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Chemical Profiles and Biological Activities of Common Medicinal Plants Artemisia vulgaris, Cyanthillium cinereum, Eleucine indica, and Gliricidia sepium

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Abstract

For centuries, people rely on medicinal plans for treatment of various ailments. Many natural products provide a wide range of various properties for medicinal properties from which other drugs content have been extracted. This study sought to determine the phytochemical components of four medicinal plants namely Artemisia vulgaris, Cyanthillium cinereum, Eleucine indica, and Gliricidia sepium and evaluate their cytotoxic, antioxidant, and antitumor activities. Thin-Layer Chromatography (TLC) was done to determine the secondary metabolites present in each plant extract. Cytotoxicity of the extracts was determined by Brine Shrimp Lethality Assay (BSLA). Antioxidant capacity was also determined through Folin-Ciocalteu method where their ascorbic acid equivalents were computed. Antitumor potential was determined against Agrobacterium tumefaciens. Results showed an array of secondary metabolites like alkaloids, phenolics, flavonoids, sugars, triterpenes, and essential oils. The plants have antioxidant capacities ranging from 220-7,300 mg AAE/g extract, where A. vulgaris showed the highest potential. Cytotoxicity assay showed that the extracts are not toxic at 6-hr exposure but exhibited toxicity at 12-hr exposure on A. salina. The extracts showed antitumor potential with C. cinereum having the highest activity at 60% tumor inhibition. Further undertakings include the isolation of the bioactive metabolites of the different medicinal plants and for further assays on pharmacological effects of the extracts.

Keywords: Antioxidant Capacity, BSLA Assay, Cytotoxicity, Thin-Layer Chromatography, A. tumefaciens tumor inhibition.

Introduction

The use of natural products in treating common illnesses is widely practice among rural areas and even urban settlers for a very long period of time. This is most commonly used by traditional healer to replace high cost industrial drugs. Also, many natural products provide a wide range of various properties of healthful components from which other drugs content have been extracted. This phenomenal practices arises the interest of many researchers to study the components present in this plants. And the next shot for new dose of medicine from natural products to cure pathogenic diseases have become a race to many researchers around the world.

The World Health Organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various diseases. These plants which are widely found in our environment enjoy wide range of acceptability through the population and serve as a cheaper alternative to orthodox medicine (Chanda et al., 2015; Chowdhury et al., 2012).

However, to develop these medicinal plants as drugs, attempts should be first made to certainly identify them and preclinical studies on them should be carried out to establish their claimed therapeutic properties (Asaduzzaman et.al., 2015). These are very important because the biological activity of a plant or its preparation will assist on determining the therapeutic target of its development. Since the chemical constituents and pharmacological actions of most of these plants are known and as they are in current use in traditional medicines, their clinical evaluation can be undertaken (Asaduzzaman et.al., 2015).

The scientific interest has now been diverted towards the natural compounds which are biocompatible, safe, and also cost-effective. Thus, efforts are continuously being made to identify such agents and to validate their scientific authenticity (Baravalia et al., 2012). Despite some beneficial effects described by users, the indiscriminate use of plants and their infusions without medical advice or scientific basis can be dangerous. Several cases of biological effects of medicinal plants have been reported and drug interactions and side effects of theses products are not completely known (Abraham, 2015; Rotblatt & Zimett, 2002).

There is an increasing public clamor for cheaper, safer, and natural alternative cure for diseases. Nowadays, with an increase in unhealthy lifestyle and oxidative stress, more diseases have surfaced. people are now inclined to the consumption of herbal medicines. However, herbal-derived remedies need a powerful and deep assessment of their pharmacological qualities.

With increasing recognition of herbal medicine as an alternative form of healthcare, screening of medicinal plants for biologically active compound has become an important source of antibiotic prototypes, cell-protecting agents, and cancer related drugs. Hence for selecting crude plant extract with potential useful properties, invitro screening methods have been used for further in-depth chemical elucidation and pharmacological investigations. Thus, this study addressed such scientific endeavor for bringing into the light the effectiveness and safety of the aforementioned medicinal plants as cure for diseases.

