellow newly emerged leaves. The differences in the leaf color of newly emerged leaves were observed in the nursery when plants were propagated through stem cuttings (Figure 1). Plants found in Mt. Cagua, Buwacag mountains, and Palau Island forests were creeping on the soil and climbing on other plant species. These plants were at the vegetative stage.

Multivariate analysis of the *Tetrastigma* species growing in northeastern Cagayan

A Principal Coordinate Analysis (PCoA) was performed to determine the dis(similarity) among the different accessions of *Tetrastigma* species found in...
various municipalities of northeastern Cagayan (Figure 2). This was based on the morphological characters, namely old stem shape and characteristics, leaflet number of matured leaves, young leaf color, leaf shape and margin, type of venation, leaf and petiole indumentum, and tendril morphology. Using Euclidean similarity index, coordinates 1 (79.507 eigenvalue) and 2 (30.015 eigenvalue) accounted for 60.05% and 22.67% variations, respectively. Meanwhile, Bray-Curtis similarity index accounted for 50.27% and 27.40% variations for coordinate 1 (0.243 eigenvalue) and coordinate 2 (0.133 eigenvalue), respectively. Scatter plots showed that T. harmandii species found in various municipalities of northeastern Cagayan showed more similarity with T. ellipticum accessions found in Mt. Cagua, Gonzaga than the T. loheri accessions found in Gonzaga (Mt. Cagua) and Sta. Ana (Buwacag mountains and Palau Island), Cagayan. The similarity between T. harmandii and T. ellipticum was evident due to some morphological characteristics where both species have compound leaves with green petioles, and more than three, light green, elliptical leaflets as opposed to the trifoliolate leaves with light green to reddish, lanceolate leaflets, and green to reddish petioles of the young leaves of T. loheri.

PcoA scatter plots also showed differences among T. harmandii and T. loheri accessions in different localities. Less similarity was shown in some T. harmandii accessions found in Gonzaga due to differences in the old stem shape and characteristics, and leaflet number of matured leaves. On the other hand, T. loheri accessions found in Palau Island forest, Sta Ana were less similar than those accessions found in Buwacag mountains, Sta. Ana and Mt. Cagua forest, Gonzaga. Less similarity was due to differences in the color of the newly emerged leaves where T. loheri accessions found in Palau Island have reddish-yellow newly emerged leaves as opposed to the light green newly emerged leaves of T. loheri accessions found in Buwacag mountains and Mt. Cagua forest. Morphological differences among the accessions within a species may be accounted to the type of habitat or locality where these accessions are growing. These data are important in selecting representative accessions to be included in further Tetrastigma investigations or studies. Further studies showing the similarity of the species based on molecular characteristics are suggested.

**Geographical distribution of Tetrastigma species and their environments**

Three species of Philippine Tetrastigma were found in various habitats of northeastern Cagayan, Philippines. In this study, we report the updated preliminary data on diversity, distribution, and growing environment characteristics of the three Tetrastigma species growing in northeastern Cagayan, Philippines. Tetrastigma species distribution in the Philippines was reported in several studies where the three species were said to be widely distributed in Luzon, Visayas, and Mindanao. Reports on the specific location of distribution for T. ellipticum were in Basilan, Panay, and Dinagat while T. loheri was found in Negros, Panay, Samar, Mindoro and Palawan.

