ORIGINAL ARTICLE

Antiproliferative and Cytotoxic Potential of Semi-purified Extract of Snake Plant (*Dracaena trifasciata*) Using HCT116 Human Colorectal Carcinoma Cell Line

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ABSTRACT

Background. Espada plant, local name for the snake plant (*Dracaena trifasciata*) in the Philippines, is characterized by its upright sword-like leaves with vibrant yellow edges under the variety of Laurentii in the Asparagaceae family. This plant has been identified as a viable candidate for cancer research.

Objective. To investigate the antiproliferative and cytotoxic capabilities of a semi-purified methanolic extract of *D. trifasciata* extracted as a basis for cancer research.

Methods. The plant extracts were subjected to (1) qualitative phytochemical analysis, (2) instrumentation analysis which includes Fourier Transform Infrared Spectroscopy (FTIR-ATR) and Total Flavonoid Content (TFC), to quantify bioactive ingredients, analyze structures, and evaluate biological chemicals, respectively, and tested to (3) biological assay on the HCT 116 human colorectal cancer cell line using the MTT Cytotoxic Assay.

Results. *D. trifasciata* extracts revealed the presence of flavonoids, saponins, sterols, triterpenes, alkaloids, and glycosides, all of which contain an OH group and have a high solubility in polar solvents. It correlates to the results of TFC, found to be within 266.8333 mg – 622.6801 mg presented as µg Quercetin per mL with a linear line of y=0.0005x + 0.023 with a coefficient R^2 value of 0.9933. This finding corresponds to FTIR-ATR data, which shows a prominent broad appearance of -OH (primary and secondary alcohol) at peak 3327.21. In MTT Cytotoxic Assay, it has a minimal IC50 than Doxorubicin, as seen in Trial 2 with IC50 = 0.8012 µg/mL, while antiproliferative activity revealed that *D. trifasciata* has minimal inhibitory activity in Trials 1 and 3 at the same concentration of 3.125 µg/mL as compared to the high antiproliferative property of positive control, as seen in Trial 2. Data showed that the *D. trifasciata* extract has minimal effectiveness even at 1.56 µg/mL concentration, implying that other extraction techniques such as fractionation and purification may be used to satisfy its antiproliferative property.

Conclusion. The *D. trifasciata* extract contains polyalcohol, phenol, polyphenol, and polyhydroxylated metabolites, which are structures that correspond to the major groups of flavonoids (structures that have antioxidant properties), contributing to the high TFC values.

Keywords: antiproliferative, MTT cytotoxic assay, Dracaena trifasciata (Prain) Mabb.

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INTRODUCTION

Cancer is a significant global concern due to its devastating impact on lives. There are numerous types of cancer prevalent worldwide, including colon and rectal cancer, which are often grouped together due to their physiological relationship. Consequently, the researcher is increasingly exploring natural remedies, such as the utilization of medicinal plants, as a cornerstone in drug discovery and product formulation. In the Philippines, there exists a wealth of indigenous and promising molecules that could serve as valuable resources for advancing cancer research, particularly in today's modern scientific landscape, by utilizing new approaches using advanced instrumentation techniques such as spectroscopy, in-vitro cell culture assay, and various other evidence-based analyses.

According to the World Health Organization (WHO), eighty percent of people around the world rely on herbal medicines.[1] Herbal medicines are gaining popularity due to public dissatisfaction with the cost of prescription medications, various side effects of synthetic medicines, non-toxic nature, more affordable with lower costs, and allows greater public access to health information. Most of these chemicals possess pharmacological properties, which allow them to be used in the treatment of acute and chronic illnesses, as well as a wide range of conditions, including cardiovascular disease, prostate problems, depression, inflammation, immune system stimulation, and antioxidant capabilities.