Objectives of the Study

This study sought to determine the phytochemical components and biological activities of four medicinal plants namely *Artemisia vulgaris, Cyanthillium cinereum, Eleucine indica, and Gliricidia sepium.*

Specifically, it aimed to

1.Determine the secondary metabolites from the plant through TLC fingerprinting,

2.Determine the cytotoxicity of the plant extract extracts through brine shrimp lethality assay (BSLA),

3.Determine the antioxidant potential through Folin-Ciocalteu method, and

4.Determine the antitumor potential using *Agrobacterium tumefaciens.*

Methodology

Collection and Extraction of Leaves of Medicinal Plants

Fresh leaves of *Artemisia vulgaris, Cyanthillium cinereum, Eleucine indica,* and *Gliricidia sepium* were collected in the farm yards of Quirino Province, Philippines. The leaves were air dried, chopped, and soaked overnight with methanol. After overnight soaking, the methanolic extracts were filtered and evaporated using water bath in a stove with temperature ranging from 40°C-50°C, the extracts were then transferred to vials and stored in a refrigerator.

Thin Layer Chromatographic Screening of the Secondary Metabolites

The samples were developed in Silica Gel F_{254} pre-coated aluminum plates using ethyl acetate and methanol which gave the best separation of the compounds, then visualized and marked using different spray reagents like Dragendorff, Potassium ferricyanide-Ferric chloride, Vanillin-H_2SO_4, alpha-naphthol sulfuric acid, Methanolic KOH to determine the class of compounds present.

Antioxidant Assay: Determination of Total Phenolics Content through Folin-Ciocalteu Method

The Total phenolics content of the crude extracts was determined with the Folin-Ciocalteu method of Hodzic et al. (2009) with modifications. A calibration curve was determined at different concentrations (0.25, 0.5, 1.0, 2,0 & 4.0 mg/mL) of Ascorbic Acid using UV-Vis Spectrophotometer (Spectronic-200, Thermofisher). The Ascorbic Acid solutions (volume of 1 mL) were place in vials. To each vial, 200 µL of Folin-Ciocalteu reagent and 1 mL of Na_2CO_3 was added. Immediately, these were transferred to cuvettes and read using UV-Vis spectrophotometer at 680 nm wavelength. After standardization, 1 mg of the crude extracts was dissolved in 1 mL distilled water.

From each of the extract, 1 mL was transferred to vials (in triplicates). To theses, 200 µL of Folin-Ciocalteu reagent was added and incubated at room temperature for 5 minutes. Then, 1 mL Na_2CO_3 was added to the mixture of crude culture extracts and FC reagent and read at 680 nm wavelength. Absorbance values of the crude extracts were compared with the calibration curve prepared using the ascorbic acid. The total phenolics content was calculated based on the standard curve of the ascorbic acid and its linear regression as shown in this equation: y=mx+b, where y represents the optical density, m represents the slope of the ascorbic acid regression line, and b represents the y-intercept (Hamidi et al, 2014).

Brine Shrimp Lethality Assay (Cytotoxicity Assay)

Cytotoxicity activity of the plant extracts were determined by brine shrimp lethality assay as described in Meyer et al. (1982).

Sample Preparation

Seven test tubes were prepared for the assay with concentrations (ppm) of 2000, 1000, 500, 250, 125, 62.5, 31.25 respectively. About 1g of each sample crude extract was weighed and mixed with 50 mL artificial sea water. Then it was diluted until the desired concentration of 4000 ppm was obtained. Two-fold serial dilution was done to obtain the concentrations above.