![Figure 2. Principal Coordinate Analysis (PCoA) scatter plots of three Tetrastigma species growing in various municipalities of northeastern Cagayan, Luzon, Philippines, based on morphological traits. PCoA was calculated using PAST (Hammer et al. 2001). PCoA plots were computed based on Euclidean (A) and Bray-Curtis (B) similarity indices, transformation exponent c=2 and eigenvalue scale](attachment:image.png)
Meanwhile, *T. harmandii* was distributed in Mindoro, Panay, and Negros (Wen et al. 2013; Pelser et al. 2016). The only report in Cagayan Province was in Bolos Point where *T. loheri* was found (Pelser et al. 2016). This study has encountered these three species of *Tetrastigma* which were distributed around northeastern Cagayan (Figure 3). This study is the first detailed report on the habitat and growing environment of the three *Tetrastigma* species. Of the 49 *Tetrastigma* species accessions encountered during the study, 36 accessions of *T. harmandii* Planch. species were widespread and found in all municipalities and found growing in various elevations ranging from 18.89 m asl. to 187.15 m asl.. This species was found in various habitats, including residential areas, near coastal areas, nipa plantations, agricultural lands planted with rice, corn, and vegetables, grasslands, near water bodies (rivers, creeks, dam, and freshwater spring), secondary forests, outside the caves, and in the community of Palaui Island (Figure 4). However, this species was not found in high elevations such as in Mt. Cagua forests, summit/grassland, and volcano, Palaui Island, and Buwacag mountain forests. Meanwhile, *T. ellipticum* Merr. and *T. loheri* Gagnep. were found in the Mt. Cagua forests with high elevations. *Tetrastigma ellipticum* species were only encountered in Mt. Cagua, Gonzaga, Cagayan (793.08 m asl. to 862.88 m asl.) and were not found in other forests such as in Santa Ana Buwacag mountains and Palaui Island mountains wherein *T. loheri* also existed. *Tetrastigma loheri* was found in the forests of Mt. Cagua (698.90 m asl. to 937.86 m asl.), Buwacag mountains, and Palaui Island (56.08 m asl. to 156.05 m asl.). *Tetrastigma loheri* and *T. ellipticum* were highland or mountain species. The same observation was reported by Pelser et al. (2016) where these two species grew mostly in various mountains in the Philippines. Moreover, *T. ellipticum* and *T. loheri* were found near the Mt. Cagua volcano crater with high sulfur and high-temperature environment, while *T. harmandii* thrived near coastal areas with saline sandy soil.

**Figure 3.** Geographical distribution of three *Tetrastigma* species in northeastern part of Cagayan, Luzon, Philippines.
The vines of *T. harmandii* were mostly growing on trees such as *Melanolepis multiglandulosa* (Reinw. Ex Blume) Reichb. & Zoll. [Alim], *Macaranga tanarius* (Linn.) Muell.-Arg [Samak], *Gliciridia sepium* (Jacq.) Kunth ex Walp. [Kakawate], *Ficus ulmifolia* Lam. [Oplas], *Ficus nota* (Blanco) Merr. [Tibig], *Antidesma bunius* (L.) Spreng [Bignay], *Hibiscus tiliaceus* Linn. [Malabago], *Kleinhovia hospita* Linn. [Biknong], *Coffea robusta* Linden [Kape], and other trees. Meanwhile, vines of *T. ellipticum* and *T. loheri* were mostly encountered as creeping on the soil with other vegetation and climbing on taller plants such as *Dillenia philippinensis* Rolfe [Katmon], *Shorea contorta* Vidal [Lauan], *Wallaceodendron celebicum* Koord. [Banuyo], *Calamus rotang* Linn. [Rattan], *Diplodiscus paniculatus* Turcz. [Balobo], *Donax canniformis* (G. Forst.) K. Schum. [Darumaka], *Dracuncomerom dae* (Blanco) Merr. & Rolfe [Dao], *Ficus nota* (Blanco) Merr. [Tibig], *Hibiscus tiliaceus* Linn. [Malabago], *Diospyros philippinensis* A. DC. [Kamagong], and other plants (Table 4). *T. ellipticum, T. harmandii, and T. loheri* were known to be distributed in the regions of Luzon, Philippines (Pelster et al. 2016). In this study, we observed that most of the plant species growing within the *Tetrastigma* environments were wild bamboo and wild ferns. Other plant species such as agricultural crops, native and endemic species were also found growing nearby and these were believed to provide a favorable growing environment by providing partial shade to the *Tetrastigma* plants (Table 3).
Table 4. Geographical distribution and environment of the three *Tetrastigma* species in the northeastern part of Cagayan, Philippines