The unchecked multiplication of aberrant cells in the colon or rectum, which are both parts of the digestive system, is the hallmark of colorectal cancer, often known as CRC. Either the colon, which is a section of the large intestine or big bowel, or the rectum, which is the tunnel that connects the colon to the anus, or both locations might be affected by cancer. Early detection of colorectal cancer typically results in a favorable prognosis for the patient. With greater screening, the incidence and mortality rates of colorectal cancer could be lowered by a significant amount.[2] In the Philippines, colorectal cancer ranks fourth among the cancer-related deaths of Filipinos.[3] According to the Philippine Cancer Society, Inc., almost 75 percent of the individuals affected were aged 50 and above while only about three percent were children 14 years old and below.[3] The number of people diagnosed with CRC increased from 5,787 in the year 2010 to 9,625 in the year 2015. The five-year and 10-year survival rates for colon cancer are 38.1 and 33.9 percent, respectively, while the five-year and 10-year survival rates for rectal cancer are 31.3 and 20.0 percent, respectively. It is estimated that one out of 1800 Filipinos will develop the cancer yearly.[3]

Doxorubicin is a chemotherapeutic agent under the class of anthracycline, which slows or stops the growth of cancer cells by blocking an enzyme called topo isomerase 2.[4] In colon cancer, nearly all patients eventually experience drug resistance and stop responding to the approved drugs, making treatment difficult. Doxorubicin, a potent anticancer drug that is widely used to fight various cancer types, including colon cancer, is cost-effective compared with other anticancer drugs.[5] The study generated a drug-resistant cell line against Doxorubicin by treating the cell line with the drug for six months.[6] Doxorubicin was utilized in this study as a positive control or the main standard, aligning with the protocol used by the Mammalian Cell Culture Laboratory in UP Diliman.

Non-Communicable Diseases such as cancer are the major causes of death in the Philippines. Cancer was the second largest cause of death recorded in 2016.[7] Colorectal cancer was identified as one of the top cancer kinds in the Philippines with the most recent cases and deaths.[8] According to data published in the Global Cancer Observatory's (GCO) online database, colorectal or colorectum cancer was the third most prevalent type of cancer worldwide in 2020, accounting for 1,931,590 cases, or 10% of all cancer cases for both sexes and all ages. Colorectal cancer claimed 935,173 lives in 2020, accounting for 9.4% of the total 9,958,133 cancer-related deaths worldwide, making it the second most lethal cancer after lung cancer. According to the same statistics, there were 17,364 instances of colorectal cancer reported in the Philippines in 2020, spanning both genders and ages. Furthermore, colon cancer was the third most prevalent type of cancer that year, accounting for at least 11.3 percent of the country's total 153,751 cancer cases. During the same year, the cancer type was more common among Filipino men, with a 23.7 percent incidence rate, and 15.1 percent among Filipino women. In 2020, there were 9,091 deaths in the Philippines related to colorectal cancer, with 6,109 deaths due to colon cancer and 2,982 deaths due to rectum cancer, according to data.[9]

The genus Dracaena contains more than 110 recognized species. Succulent shrubs and trees can be found in Africa, Australia, India, and Southeast Asia. *Dracaena trifasciata* (Prain) Mabb., often known as snake plant (Family Asparagaceae), is one of these species. In the Philippines, it is one of the most popular and hardy houseplants, also called "espada" plants. It is an evergreen, succulent, perennial plant with long, narrow, upright or slightly spreading sword-shaped leaves up to 75 cm long that grow from a rhizomatous rootstock.[10] It was given the formal name *Sansevieria trifasciata* in 2017, however, its similarities to Dracaena species were too numerous to ignore. Sansevieria was found to be nested within Dracaena in recent molecular phylogenetic analyses, making the latter paraphyletic unless Dracaena was enlarged to include species formerly classified as Sansevieria.[11]

The taxonomic limits of the dracaenoid genera Dracaena and Sansevieria have been a point of contention for a long time. A study revealed the genetic distance between Sansevieria and Dracaena is between 0,003 and 0,006. The closer the kinship between the species being compared, the lower the genetic gap. If no genetic gap exists between the creatures being compared, they are of the same species. *Sansevieria trifasciata* is monophyletic, according to this study, and there is no intraspecific variation within it.[12]

After extensive research, no available studies on Dracaena trifasciata were discovered, both locally and internationally. Due to numerous confusions about its correct scientific name, the sample plant was subjected to authentication at the DOST – Forest Products Research and Development Institute in UP Los Baños, Laguna. Based on the test results and certification, the institution declared the scientific name as Dracaena trifasciata, referencing Plants of the World Online.

