

Antioxidant and Cytotoxic Activity of the Leaf Ethanolic Extracts of *Tithonia diversifolia* and *Gliricidia sepium* from Bukidnon, Philippines

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ABSTRACT

Introduction: Nature has been an attractive source of new therapeutic candidate compounds. The plants produce naturally occurring secondary metabolites which are being investigated for their anticancer and antioxidant activities. *Tithonia diversifolia* and *Gliricidia sepium* are commonly growing in Bukidnon, Philippines. These plants have long list of ethno-medicinal uses. Thus, there is a need to further investigate their phytochemical constituents as well as their biological activities.

Objectives: The study focuses on the phytochemicals in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* collected from Bukidnon, Philippines. Moreover, cytotoxic and antioxidant properties of the extracts were also determined through Brine Shrimp Lethality Assay (BSLA) and Total Antioxidant Capacity, respectively. **Materials and Methods:** Qualitative phytochemical screening and determination of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Antioxidant Capacity (TAC) and cytotoxicity of the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* were conducted. **Results and Discussion:** Results of the study showed the presence of flavonoids, phenolics, tannins and terpenoids in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium*. The TPC of *T. diversifolia* (15.20 mg GAE/g dry sample) was found higher than that of *G. sepium* (14.43 mg GAE/g dry sample). Similar trend was also observed in the TFC where *T. diversifolia* and *G. sepium* recorded 12.50 and 9.00 mg QE/g dry sample, respectively. *T. diversifolia* also gave higher TAC of 302.8 mg AAE/ g dry sample than *G. sepium* (200.2 mg AAE/g dry sample). For cytotoxicity test, *T. diversifolia* ($LC_{50} = 14.57$ mg/L) exhibited more potent toxicity as compared to *G. sepium* ($LC_{50} = 15.85$ mg/L). The results indicate that the detected phytochemicals may account for the exhibited biological activities of *T. diversifolia* and *G. sepium*. **Conclusion:** This study uncovers the promising antioxidative and cytotoxic property of *G. sepium* and *T. diversifolia* that warrants further investigation.

Key words: *Gliricidia sepium*, *Tithonia diversifolia*, Cytotoxicity, Antioxidant activity.

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INTRODUCTION

Nature has been an attractive source of new therapeutic candidate compounds. Due to emergence of life-threatening diseases, a demand for new therapies has continued to grow. Scientific researchers are drawing

its attention towards naturally-derived compounds as they are considered to have less toxic side effects.^[1] The plants produce naturally occurring secondary metabolites which are being investigated for their anticancer and antioxidant activities leading to the development of new lead drugs.

Antioxidants significantly delay or prevent oxidative damage of cellular components such as DNA, proteins and lipids.^[2] The antioxidant potential of plants has received a great deal of interest because increased oxidative stress has been identified as a major causative factor in the development and progression of neurodegenerative and cardiovascular diseases. Moreover, it

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is believed that supplementation with exogenous antioxidants or boosting of the endogenous antioxidant defenses of the body is a promising method to counteract the undesirable effects of oxidative stress.^[3]

Apart from antioxidant properties, plants have undeniably long history of use in the treatment of cancer. It is significant to note that over 60% of currently used anti-cancer agents come from natural sources.^[4] In fact, half of all anti-cancer drugs approved internationally were either natural products or their derivatives and were developed on the basis of knowledge gained from small molecules or macromolecules that exist in nature.^[5] With this, plants offer a great opportunity for research studies on discovering new chemical entities. Of the approximately 250,000 plant species, only 6% have been reportedly screened for biological activity and about 15% have been screened for phytochemical activity.^[6] Thus, plants are worth investigating.

T. diversifolia is an invasive weed, growing aggressively along road path, abandoned farmlands and hedges. *T. diversifolia* tolerates heat and drought and can quickly form large herbaceous shrubs. It has been used successfully to improve soil fertility and crop yields,^[7] animal forages,^[8] and compost.^[9] In addition, this plant is important due to its substantial use in traditional medicine in several countries.^[10] The plant was also proven to exhibit anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, antidiarrheal and antimicrobial activities.^[11,12]

Gliricidia sepium, on the other hand, is an exotic plant belonging to the family Fabaceae and is locally known as madre de cacao. The plant parts of *G. sepium*, i.e. tree-barks, roots and leaves, have ethno-medicinal properties and are used as mosquito repellent, fumigants, treatment of dysentery, wound-dressing, antibacterial, antifungal, antiviral as well as central nervous system depressant.^[13] *G. sepium* has been reported to have larvicidal activity, insecticidal, nematicidal and antibacterial activity.^[14]

The present study reports on the phytochemicals present in the leaves of *T. diversifolia* and *G. sepium* collected from Bukidnon, Philippines. Moreover, their cytotoxic and antioxidant properties were also determined through Brine Shrimp Lethality Assay (BSLA) and Total Antioxidant Capacity (TAC).