| Species                  | Location                  | Habitats and ecology                                                                 | Latitude-longitude and elevations (m asl.) | Other plants growing within the *Tetrastigma* environments                        |
|--------------------------|---------------------------|---------------------------------------------------------------------------------------|-------------------------------------------|-------------------------------------------------------------------------------------|
| *Tetrastigma ellipticum* | Mt. Cagua, Santa Clara,   | High elevation mountain forests (793.09-862.89 m asl.),                               | 18.21°N, 122.11°E to 18.22°N, 122.10°E  | *Dillenelia philippinensis* Rolfe [Catmon/Katmon]                                      |
|                          | Gonzaga                   | Primary growth forests,                                                               | 793.08 to 862.88 m asl.                  | *Shorea contorta* Villal [Lauan]                                                      |
|                          |                           | Near the Mt. Cagua creek,                                                             |                                           | *Wallaceodendron celebicum* Koord. [Narang-dawer, Derahm mahogany/Banuyo]            |
|                          |                           | Near the Mt. Cagua volcano (approx. 100 meters below the volcano crater),             |                                           | *Pandanus tectorius* Parkinson ex Du Roi [Screw pine/Pandan]                         |
|                          |                           | Above the Mt. Cagua falls                                                             |                                           | *Calamandra rotang* Linn. [Rattan]                                                   |
|                          |                           |                                                                                      |                                           | *Dinocloa dielsiana* Pilger [Bikal-boboi]                                            |
|                          |                           |                                                                                      |                                           | *Bambusa spp.* [Wild bamboo]                                                        |
|                          |                           |                                                                                      |                                           | *Asplenium nius* Linn. [Bird’s nest fern/pakpak-lauin]                               |
|                          |                           |                                                                                      |                                           | [Wild ferns]                                                                         |
|                          | Bulala Norte, Aparri       | Residential area,                                                                     | 18.22°N, 122.10°E to 18.23°N, 122.09°E  | *Dirig*                                                                             |
|                          |                           | Only from coastal areas (approx. 200 meters away from the sea),                       | 85.95 to 107.59 m asl.                   | *Melanolepis multiglandulosa* (Reinw. Ex Blume) Reichb. & Zoll. [Alim/Alem]        |
|                          | Babaywan Creek, Juaquim    | Grasslands,                                                                           | 18.22°N, 122.10°E to 18.23°N, 122.09°E  | *Macaranga tanarius* (Linn.) Muell.-Arg [Elephant’s ear/Binunga,Samak]              |
| Dela Cruz, Camalanianuan,|                           | Agricultural lands/Agroecosystem,                                                    | 18.37°N, 122.15°E to 18.38°N, 122.14°E  | *Antidesma bunius* (L.) Spreng [Currant tree/Bignay]                                 |
|                           |                           | Grasslands,                                                                           | 18.38°N, 122.15°E to 18.39°N, 122.14°E  | *Antidesma glaesemille* Gaertn [Black Currant tree/Arosep,Binayayo]                 |
|                           | Cagayan river, Camalanianuan,|                                    | 18.42°N, 122.17°E to 18.43°N, 122.16°E  | *Phragmites vulgaris* (Lam.) Trin. [Common Reed/Tambo,Tanobong]                     |
|                           |                           | Secondary growth forest                                                               | 18.43°N, 122.17°E to 18.44°N, 122.16°E  | *Pterocarpus indicus* Willd. [Rosewood/Narra]                                        |
|                           | Nangatanal Creek, Villa    | Swamp, spring, and irrigation canals,                                                 | 18.45°N, 122.20°E to 18.46°N, 122.20°E  | *Kleinhovia hospita* Linn. [Timanga tree/Biknong]                                   |
|                           | Cielo, Buguey,             | Outside the cave,                                                                     | 18.56°N, 122.21°E to 18.57°N, 122.21°E  | *Hibiscus tiliaoeus* Linn. [Sea Rosemallow/Malabago]                               |
|                           | Villa Leonora, Buguey,     | Island shorelines,                                                                    | 18.66°N, 122.22°E to 18.67°N, 122.22°E  | *Donax canniformis* (G. Forst.) K. Schum. [Common donax/Darumaka]                  |
|                           |                           |                                                                                      | 35.96 to 41.14 m asl.                    | *Harpallia arborea* (Blanco) Radik. [Tulip wood tree/Uas]                           |
|                           |                           |                                                                                      |                                           | *Thapathodea campandata* P.Beauv. [African tulip tree/Sirit-sirit]                   |