Hatching the Shrimp

Brine shrimp (*A. salina*) eggs were hatched in a shallow petri dish filled with artificial "sea water" (19 g sea salt pure NaCl was weighed, dissolved in 500 mL of distilled water). A paper divider with holes was place in the middle of the dish to make two un equal chambers. The shrimp eggs were sprinkled into the larger chamber which was darkened (covered) while the smaller chamber was illuminated. After 48 hours the phototropic nauplii were collected using Pasteur pipettes from the illuminated chamber.

Bioassay

Minimum of 10 larvae of brine shrimps (nauplii) were transferred to each of the prepared test tubes of different concentration using a Pasteur pipette with a long tip and the total volume I the test tubes were adjusted to 2 mL with artificial sea water. The vials were maintained under illumination. The shrimps that survived were counted after every 3 hours within 24 hours. The total death and percentage mortality at each dose level were determined.

Determination of the Antitumor Potential Against *A. tumefaciens*

The antitumor potential of the isolates were analyzed by following the protocol of Mc Laughlin and Rogers (1998). *A. tumefaciens* were grown in culture in sucrose, nutrient broth, and yeast extract (SNBYE) medium autoclaved for 15 minutes at 121°C and 15 psi pressure. The culture was kept in a flask and constantly for 48 hours at 30°C. a 1.5% agar was prepared by adding 100 mL of distilled water to 1.5 g of Bacto-agar. This was autoclaved, including all instruments for the microbiological assay. After autoclaving, the agar was poured in petri plates and allowed to cool and solidify. Then sugar beets were washed in running water to remove dirt and then soaked in sodium hypochlorite solution. Four milligrams of the isolates were also dissolved in 1 mL of Dimethyl sulfoxide (DMSO) in preparation for the assay. A bacterial inoculum was prepared by adding 1.5 mL of water, 2.0 mL of the 48-hour bacterial culture, and 0.5 mL of the extract dissolved in DMSO in a tube. A negative control was prepared by adding DMSO only instead of sample. Antitumor assay was done by cutting sugar beets and cylinders were bored out. These were cut into discs which were placed on top of the solidified agar (5 discs per plate). Then 0.5 mL of the inoculum was dropped in each disc aseptically. The plates were kept in the dark at 27°C for 15 days. Then the percent inhibition of the crown gall tumor was calculated using the equation:

$$\%\ inhibtion = 100 - \frac{Average\ number\ of\ tumors\ of\ sample}{Average\ number\ of\ tumor\ of\ control} x\ 100$$

Data Analysis

Analysis and processing of results of the experiments on cytotoxicity assay, antitumor assay, and antioxidant assay was done using Microsoft Excel for linear regression, mean, and percentage computation, and graphical representations.

Results and Discussion

Thin Layer Chromatographic Screening of the Secondary Metabolites

The different crude extracts contain a wide array of secondary metabolites. Table 1 shows the type of compounds present in each plant material being studied.

Table 1. Phytochemical Constituents of the Different Plant Extracts

Sample	Alkaloids	Flavonoids	Phenols	Triterpenes	Essential Oils	Sugars
A. vulgaris	+	+	+	+	+	-
C. cinereum	+	+	+	+	+	+
E. indica	+	-	+	-	-	-
G. sepium	+	-	+	-	+	-

Antioxidant Activity (Total Phenolics Content)

The crude methanolic extracts were subjected to antioxidant assay to determine the antioxidant activity of the medicinal plants. Analysis of the total phenolics content (TPC) of the crude extracts were done using Folin-Ciocalteu reagent. The TPC was established using the ascorbic acid equivalence (AAE). The calibration curve of ascorbic acid (1000 ppm) was first established and is shown in Figure 1.