Researchers in Batangas City, Philippines in 2018, investigated the phenolic content of *S. trifasciata* extracts. The plant contains alkaloids, phenols, terpenoids, terpenoids, flavonoids, saponins, steroids, and glycosides, which are responsible for its numerous therapeutic effects. In this study, the *S. trifasciata* leaves were extracted in ethanol or water which then revealed a variety of phytochemical components such as tannins, proteins, carbohydrates, and polyphenols.[13] Moreover, a study in 2021 investigated the phenolic and flavonoid content of *Suffruticosa suffruticosa* and *Suffruticosa trifasciata* Prain, a member under the family of Asparagaceae. The total flavonoid concentration was found to be 2 2.81 ± 0.26 mg RE/g fresh parts [milligrams rutin equivalent (RE) per gram of fresh parts]. The coefficient of determination was r = 0.9946.[14]

The total flavonoid content (TFC) of *S. trifasciata* using two different extraction method (maceration and Soxhlet extraction).[15] It was found that the maceration extraction method had 13.934 mgQE/g of flavonoids, which is 1.39 percent more than the Soxhlet extraction method, which had 8.117 mgQE/g, which is 0.81 percent. The results of statistical tests showed that the sig value was between 0.001 and 0.05, which means that there is a significant difference between the levels of total flavonoids in the maceration and Soxhlet extraction methods.[15]

Flavonoids are important class of natural product found in fruits, vegetable and certain beverages. In nature, these compounds are the one responsible for the color, pigment, and aroma of the medicinal plant. They are also known for their antioxidant properties and they have several subgroups, which include chalcones, flavones, flavonols, and isoflavones. These subgroups have unique major sources. For example, onions and tea are major dietary sources of flavonols and flavones. Flavonoids have been studied extensively for their potential health benefits, including their role in reducing inflammation, improving heart health, and possibly even lowering the risk of certain chronic diseases like cancer.[16]

Despite the structural differences between Sansevieria and Dracaena species, they are the only flavonoids that can be extracted from both of these genera. In point of fact, when compared to Dracaena homoisoflavanones, the structures of Sansevieria spp. derivatives often have a greater number of O- and C-alkyl substituents. In addition to this, a hydroxy group is frequently connected to the carbonyl group (C3). Congeners that are antipodal to one another include enantiomeric trifasciatines B and C, in addition to homoisoflavanones, and can be found in different species of the same genus.[11]

Properties such as antioxidant, cytotoxicity, anti-inflammatory, antiallergic, anti-anaphylactic, antidiabetic and thrombolytic, and analgesic activities have been proven by previous researchers yet no published study or journal regarding any of the Dracaena species (Asparagaceae) that were found in the Philippines, particularly for the cancer study.

Multiple international studies[8,9,17] showed the antioxidant effect of the plant using ethanol as a solvent in various parts such as rhizomes and leaves. While the preliminary antioxidant determination of *S. trifaciata* have been proven by the previous researchers in Batangas City, Philippines. Study about the extracts of *S. trifasciata* leaves and rhizomes cause no significant effect on the viability of A549 lung cancer tumor cells.[13] Few studies of the snake plant such as antiproliferative and cytotoxicity were not specifically mentioned.

In vivo pharmacological studies of the actions and underlying mechanisms of Dracaena and Sansevieria species are still lacking or have taken an ineffective scientific approach.[11] Based on the previous studies and gaps, the researcher focused on new bioactive constituents, discovering possible structures, assessing their therapeutic potential in CRC using semi-purified extract of *D. trifasciata* using 95% methanol as a solvent, and determine its potential antiproliferative and cytotoxic properties using the MTT assay.