|                           |                           |                                                                                      |                                           | *Ficus nola* (Blanco) Merr. [Sacking tree/Tibig]                                     |
|                           | Luga, Santa Teresita,      |                                                                                      |                                           | *Ficus septica* Blanco [Hauli fig tree/Hauli, Ria-ria]                             |
|                           |                           |                                                                                      |                                           | *Ficus ulmifolia* Lam. [Isis, Oplas]                                                |
|                           | Tabaco Cave, Luga, Santa   |                                                                                      |                                           | *Ficus benjamina* Linn. [Weeping fig/Balete]                                        |
| Teresita,                 |                           |                                                                                      |                                           | *Gnetum gnemon* Linn. [Bago]                                                        |
|                           |                           |                                                                                      |                                           | *Gnetum niius* Linn. [Timanga tree/Biknong]                                         |
|                           |                           |                                                                                      |                                           | *Gmelina arborea* Robx. [White tea/Gmelina]                                        |
|                           |                           |                                                                                      |                                           | *Swietenia mahogani* (L.) Jacq. [Mahogany]                                          |
|                           |                           |                                                                                      |                                           | *Ceiba pentandra* (L.) Gaertn [White silk cotton tree/Kapok]                       |
|                           | Aroidowen, Santa Teresita, |                                                                                      |                                           | *Eucalyptus globulus* Labill [Blue gum tree/Eucalyptos]                             |
|                           |                           |                                                                                      |                                           | *Areca catechu* L. [Areca nut palm/Buñga, Boa]                                     |
|                           | Mission, Santa Teresita,   |                                                                                      |                                           | *Syzygium cumini* (L.) Skeels [Java plum/Duhat]                                     |
|                           |                           |                                                                                      |                                           | *Saribus rotundifolius* (Lam.) Blume [Fan palm/Anahaw]                             |
|                           |                           |                                                                                      |                                           | *Jatropha curcas* Linn. [Phyic nut tree/Tubang-bakod]                               |
|                           |                           |                                                                                      |                                           | *Ehretia microphylla* Lam. [Wild tea/Tsang gabat]                                  |
|                           |                           |                                                                                      |                                           | *Piper sarmentosum* Roxb. [Betel leaf/Gawed]                                        |
|                           |                           |                                                                                      |                                           | *Pandanus tectorius* Parkinson ex Du Roi [Screw pine/Pandan]                        |
|                           |                           |                                                                                      |                                           | *Nypa fruticans* Wurm. [Sasa/Nipa]                                                  |
|                           |                           |                                                                                      |                                           | *Morus alba* Linn. [Mulberry/Morera]                                                |
|                           |                           |                                                                                      |                                           | *Bambusa spp.* [Bamboo]                                                            |
|                           |                           |                                                                                      |                                           | *Coffee robusta* Linden.[Robusta Coffee/Kape]                                      |
|                           |                           |                                                                                      |                                           | *Musa sp.* [Banana/Saging]                                                          |
|                           |                           |                                                                                      |                                           | *Cocos nucifera* (L.) [Coconut/Niyog]                                               |