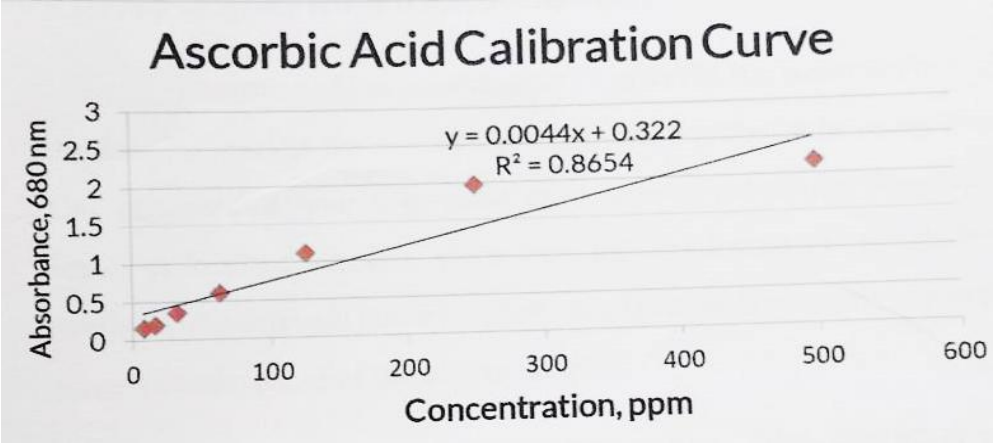

Fig. 1. The regression line equation of the calibration curve of Ascorbic acid

The mean absorbance of the crude extracts (1000 ppm) at 680 nm were computed. These mean absorbance values were then computed from the equation of the calibration curve to determine their AAE. Results are shown in Table 2.

Table 2. Ascorbic Acid Equivalents of the different plant samples in Folin-Ciocalteu Assay for Total Phenolics Content

Plant Extract	Total Phenolic Content, *mg AAE/g*
A. vulgaris	7, 340.91
C. cinereum	7, 727.30
E. indica	2, 909.09
G. sepium	227.27

It can be gleaned from the table that only *G. sepium* exhibited the lowest AAE. All the rest of the medicinal plants have very high ascorbic acid equivalence. A gram of each extract is tantamount to consumption of 6-14 Vitamin C (500 mg) tablet. With the presence of high phenolics content, we can conclude that it has high antioxidant power. Thus, consumption of the plant leaves can fight oxidative stress, diseases, and tissue ageing and can promote cell renewal and cell protection.

Brine Shrimp Lethality Assay (Cytotoxicity Assay)

Because of the potential of the extracts, it is worthwhile to determine the toxicity level of the extracts through cytotoxicity assay using brine shrimp, *A. salina*. The organism is now suggested as a convenient probe for pharmacologic activities in plant extracts which may be manifested as toxicity towards the newly hatched nauplii (Sarah et al., 2017). Table 3 presents the median lethal concentration (LC_{50}) of the plant samples.

Table 3. Median Lethal Concentration **(LC_{50}) of the Different Plant Samples on Brine Shrimp (*A. salina*) Cytotoxicity Assay**

Plant Extract	LC_{50} (6hrs)	LC_{50} (12hrs)
A. vulgaris	8, 082.541 (Not Toxic)	836.77 (Low Toxicity)
C. cinereum	6, 185.32 (Not Toxic)	978.53 (Low Toxicity)
E. indica	1, 032.254 (Not Toxic)	539.21 (Low Toxicity)
G. sepium	3, 078.85 (Not Toxic)	2153.63 (Not Toxic)

From the results obtained, it can be noted that the extracts are not toxic at 6 hour exposure with LC_{50} value of >1000 µg/mL which is supported by Meyer's test stating that LC_{50}<1000 µg/mL is considered toxic and also with Clarkson's toxicity

assessment which he classified LC_{50} >1000 µg/mL are not toxic, LC_{50} of 500-1000 µg/mL are low toxicity and LC_{50} of 0-100 µL are highly toxic. It is interesting to note that *G. sepium* is not toxic at 12-hr and even in 24-hr exposure. Therefore, the plant extracts can be used for pharmacological purposes with proper dosage (Musa, AA., 2012).

Antitumor Potential of the Medicinal Plants

The anti-tumor potential of the plant extracts were also determined using the potato tumorigenic agent, *A. tumefaciens.* Figure 2 shows the inhibition of the plant extracts against tumor.