MATERIALS AND METHODS

Plant Sample Preparation and Extraction

Collection and Authentication of Plant Sample

The Snake Plant (leaves) was obtained from a flower farm located at San Andres, Romblon, Philippines. It was authenticated in November 2021, by the Forest Product Research and Development Institute of the Department of Science and Technology at the University of the Philippines - Los Baños, Laguna.

Test for Impurities

The procedures used for Moisture Content were based on the protocol by Central Instrumentation Facility, De La Salle University – Laguna Campus, while Total Ash Content was based on Industrial Technology Development Institute Standard and Testing Division – Department of Science and Technology. Both tests utilized fresh snake plant sample without roots.

Plant Extraction

The 2.0 kilogram of fresh plant leaves were blended/osterized and immersed for 48 hours with intermittent stirring in 6.0L of methyl alcohol. Following the maceration step, the mixture was filtered, and then the filtrate was concentrated using a rotary evaporator at a temperature of

sixty degrees Celsius and a vacuum for a period of four hours. To produce a semi-purified extract, the concentrated extract was transferred to an evaporating dish and subsequently concentrated in a 60˚C water bath. The concentrated crude extract was collected and labeled in an amber bottle. The percentage yield of the extract was calculated, and the extract was kept in a refrigerator until use.

Physical Tests and Phytochemical Screening

Organoleptic Evaluation

The physical properties of semi-purified extract were evaluated for its appearance, color, odor, consistency, and taste.

pH Determination

The pH determination was performed using the standard procedure by Central Instrumentation Facility, De La Salle University – Laguna Campus.

Solubility Test

The solubility of semi-purified extract was tested by dissolving in a 1:1 ratio of the extract and the following polar solvents such as purified water, normal saline solution, calcium chloride, ethanol, and non-polar solvents such as acetone, carbon tetrachloride and hexane.

Phytochemical Screening

The phytochemical tests were conducted using the standard procedures at the Department of Science and Technology, Industrial Technology Development Institute (DOST) – Standards and Testing Division. The tests were performed to identify the plant constituent present in *D. trifasciata*.

Instrumentation Analysis

Fourier Transform Infrared Spectroscopy

The test for FTIR-ATR analysis was conducted at the University of Santo Tomas, Analytical Services Laboratory – Research Center for the Natural and Applied Science with the SHIMADZU IRPrestige-21 using ATR or Attenuated Total Reflectance.

Total Flavonoid Content

The Total Flavonoid Content Assay was conducted at the University of the Philippines, Diliman – Institute of Biology and the protocol was adapted from Sanchez.[18] Quercetin was prepared in 1, 10, 100 and 1000 ppm solutions. 1 mg/mL of the sample was also prepared. In a 96-well plate, the following were added to each well: 50 uL of 6 g/L $NaNO_2$, 50 uL of quercetin/sample, 50 uL $AlCl_3$ (22 g/L), and 50 uL 0.8 M NaOH. After a 3-minute incubation, absorbance was read at 510 nm. A standard curve was calculated from the absorbance readings. The total flavonoid content of the sample will be presented as µg quercetin equivalent (QE)/mL. Three trials were performed in triplicate.

Biological Tests

MTT Cytotoxic Assay

For the analysis of the cytotoxic potential of the *D. trifasciata* semi-purified plant extract, MTT Assay was used. It was performed at University of the Philippines – Diliman, Mammalian Cell Culture Laboratory – Institute of Biology using their protocol as stated below:

The MTT cytotoxicity assay performed in this study was adapted from Mosmann.[19] In detail, cells were seeded at 4 or 6 x 10^4 cells/mL (depending on the cell culture used) in sterile 96-well microtiter plates. The plates were incubated overnight at 37˚C and 5% CO_2.

Eight two-fold dilutions of the sample were used as treatments starting from 100 µg/mL down to 0.78 µg/mL. Doxorubicin served as positive control while dimethyl sulfoxide (DMSO) served as negative control. Following incubation, cells were treated with each extract dilution. The treated cells were again incubated for 72 hours at 37˚C and 5% CO_2.