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts

Leaf samples of *T. diversifolia* and *G. sepium* were collected from Bukidnon, Philippines particularly in Barangay Barobo, Valencia City and Barangay Camp 1, Maramag, respectively, in February 2018. The plant

samples were taxonomically identified by Central Mindanao University (CMU) Museum, CMU, Musuan, Bukidnon. The fresh leaf samples were sterilized using 10% sodium hypochlorite and rinsed with distilled water at least three times. The washed leaf samples were air-dried for three to four weeks under ambient temperature, 25°C. The dried leaf samples were then powdered using a heavy duty blender and stored in an airtight glass container until extraction.

Two hundred fifty grams (250 g) of powdered dry plant leaf samples were soaked in sufficient amount of absolute ethanol for 48 hrs in an ambient room temperature. Then, it was filtered twice using cheese cloth and Whatman filter paper. The collected filtrates were rotary-evaporated under vacuum at 40°C to remove ethanol. The dried crude ethanol extracts were kept in -20°C for further analyses.

Phytochemical Analysis

A. Qualitative Phytochemical Screening

Test for Alkaloids: To 3 mm sample extract, 1 mm of 1M H₂SO₄ was added. The mixture was well-shaken and allowed to stand. Two to three drops of Dragendorff's reagent was further added. A yellow-orange precipitate confirmed the presence of alkaloids.^[15]

Test for Saponins (Foam Test): To half a gram of sample extract, 2 mm of water were added and the mixture was shaken. Positive result was indicated by foaming which persisted for ten mins.^[15]

Test for Tannins: The method of Guevarra was employed with slight modification on the concentration of FeCl₃. To 1 mm sample extract, three drops of 5% FeCl₃ was added. A green-black precipitate indicated the presence of tannins.^[15]

Test for Terpenoids: 2 mm of the sample extract were added with 1 mL of the mixture containing 2,4-dinitrophenylhydrazine in 100 mL of 2 M HCl. A yellow-orange coloration indicated the presence of terpenoids.^[16]

Test for Flavonoids: To 3 mm sample extract, three pieces of magnesium turnings were added and the mixture was warmed. Three drops of concentrated HCl were added. An orange-pink color observed indicated the presence of flavonoids.^[15]

Test for Phenolics: Three milliliters of sample extract were added with 3 drops of ferric chloride-ferrocyanide solution (1 mL of 1% ferric chloride – 1 mL 1M ferrocyanide). A deep blue color indicated the presence of phenolics.^[17]

Test for Anthraquinone: The method of Guevarra was employed with slight modification, that is, extraction of

the aqueous filtrate with benzene was omitted. An aliquot of the sample extract was dried over a steam bath. The residue was taken up with 10 mL distilled water and filtered, discarding the residue. The mixture was treated with five mL ammonia solution and shaken. A red coloration indicated the presence of anthraquinones.^[15]

B. Quantitative Phytochemical Test

Total Phenolic Content (TPC): A modified 96-well microplate Folin-Ciocalteu method was employed in the determination.^[18,19] In a 2 mL Eppendorf tube, 200 μ L (2.5 mg/mL) leaf ethanol extract, 200 μ L 10% Folin-Ciocalteu reagent and 800 mM sodium carbonate were mixed by inverting the tubes 5 times. After incubating for 2 hrs, 200 μ L of the reaction mixture was loaded into the microplate and the absorbance was measured at 750 nm. The standard calibration curve (2.5-15.0 mg/L) was plotted using gallic acid as standard. TPC results were expressed as milligram gallic acid equivalents per gram dry sample (mg GAE/g dry sample). The estimation was performed in four trials and the results were expressed in mean \pm SD.