*Note: Some of the plant species are mentioned in a simplified form, and their scientific names are not consistently represented.*
| Tetrastigma harmandii Planch. | Calayan, Gonzaga, Ipil, Gonzaga, Baua, Gonzaga, Cabiraoan, Gonzaga, Casambalangan, Santa Ana, Racat, Santa Ana, Zinungan, Santa Ana, Punta Verde, Palau Island, San Vicente, Santa Ana, | Artocarpus heterophyllus Lam [Jackfruit/Langka] Mangifera indica L. [Mango/Mangga] Carica papaya L. [Pawpaw/Papaya] Sandoricum koetjape (Burm.f.) Merr. [Lolly fruit/Santol] Psidium guajava Linn. [Guava/Bayabas] Tamarindus indica Linn. [Tamarind/Sampalok] Gliciridia sepium (Jacq.) Kunth ex Walp. [St. Vincent Plum/Kakawate] Tabernaemontana pandacaqui Poir. [Banana bush/Kampupot] Chromolaena odorata (L.) R.M. King & H. Rob. [Siam weed/Hagonoy] Leucaena leucocephala (Lam.) de Wit [Lead tree/Ipil-ipil] Imperata cylindrica (L.) Raeusch [Cogon grass, Speargrass/Kogon,Pan-au] Dinoclooa dielsiana Pilger [Bikal-boboi] Ipomoea aquatica Forsk. [Water spinach/Kangkong] Panicum maximum Jacq. [Guinea grass] Passiflora foetida Linn. [stinking passionflower/Prutas-Baguio] [Marapapaya] Capsicum sp. [Wild capsicum] Solanum torvum Sw. [Turkey berry, Devil’s Fig/Tandang-aso] Colocasia esculenta Linn. [Taro/Gabi] Tradescantia spathacea Sw. [Moses-in-the-cradle, boat lily/Banga-bangkaan] Monstera deliciosa Liebm. [Swiss cheese plant, split-leaf philodendron] Mucuna pruriens (L.) DC. [Velvet bean/Sabawil] Centrosema pubescens Benth. [Butterfly pea, Centro/Dilang-butiki] Mimosa pudica L. (Lajavinti) [Sensitive plant/Makahiya] [Wild ferns] |
| Tetrastigma loheri Gagnep. | Mt. Cagua, Santa Clara, Gonzaga Buwacag Falls, Santa Clara, Santa Ana Punta Verde, Palau Island, San Vicente, Santa Ana | [Daer] Diplocyclis paniculatus Turcz. [Bagobo, Balobo] Donax canniforis (G. Forst.) K. Schum. [Common donax/Darumaka] Shorea contorta Vidal [White lauan] Dracantomelon dao (Blanco) Merr. & Rolfe [Pacific Walnut/Dao] Ficus nota (Blanco) Merr. [Sacking tree/Tibig] Hibiscus tilicosis Linn. [Sea Rosemallow/Malabago] Diospyros philippinensis A. DC. [Kamagong gubat] Pterocarpus indicus Willd. [Rosewood/Narra] Mangifera indica L. [Mango/Mangga] Artocarpus sp. [Artocarpus] Citrus limon (L.) Burm. [Lemon] Calamus rotang Linn. [Rattan] Dinoclooa delsiiana Pilger [Bikal-boboi] Bambusa spp. [Wild bamboos] Thysanolaena maxima (Roxb.) [Tiger grass] Caladium sp. [Wild caladium] Begonia sp. [Wild begonia] Spathoglottis plicata Blume [Wild ground orchids] [Wild ferns] |