After incubation, the media was removed and 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye at 0.5 mg/mL PBS was added. The cells were again incubated at 37˚C and 5% CO_2 for four hours. After which, DMSO is used to dissolve the formazan crystals formed by the reduction of the dye by the live cells. Absorbance was read at 570 nm.

Inhibitory Concentration Determination

IC50 represents the concentration at which a substance exerts half of its maximal inhibitory effect. This value is typically used to characterize an antagonist of a biological process. In pharmacology, it is an important measure of potency for a given agent.[20] Using GraphPad Prism 6, the Inhibition Concentration 50 (IC50) was obtained. GraphPad Prism 6 computes the IC50 of the sample by fitting a non-linear regression curve to the sample's calculated percent inhibition per concentration. Active samples are those with IC50 values less than 30 µg/mL.[21]

Antiproliferative Potential

For the assessment of antiproliferative level of the *D. trifasciata* semi-purified plant extract, MTT Cytotoxic Assay (HCT 116 human colorectal carcinoma cell line) was used. The antiproliferative potential was determined by the standard procedures of Mammalian Cell Culture Laboratory, University of the Philippines – Diliman, Quezon City.

Statistical Treatment

One Way Analysis of Variance (ANOVA) is used to assess whether there are any statistically significant differences between the MTT Assay of the standard drug –

Doxorubicin, negative control (DMSO), and semi-purified plant extract of *D. trifasciata* and to identify if there is a significant difference in antiproliferative activity between the different concentrations of *D. trifasciata* semi-purified plant extract and the negative control, as well as if there was a significant difference in antiproliferative and cytotoxic activity of the plant extract compared to the standards. Statistical significance was acceptable to a level of p <0.05.

RESULTS

Percentage Yield of the Semi-purified Extract from the Snake Plant Leaves

The total amount of *D. trifasciata* semi-purified extract obtained after a series of extraction with 95% methanol from 2000 grams fresh leaves was 43 grams which resulted in an average percentage yield equal to 2.15%.

Test for Impurities

Moisture Content (Gravimetric Method)

From 5 g of the fresh plant sample, the total moisture content of the plant sample has been conducted within three trials with the use of gravimetric oven drying method. It was found to have a moisture content of 91.18 ± 0.37 % (Table 1).

Total Ash Content

After incinerating the fresh plant (2.9 - 3.5 grams), total ash obtained was 0.0701 g with an average percentage of 1.40% w/w (Table 2).

Physical Tests and Phytochemical Screening

Organoleptic Evaluation

The *D. trifasciata* extract obtained was a semi-solid, dark greenish brown, thick viscous extract with a salty-bitter taste, and anise-like odor.

pH Determination

Using Jenco VisionPlus pH6175 pH meter, the semi-purified extract obtained from *D. trifasciata* is found acidic with pH = 3.94 ± 0.02. The pH of the neutralized water is 7.08 at 23.7°C.

Solubility Test

Using 1:1 ratio, the *D. trifasciata* semi-purified extract was soluble in polar solvents (purified water, normal saline solution, and ethanol) and non-polar solvents (acetone, carbon tetrachloride, and hexane). The extract was found soluble in polar solvents and insoluble in non-polar solvents.

Phytochemical Screening

The phytochemical tests of the snake plant *D. trifasciata* (Family Asparagaceae Mabb.) semi-purified extract contain the following traces of plant constituents such as sterols, triterpenes, flavonoids, alkaloids, saponin and glycosides, and no presence of tannins (Table 3).