Total Flavonoid Content (TFC): The TFC of the samples was measured through aluminum chloride method using 96-well microplate reader.^[20,21] 50 μ L (50 μ L, 0.4 mg/mL) of the ethanol extract was loaded into 96-well microplate and added with 10 μ L 10% aluminum chloride, 130 μ L 95% ethanol and 10 μ L sodium acetate. The mixture was then mixed, incubated in the dark for 40 mins and the absorbance readings were taken at 415 nm. The standard calibration curve (5.0 - 20.0 mg/L) was plotted using quercetin as standard. TFC results were expressed as milligram quercetin equivalents per gram dry sample (mg QE/g dry sample). The determination was performed in four trials and the results were expressed in mean \pm SD.

Total Antioxidant Capacity (TAC)

Phosphomolybdenum method was carried out to measure the TAC with slight modification appropriate for spectrophotometric 96-well microplate reader.^[22,23] In the Eppendorf tube, 100 μ L (2.5 mg/mL) of ethanol extract was combined with 0.9 mL phosphomolybdenum reagent (28 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid). The mixture was incubated for 90 mins in 95°C dry oven and cooled at room temperature. An aliquot of the mixture, that is, 200 μ L was loaded into 96-Well microplate and the absorbance was read at 695 nm. The standard calibration curve (10.0-400 mg/L) was plotted using ascorbic acid as standard. TAC results were expressed as milli-

gram ascorbic acid equivalents per gram dry sample (mg AAE/g dry sample). The estimation was performed in 4 trials and the results were expressed in mean \pm SD.

Cytotoxic Activity

Brine Shrimp Lethality Assay (BSLA) was employed in the determination of possible cytotoxic activity of *T. diversifolia* and *G. sepium* ethanolic leaf extracts. A 10000 mg/L stock solution for each sample extract was prepared. Appropriate amounts (2.5, 5.0, 25.0, 50.0, 250.0, 500.0 μ L) of the stock solution were transferred into separate test tubes. The ethanol was allowed to evaporate overnight under the hood. For the test organism, *A. salina* eggs were acquired from the New Aqua Laboratory in Naawan, Misamis Oriental and were hatched in an artificial sea water (38 g per 1L of sterilized double distilled water) placed in a 20x30cm rectangular chamber for 24 hours. The chamber consists of 2 unequal compartments (light and dark) with several whole divider in between. The eggs were added in the dark compartment. After 24 hrs, 10 nauplii were transferred using micropipette into the test tube containing the test solutions and then adjusted to 5.00 mL using artificial sea water to give six different concentrations (5, 10, 50, 100, 500 and 1000 mg/L) of test solutions. Negative and positive controls were prepared using sterilized artificial sea water and aqueous solutions of potassium dichromate, respectively. The test tubes were placed under illumination for 24 hrs and survivors were counted.^[24] The percentage mortality of nauplii was calculated for each concentration of the sample using Equation 1.

$$\% \text{ mortality} = \frac{\text{number of dead nauplii}}{\text{initial number of live nauplii}} \times 100 \quad \text{Eq.1}$$

Probit analysis was used to determine the LC_{50} .^[25] Plant extracts with LC_{50} less than 200 ppm are considered significantly active and had the potential for further investigation.^[26] There were 4 replicates for each concentration of test solution.

RESULTS

Phytochemical Analysis

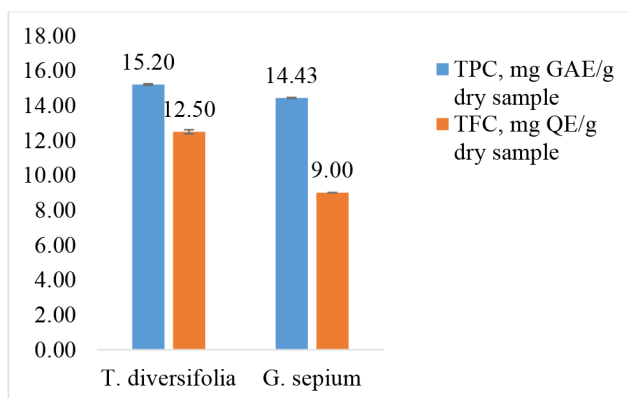
A. Qualitative Phytochemical Screening

The results of the phytochemical screening of the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* are presented in Table 1.

Table 1: Results of phytochemical screening of the ethanolic leaf extracts of *T. diversifolia* and *G. sepium*.