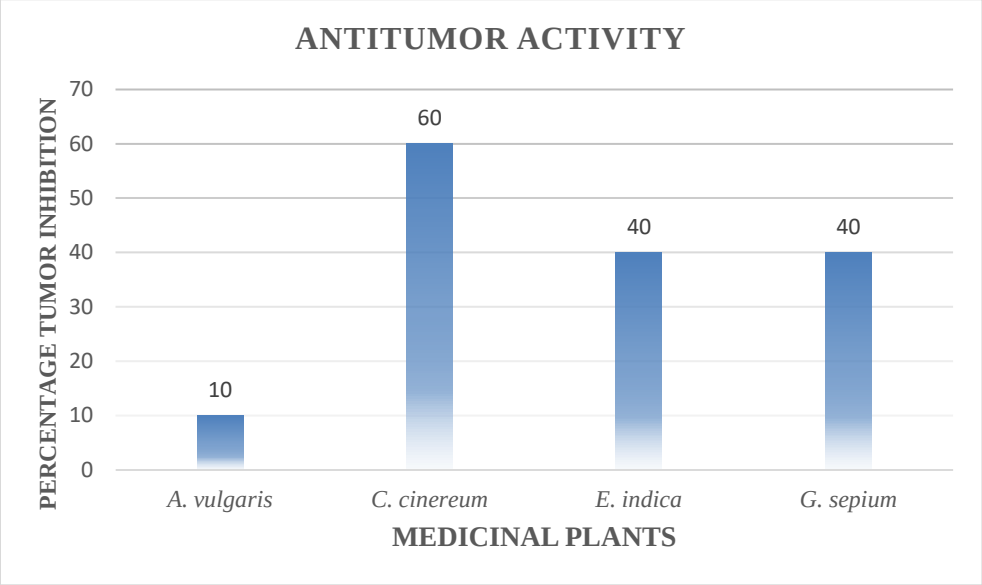

Fig. 2. Percent Inhibition of A. tumefaciens tumor growth on sugar beets discs

The results of this study show the potential of the plants for varied pharmacological activities and in agreement with several studies. The presence of phenolic compounds from the different plants contributed to this tumor inhibitory activity demonstrated by the plants. In several studies, plant phenolics have demonstrated to be very effective antitumor agents; some of them have been determined to be flavonoids, polyphenols, anthraquinones, coumarins (Chairez-Ramirez, et al., 2021). Flavonoids are polyphenolic compounds that are ubiquitously found in plants that accounts for their ability to prevent cancer and tumor growth as with the case of *C. cinereum* (Roy, Julius, and Chinnapan, 2022). In the case of *A. vulgaris*, eudesmane-type sesquiterpene, morin, luteolin, triterpenes, coumarin, flavonoids, eriodictyol were found to be the contributing compounds for its wide array

of activities including antioxidant and analgesic agent (Blishchenko et al., 2005; Nigan et al., 2019).

Conclusion

With the foregoing results obtained from this study, it can be concluded that compounds from the leaves of *Artemisia vulgaris, Cyanthillium cinereum, Eleucine indica*, and *Gliricidia sepium* have antioxidant and antitumor potentials due to the presence of phenolic compounds and other secondary metabolites obtained from the phytochemical analysis. However, due to the presence of toxic components in the plants like alkaloids, careful use of decoctions or use of the crude extracts must be taken into consideration.

Recommendation

In view of the result and conclusion derived from this study, future undertakings should be done to isolate only the active components of the plants and not use the whole leaves of the different herbs since they exhibited low cytotoxic properties at longer exposure. In the isolation of the drug pharmacophores, removal of the toxic components must be done through gravity column chromatography. Future studies could look into other pharmacological properties can also be delved into like diuretic, anti-inflammatory, anti-pyrexia, and anti-cancer activities using cancer cell lines.

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