Table 1. Results of the Moisture Content Determination (Gravimetric)

	Trial 1	Trial 2	Trial 3
Mass of Aluminum pan, g	0.8310	0.8057	0.8189
Mass of fresh sample, g	5.0390	5.0722	5.0690
Mass of fresh sample + Al pan, g	5.8700	5.8779	5.8879
Mass of dried sample + Al pan, g	1.2639	1.2428	1.2874
Mass of dried sample, g	0.4329	0.4371	0.4685
% Moisture	91.41	91.38	90.76
% Moisture, Average ± SD		91.18 ± 0.37	

Table 2. Result of the Total Ash Content Determination

	Trial 1	Trial 2
Weight of tared crucible with cover, g	18.5751	18.3982
Weight of tared crucible with cover + sample, g	22.0952	21.3356
Weight of sample, g	3.5201	2.9374
Weight of ash, g	0.0497	0.0408
Percentage of Total Ash	1.4119%	1.3890%
Average Percentage of Total Ash	1.4004% or 1.40% w/w	

Table 3. Results of Phytochemical Tests on *D. trifasciata* Semi-purified Extract

Plant Constituents	Inference	Test Method
Sterols	(+)	Lieberman-Burchard Test
Triterpenes	(+)	Lieberman-Burchard Test
Flavonoids	(+)	Shinoda Test
Alkaloids	(+)	Mayer's Test
Saponin	(+)	Froth Test
Glycosides	(+)	Fehling's Test
Tannins	(-)	Ferric Chloride Test

DISCUSSION

Properties of *D. trifasciata* Semi-purified Extract

The sample yielded a total of 43 grams of extract out of 2000 grams used in the extraction. From the data obtained, the extraction resulted into an average percentage yield equal to 2.15%. It tells us how much of the desired compound we have successfully extracted from the plant material, helping us gauge the effectiveness and efficiency of our extraction process.

Moisture content analysis, utilizes gravimetric methods due to the unsuitability of thermogravimetric techniques for semi-purified extracts, revealed a moisture content of 91.18 ± 0.37%. This determination is critical for assessing the stability and quality of the extract.

Ash content analysis indicated a total ash content of 0.0701 g, with an average percentage of 1.40% w/w.

This measurement provides insights into the presence of inorganic materials within the extract, which can impact its pharmacological properties and purity.

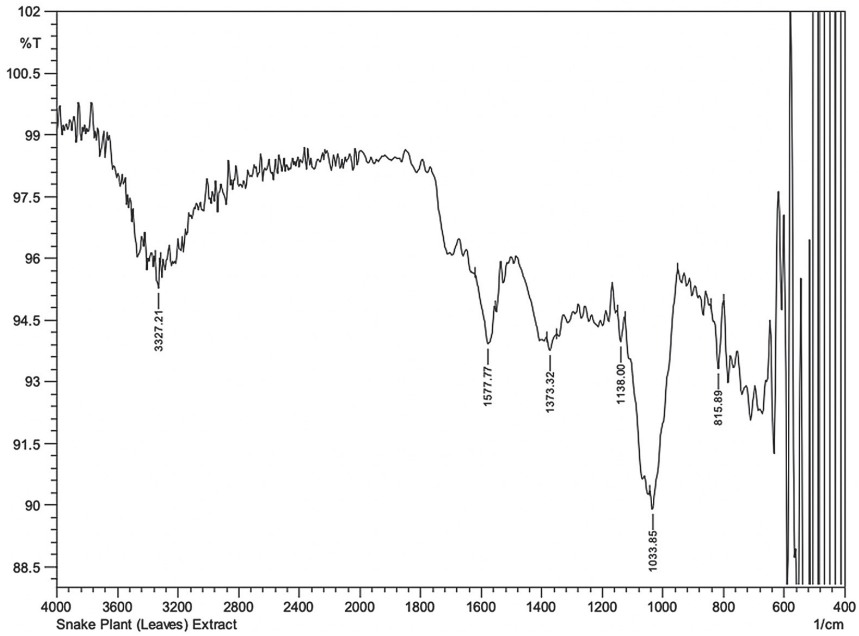
The *D. trifasciata* plant extract's organoleptic has a semi-solid, dark greenish brown, with a thick viscous texture, salty-bitter taste, and anise-like odor, with an acidic pH of 3.94. It is soluble in polar solvents but insoluble in non-polar solvents. These results will be a good basis if the study will be used for formulation by future researchers. It may also contribute to predict what type of solvent might be used for extraction and even acceptability and compatibility of extract to different physiological environments.