Phytochemical	<i>T. diversifolia</i>	<i>G. sepium</i>
Alkaloid	-	-
Anthraquinone	-	-
Flavonoids	+	+
Phenolics	+	+
Tannins	+	+
Terpenoid	+	+
Saponin	-	-

+ : present - : absent

**Figure 1: Total phenolic and flavonoid content of the ethanolic extracts of *T. diversifolia* and *G. sepium*.****Table 2: Total Antioxidant Activity of crude ethanol leaf extract of *H. conoidea*, *T. diversifolia* and *G. sepium*.**

plants	Mean±SD mg AAE/g dry sample
<i>T. diversifolia</i>	302.8 ± 8.95
<i>G. sepium</i>	200.2 ± 4.37

TAC values are expressed as mean ± standard deviation (SD), n=4.

B. Quantitative Phytochemical Test

Total Phenolic and Total Flavonoid Content

The TPC and TFC in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* are presented in Figure 1.

Total Antioxidant Capacity

Results of the TAC determination are presented in Table 2.

Cytotoxic Activity

The cytotoxicity of the ethanolic extracts of *T. diversifolia* and *G. sepium* expressed as median lethal concentration (LC_{50}) is shown in Table 3.

Table 3: LC_{50} (mg/L) of the ethanolic extracts of *T. diversifolia* and *G. sepium* against *A. salina* nauplii.

Sample	LC_{50} , mg/L	Inference*
<i>T. diversifolia</i>	14.57 ± 3.59	Cytotoxic
<i>G. sepium</i>	15.85 ± 3.49	Cytotoxic
$K_2Cr_2O_4$	9.68 ± 1.74	Cytotoxic

* LC_{50} < 200 ppm (mg/L) is cytotoxic (Alali et al. 2006)Median lethal concentration, LC_{50} values in mg/L, are expressed in mean ± standard deviation (SD), n=3.

DISCUSSION

Phytochemical Analysis

A. Qualitative Phytochemical Screening

In this study, flavonoids, phenolics, tannins and terpenoids were detected in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium*. Meanwhile, alkaloid, anthraquinone and saponin were found absent among the extracts (Table 1). The results indicate that these detected phytochemicals may account for the reported medicinal properties of *T. diversifolia* and *G. sepium*.

T. diversifolia has been used by the Ugandan farmers for pest management in the fields, cure for constipation, stomach pains, indigestion, sore throat, liver pains and malaria and has been reported to exhibit anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, antidiarrheal, antimicrobial, antispasmodic, vasorelaxant and cancer-chemopreventive.^[27] On the other hand, *G. sepium* has been used as mosquito repellent, fumigant, treatment for dysentery, wound dressing, antibacterial, antifungal and antiviral agent.^[28]

Related studies on phytochemical screening have shown presence of different metabolites in the plant samples. The findings of this present study are consistent with the results of a previous study which reported the presence of phenolics, flavonoids and tannins and the absence of alkaloids and saponins in the *T. diversifolia* ethanolic flower extract.^[29]

Phenolics are well-known for its antioxidative^[30] and antimicrobial properties.^[31] These physiological properties are responsible for the preventive activity of phenolic compounds against infectious and degenerative diseases, inflammation and allergies via antioxidant, antimicrobial and protein or enzyme neutralization or modulation mechanisms.^[31] Flavonoids and tannins are among the classes of phenolic compounds. Accordingly, flavonoids have many favorable medicinal and physiological properties and are proven to exhibit antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties. They are also known to be modulators of key cellular enzyme functions and potent inhibitors of

xanthine oxidase, cyclo-oxygenase, lipoxygenase and phosphoinositide 3-kinase.^[32]

Tannins, on the other hand, are found to show antioxidative, anticarcinogenic and antimutagenic potentials. Previous studies revealed that tannins exert other physiological effects, such as acceleration of blood clotting, lowering of blood pressure, decreasing the serum lipid level and modulating immunoresponses.^[33] Terpenoids act as antibiotics.^[34] These compounds are also reported to possess chemopreventive and therapeutic effects on cancer and cell signaling activities and have been used in the prevention, inhibition and therapy of several illnesses including cancer and they also exhibit other interesting biological properties such as antiviral,^[35-38] antimicrobial,^[39-42] anti-allergenic,^[43,44] antispasmodic, antidiabetic,^[45,46] anti-inflammatory,^[47,48] anticancer^[49] and immuno-modulatory properties.^[50]

The wide-array of physiological functions associated to the phytochemicals present in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* may support their recorded ethno-medicinal uses.