Note: Observations were done on June-October 2020. Names inside brackets [ ] are common names of plant species.
Soil characteristics of the Tetrastigma species habitats

The chemical composition of soils where the Tetrastigma species are varied (Table 5). Tetrastigma ellipticum species grew in soils with extremely acidic pH with medium to high organic matter content, low to medium phosphorus content, medium to high potassium content, medium to high copper content, very low to low zinc content, very high iron content, and medium to very high manganese content. On the other hand, T. harmandii species grew in soils with strongly acidic to slightly acidic pH with low to medium organic matter content, high to excessive phosphorus content, high potassium content, low to high copper content, very low to medium zinc content, high to very high iron and manganese contents. Tetrastigma loheri species grew in soils with extremely acidic to moderately acidic pH with low to high organic matter content, low to medium phosphorus content, high to excessive potassium content, medium to very high copper content, very low to high zinc content, very high iron and manganese contents.

To compare the species, T. ellipticum and T. loheri grew in soils with very low pH, while T. harmandii grew in soils with higher pH. The soils where T. ellipticum and T. loheri were found also had higher organic matter content than those of T. harmandii. However, the soils where T. harmandii grew contained the highest phosphorus while those of T. loheri contained the highest potassium. In terms of the micronutrient content, the soils where T. loheri was found had the highest copper, zinc, and moderately high manganese content while T. ellipticum grew in soils with the highest iron content but lowest copper, zinc, and manganese contents.

Phytochemical screening of the Tetrastigma species

Phenols, tryptophan, and tannins are commonly present while steroids were commonly absent in the three Tetrastigma species (Table 6). Secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthocyanin, and tannins were detected in the leaves of T. ellipticum. Leaves of T. harmandii and T. loheri contain phenols, terpenoids, and tannins. However, saponins were not detected in T. loheri species. Tetrastigma ellipticum differed from other species due to the presence of alkaloids, flavonoids, and anthocyanins, while T. loheri and T. harmandii lack the aforementioned secondary metabolites. Also, T. ellipticum and T. loheri lack saponins. Among the three Tetrastigma species, only the phytochemical property of T. harmandii was investigated (Agnaryangay et al. 2015, unpublished data). With the above-mentioned findings, the extraction and utilization of secondary metabolites in the Tetrastigma species for pharmaceutical and industrial purposes would be easier since their potential sources were already identified.

Antibacterial properties of the Tetrastigma species

The antibacterial properties of the three Tetrastigma species in northeastern Cagayan against various gram-negative and gram-positive pathogenic bacteria are presented in Table 7. This is the first study on the antibacterial profiles of the three Philippine Tetrastigma species. Among the three species, only the T. harmandii demonstrated an inhibitory effect on the growth of some bacterial strains such as Staphylococcus aureus and Klebsiella aerogenes with 10 mm and 9 mm mean zones of inhibition, respectively. Antibacterial activity of the leaf ethanolic extract of T. harmandii demonstrated a partially active reaction against Staphylococcus aureus and a least active reaction in Klebsiella aerogenes. Mountain forest species of Tetrastigma did not show any antibacterial activity against the tested microorganisms. Among the three Tetrastigma species, only the T. harmandii contained saponins which were reported to have antibacterial mechanism against Staphylococcus aureus (Avato et al. 2006; Khan et al. 2018).

Nutritional and mineral profiles of the edible Tetrastigma species

Tetrastigma harmandii and T. loheri species are utilized for food because of their edible sour leaves (Morton and Collectanea, 1968). This study is the first report on the nutritional profiles of these two edible species (Table 8). Tetrastigma harmandii was found to be deficient in nitrogen, low phosphorus, high potassium, and sufficient calcium, manganese, iron, zinc, and copper contents. On the other hand, T. loheri was found to be deficient in nitrogen and phosphorus, high potassium, low calcium, sufficient manganese, iron, and zinc, and high copper content.

Per 100 g dry weight of Tetrastigma leaves, T. loheri contained higher crude protein, crude fat, and crude fiber than T. harmandii with 6.91%, 0.94%, and 22.2% more crude protein, crude fat, and crude fiber, respectively. In contrast, T. harmandii contained more macronutrients and micronutrients compared to T. loheri. Tetrastigma harmandii contained 0.72% more nitrogen, 0.04% more phosphorus, 0.10% more calcium, 3.0 ppm higher manganese, 84.0 ppm more iron, and 19.0 ppm more zinc content than T. loheri. However, T. loheri contained 0.26% more potassium and 37.0 ppm more copper than T. harmandii.
### Table 5. Soil chemical characteristics of the *Tetrastigma* species growing environment