Phytochemical screening showed that *D. trifasciata* contain various plant constituents, including sterols, triterpenes, flavonoids, alkaloids, saponins, and glycosides, with the absence of tannins.

These bioactive compounds contribute to the extract's potential pharmacological effects and therapeutic applications, and the findings of physical and chemical tests may help determine what and how much of this plant should be properly formulated for future analysis.

Instrumentation Analysis: Fourier Transform Infrared Spectroscopy

The plant *D. trifasciata* showed a promising result. It was found out that the bands located at 3327.21 exhibited two possible stretches which are the -OH and NH, however, the -OH appearance was broad while the NH was not so much (Table 4). It indicates a strong peak compound particularly the hydroxyl group -OH, which is a highly flammable and volatile organic compound called alcohols, usually denoted as -OH in chemical structures. When -OH exists by itself we call it a hydroxyl, but when it is incorporated in a molecule as a functional group, it is called an alcohol. At peak number 1577.77, it indicates many possibilities such as straight chain alkenes that have weak stretches. The probability can be the sample may be a conjugated, aromatic, or cyclic alkene, but data proved that it is not conjugated or aromatic since it is not found in higher weight numbers. According to Figure 1, it shows a medium appearance that may contribute to a cyclic alkene. At peak number 1373.32, there are two possibilities, it shows an overlap of peaks that indicates the presence of C-H and O-H bend. Data for 1033.85 and



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	815.89	93.3	1.6	842.89	800.46	1.1	0.1
2	1033.85	89.9	1.0	1043.49	950.91	3.2	0.3
3	1138.00	94.0	0.7	1151.50	1126.43	0.6	0.0
4	1373.32	93.8	0.3	1384.89	1350.17	0.9	0.0
5	1577.77	93.9	1.2	1622.13	1554.63	1.6	0.2
6	3327.21	95.3	0.7	3352.28	3315.63	0.7	0.1

Figure 1. ***FTIR Analysis (SHIMADZU IRPrestige-21 using ATR).*** The broad peak intensity of *D. trifasciata* extract corresponds to the results of phytochemical tests indicating the presence of phytochemicals such as saponin, flavonoids, sterols, triterpenes, alkaloids, and glycosides, which all contain OH groups.

Table 4. FTIR Peaks and Relative Intensities of *D. trifasciata* Plant Extract

Peak	Appearance/ Intensity	Origin (Functional Groups)
3327.21	Board	Indicative of OH (broad) or NH (not so much) stretch
1577.77	Medium	C=C stretch (cyclic alkene)
1373.32	Medium	C-H, O-H Bend
1138.00	Shoulder-like-appearance	C-O stretch
1033.85	Strong	C-O stretch
815.89	Weak	C-H Bend

Table 5. Results of the Total Flavonoid Content (TFC)

Abs	Trial	Average Abs	µg QE/ mg extract	Mean	Std. Dev
0.2822	1	0.2681	511.2812	444.7567	59.3078
0.2099					
0.3122					
0.2272	2	0.2135	397.4130		
0.2072					
0.2062					
0.2547	3	0.2270	425.5758		
0.2022					
0.2242					

1138.00 is somehow similar having C-O stretch. The first peak 1033.85 revealed a prominent appearance that is most likely attributable to primary or secondary alcohol only, tertiary alcohol is not included because it requires a higher weight number, while 1138.00 is like a strange peak due to its shoulder-like-appearance that is found around the edge of 1033.85. Finally, for peak number 815.89, supposing it has a S=O (sulfoxide) but the strong peak was inadequate and absence of C=O at 1700 cm, as a result, it appears to have a weak appearance of C-H bend.

Data showed the presence of functional groups present in *D. trifasciata* such as alcohol, alkene, alkane, phenol, no ketone, and carboxylic acid. The appearance of strong intensity for C-O stretch bond and broad intensity of OH group indicates high number of flavonoids as shown in the results of total flavonoid content of the extract.