B. Quantitative Phytochemical Test: Total Phenolic and Flavonoid Content

TPC and TFC

The TPC values in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* are 15.20 and 14.43 mg GAE/g dry sample, respectively (Figure 1). The detection of phenolics in the leaves of *T. diversifolia* and *G. sepium* is consistent with the data in the literature.^[51,52] It was reported that the aqueous leaf extract of *T. diversifolia* showed TPC of 55.92 ± 4.45 GAE mg/g dry weight.^[53] The present findings provide scientific evidence to support medicinal value of *T. diversifolia* and *G. sepium* as potential source of antioxidant compounds.

Between the ethanolic leaf extracts, *T. diversifolia* gave a higher TPC than *G. sepium*. These findings suggest that the TPC values of *T. diversifolia* and *G. sepium* are influenced by their taxonomic classification. Accordingly, the pharmacological potential of any plant is dependent on the composition of secondary metabolites, which is unique for the individual taxa.^[54] Different taxa of plants differ in genomic sequence.^[55] Transcriptions of these genes which encode biosynthetic enzymes lead to the production of phytochemicals which are usually restricted to a few families or genera.^[56]

Flavonoids, on the other hand, are polyphenolic molecules known for their antioxidant and anti-inflammatory health benefits as well as support to the cardiovascular and nervous system.^[57] The TFC values in the ethanolic

leaf extracts are higher in *T. diversifolia* compared to *G. sepium* (Figure 1). The results imply that the TFC in the plant leaf samples maybe influenced by its taxonomic classification.

Total Antioxidant Capacity

As shown in Table 2, the TAC of *T. diversifolia* was found to be higher than that of *G. sepium*. Finding on the TAA of *T. diversifolia* is in coherence with the previous report. The aqueous leaf extracts of *T. diversifolia* was found to have TAC of 93.09 ± 37.91 μ M TEAC/mg dry weight as measured by the ABTS radical cation decolorization assay. Further, it was confirmed that *T. diversifolia* is an interesting source of antioxidant, reduces the elevation of lipid profile and lipid peroxidation and improves glucose metabolism.^[53] *T. diversifolia* is a widespread plant in Vietnam and its extracts displayed antimicrobial, antimalarial, larvicidal activity against *Aedes aegypti* and was found to contain the anti-inflammatory chlorogenic acid and some anti-hyperglycemic compounds such as cerebrosides.^[58] The aqueous leaf extract of *T. diversifolia* had N-acetyl cysteine equivalent antioxidative capacity of 32.62 ± 1.87 and 20.99 ± 2.79 mg N-acetyl cysteine/g extract, respectively determined by the ABTS-radical and DPPH-radical assay.^[59]

Given the ecological adaptability and wide distribution features as an invasive weed and the proficiency to yield promising therapeutic natural products of *T. diversifolia*,^[60] results of this present study suggest the potential of *T. diversifolia* plants growing in Bukidnon, Philippines.

G. sepium was valued in Nigeria for its medicinal, ornamental, insect repellent and for soil fertility improvement purposes. In a previous study, *G. sepium* and *Spathodea campanulate* were compared. *S. campanulate*, known as African tulip tree belonging to the Bignoniaceae family, were traditionally used in Nigeria against urethra inflammation, kidney diseases and antidote for animal poisons. Its stems were employed for treatment of stomach aches, herpes, diarrhea and fungal skin diseases. The said study revealed that extracts of these plants revealed appreciable ferric reducing antioxidant activity as well as effective DPPH radical scavenging activity. In particular, the leaf ethanol extract of *G. sepium* had the highest ferric reducing antioxidant activity than its methanol and petroleum ether extracts. Moreover, leaf ethanol extract of *G. sepium* had higher antioxidant activity compared to the methanol, ethanol and petroleum ether extracts of *S. campanulate*.^[61]

Cytotoxic Activity

The brine shrimp lethality assay (BSLA) is considered a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities in plant extracts. BSLA relies on the principle that most active plant constituents are toxic in high doses. Thus, evaluating their toxicity to zoological systems is an indicator of their bioactivity. Moreover, it is important to note that BSLA appears to be predictive of cytotoxicity and pesticidal activity.^[62]

LC₅₀ is a statistical index which indicates the concentration of a chemical agent capable of causing death in 50% of organism's population.^[63] The lethal concentration for 50% mortality after 24 h of exposure (the chronic LC₅₀) is determined as the measure of toxicity of the extract or compound.