| Species       | pH       | OM (%)  | Available phosphorus (ppm) | Available potassium (ppm) | Trace elements (ppm) |
|---------------|----------|---------|---------------------------|--------------------------|---------------------|
| *T. ellipticum* | 3.17-4.44 | 3.85-4.0 | 7.90-14.0                  | 190.0-288.0              | Cu: 0.44-1.76, Zn: 0.38-0.60, Fe: 19.20-126.40, Mn: 2.40-6.20 |
| Merr.         | ± 0.401  | ± 0.050 | ± 1.845                   | ± 28.449                 | ± 0.070, ± 0.416, ± 1.097, ± 31.085 |
| *T. harmandii* | 5.25-6.71 | 0.41-3.99 | 20.80-100.0               | 296.0-707.0              | ± 0.28-2.28, ± 0.48-1.38, 7.60-26.20, 5.20-37.60 |
| Planch.       | ± 0.428  | ± 1.059 | ± 25.668                  | ± 128.686                | ± 0.282, ± 0.616, ± 9.565, ± 5.370 |
| *T. loheri*   | 4.34-6.46 | 1.79-4.02 | 2.90-16.40                | 311.0-815.0              | ± 0.72-3.90, ± 0.42-3.46, 10.80-47.80, 6.20-31.40 |
| Gagnep.       | ± 0.676  | ± 0.712 | ± 3.962                   | ± 152.998                | ± 0.973, ± 0.940, ± 7.545, ± 12.170 |

Note: n: 5, mean ± Std. Error

### Table 6. Phytochemical screening of three *Tetrastigma* species leaf crude extracts

| Species                        | ALK | FLV | PHE | TRP | ANT | TNS | STD | SPN |
|--------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| *Tetrastigma ellipticum* Merr. |  +  |  +  |  +  |  -  |  +  |  +  |  -  |  -  |
| *T. harmandii* Planch.         |  +  |  -  |  +  |  -  |  +  |  +  |  -  |  -  |
| *T. loheri* Gagnep.            |  -  |  -  |  +  |  -  |  +  |  -  |  -  |  -  |

Note: *ALK: alkaloids, FLV: flavonoids, PHE: phenols, TRP: terpenoids, ANT: anthocyanin, TNS: tannins, STD: steroids, SPN: saponins

### Table 7. Antibacterial activity of leaf ethanolic extracts of three *Tetrastigma* species

| Species                        | SA | EC | PA | EF | KP | KA | AB |
|--------------------------------|----|----|----|----|----|----|----|
| *Tetrastigma ellipticum* Merr. |  6.0 | 6.0  | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| *T. harmandii* Planch.         | 10.0 | 6.0  | 6.0 | 6.0 | 6.0 | 9.0 | 6.0 |
| *T. loheri* Gagnep.            | 6.0  | 6.0  | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |

Note: Control (distilled water): 6.0 mm, n = 3, *(< 10 mm) may be expressed as inactive, (10-13 mm) partially active, (14-19 mm) active, (> 19 mm) very active. SA: *Staphylococcus aureus*, EC: Escherichia coli, PA: *Pseudomonas aeruginosa*, EF: *Enterococcus faecium*, KP: *Klebsiella pneumoniae*, KA: *Klebsiella aerogenes*, AB: *Acinetobacter baumannii*

### Table 8. Nutritional and mineral composition of *Tetrastigma harmandii* and *T. loheri* growing in northeastern Cagayan, Philippines

| Species                        | CP (%) | CF (%) | CFb (%) | N (%) | P (%) | K (%) | Ca (%) | Mn (ppm) | Fe (ppm) | Zn (ppm) | Cu (ppm) |
|--------------------------------|--------|--------|---------|-------|-------|-------|--------|----------|----------|----------|----------|
| *Tetrastigma harmandii* Planch.| 3.80   | 1.37   | 5.20    | 1.99  | 0.18  | 3.28  | 0.30   | 28.0     | 207.0    | 41.0     | 9.0      |
| *T. loheri* Gagnep.             | 10.71  | 2.31   | 27.40   | 1.27  | 0.14  | 3.54  | 0.20   | 25.0     | 123.0    | 22.0     | 46.0     |

Note: **CP: crude protein, CF: crude fat, CFb: crude fiber

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