The results of the BSLA indicated that the ethanolic leaf extracts and the positive control (K₂Cr₂O₄) produced dose-dependent cytotoxicity effect to brine shrimp nauplii. The LC₅₀ of the ethanolic leaf extracts were 14.57 and 15.85 mg/L for *T. diversifolia* and *G. sepium*, respectively. The positive control, on the other hand, gave the lowest LC₅₀ value of 9.68 mg/L. Since cytotoxicity and LC₅₀ values have inverse relationship, the findings of the study imply that between the plant sample extracts, *T. diversifolia* exhibited higher toxicity, as compared to *G. sepium*. Plants having LC₅₀ values < 200 ppm (extract) are considered as highly active.^[26] Thus, all of the plant extracts are potentially cytotoxic and may exhibit a potential anti-tumor or anti-cancer activity.

Previous studies have reported a positive correlation between brine shrimp toxicity and 9KB (human epidermoid carcinoma of nasopharynx) cytotoxicity ($p=0.036$ and Kappa=0.56). Moreover, the usefulness of brine shrimp as prescreen for antitumor activity was confirmed in a blind comparison with *in vitro* cytotoxicity and 3PS (*in vivo* P388 murine leukemia) activity ($p=0.033-0.0334$).^[26,64] Furthermore, studies on *T. diversifolia* and *G. sepium* have reported their various bioactivities. The ethanolic flower extract and aqueous leaf extract of *T. diversifolia* has exhibited antioxidant and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, respectively.^[29] Screening of the dichloromethane partition of the ethanol extract of over 100 Puerto Rican plants has found that *T. diversifolia* is potentially active (LC₅₀ < 1000 µg/ml) against brine shrimp nauplii. Further testing has revealed cytotoxicity of *T. diversifolia* against HeLa and CHO (Chinese hamster ovary) cells.^[65] *T. diversifolia* also exhibited allelopathic properties. The ethyl acetate

extracts of the leaves, stems and roots showed significant inhibition of wheat coleoptile growth and the leaf extract had similar inhibitory effects to a commercial herbicide.^[66]

The larvicidal activity of *G. sepium* leaves against the fourth instar larvae of Anopheles mosquitoes has been observed for the petroleum ether extract.^[67] Maximum mosquito (*Aedes aegypti*) repellency of 78% for the leaf ethanolic extracts of *G. sepium* have been also recorded using arm-in-cage studies as compared to citronella oil with 74% repellent activity.^[14] Moreover, the ethanolic leaf extracts of *G. sepium* have shown high antibacterial activity against *E. coli*^[14,68] and *P. aeruginosa*.^[68] Good nematocidal property of *G. sepium* has been also reported as proven by a 60% mortality of *Meloidogyne incognita* nematode in different concentrations of its ethanolic leaf extract.^[14]

Lastly, the presence of phytochemicals, i.e. flavonoids, phenolics, tannins and terpenoids, in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* may provide additional scientific evidence to their cytotoxic property.

CONCLUSION

Flavonoids, phenolics, tannins and terpenoids were detected in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium*. *T. diversifolia* showed the highest TPC, TFC and TAC. The plant leaf samples exhibited cytotoxic property. These findings support that the *T. diversifolia* and *G. sepium* grown in Bukidnon, Philippines possess the reported beneficial uses and can be potential sources of natural bioactive compounds.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the subject matter or materials discussed in this manuscript.

ABBREVIATIONS

BSLA: Brine Shrimp Lethality Assay; **TAC:** Total Antioxidant Capacity; **TPC:** Total Phenolics Content; **TFC:** Total Flavonoids Content; **GAE:** Gallic Acid Equivalent;

QE: Quercetin Equivalent; **AAE:** Ascorbic Acid Equivalent; **LC₅₀:** Median Lethal Concentration.

SUMMARY

This study reports on the phytochemical constituents in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium* collected from Bukidnon, Philippines. The cytotoxic and antioxidant properties of the extracts were also determined. Results of the study showed the presence of flavonoids, phenolics, tannins and terpenoids in the ethanolic leaf extracts of *T. diversifolia* and *G. sepium*. On the other hand, the TPC, TFC, TAC and cytotoxicity of *T. diversifolia* ethanolic leaf extract were consistently higher than that of the *G. sepium* ethanolic leaf extract. The exhibited biological activities of *T. diversifolia* and *G. sepium* may be attributed to the phytochemicals detected in the extracts. However, both plants can be promising sources of natural antioxidative and cytotoxic compounds.

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