The FTIR spectra showed the presence of functional groups in *D. trifasciata*. The highest peak of 3327.21 indicating broad intensity of FTIR peak revealed the presence of OH group, which suggests high polarity of the semi-purified extract in polar solvents. Moreover, other functional groups found in the semi-purified extract include medium peak intensity of 1577.55 for C=C cyclic alkene, and 1373.32 for C-H and O-H groups which are all present in the identified phytochemical tests. On the other hand, the appearance of strong intensity for C-O stretch bond and broad intensity of OH group indicates high number of flavonoids as shown in the results of total flavonoid content of the extract.

Figure 1 shows the presence of the -OH group which indicates the functional group of alcohols. At peak 3327.21, it shows a prominent broad appearance of primary and secondary alcohol, therefore when -OH is present, it can easily attach to either primary or secondary alcohol. The presence of an -OH stretch is indicative of alcohols or polyhydroxylated metabolites in snake plant samples. The data proven by Kanimozhi M about the physical characteristics of *Sansevieria trifasciata* (a monophylogeny of *D. trifasciata*, same taxon that share a common recent ancestor) fiber was shown that the broad and strong IR peak in the band 3421 cm-1 was attributed to the hydrogen bonded OH stretching of polysaccharides present in the fiber of *Sansevieria trifasciata*.[22] Furthermore, the results have proven that the *D. trifasciata* extract contains polyalcohol, phenol or polyphenol, which are structures responsible for antioxidant properties and this hallmark can affect or relate to anticancer properties.

Total Flavonoid Content (TFC)

The Total Flavonoid Content Assay was conducted at the UP Diliman – Institute of Biology and the protocol was adapted from Sanchez.[18]

The *D. trifasciata* plant sample solutions were performed in three trials. The total flavonoid content of the sample solution yielded 444.7567 µg quercetin per mg of sample read at 510 nm absorbance, based on the raw absorbance readings from the sample and controls.

TFC in trial 1 resulted in 511.2812 µg quercetin per mg of sample at an average of 0.2681 nm absorbance, while trial 2 obtained 397.4130 µg quercetin per mg of sample at an average of 0.2135 nm absorbance, and in Trial 3, results showed 425.5758 µg quercetin per mg of sample at an average of 0.2270 nm absorbance (Table 5). Overall, the TFC of snake plant sample solution was found to be within 266.8333 mg - 622.6801 mg presented as µg Quercetin per mL.

A study in 2021 under a common plant family in Asparagaceae were evaluated, namely *Sansiviera suffruticosa* and *S. trifasciata* Prain, were reported with significant phenolic and flavonoid contents.[14] It was shown that the total flavonoid content was 2.81 ± 0.26 mg RE/g fresh parts [expressed as milligrams rutin equivalent (RE) per gram of fresh parts]. The coefficient of determination was R^2 = 0.9946. Furthermore, this amount of quercetin may be a contributing factor to the antioxidant content of *D. trifasciata*.

The result is correlated to Agustien G. et al. wherein the absorbance values obtained in the maceration technique of the *S. trifasciata* leaves extract were 0.376, 0.369 and the soxhlation method were 0.230, 0.227.[23] The equation of the linear line y=0.025x + 0.0241 is obtained with coefficient R^2 value of 0.9947, which was found to be close with the R^2 of *D. trifasciata* TFC as shown in Figure 2.

Biological Tests

MTT Cytotoxic Assay

The semi-purified extract of snake plant (*D. trifasciata*) was prepared in eight concentrations within two-fold serial dilutions (Table 6). The concentration for Doxorubicin begins with 12.5 µg/mL down to 0.09765625 µg/mL, while the negative control DMSO and *D. trifasciata* plant extract have the same concentration starting from 100 µg/mL down to 0.78 µg/mL. MTT Cytotoxicity IC50 results show that the positive control Doxorubicin produced good data for its inhibitory activity, specifically observed in Trial 2 with IC50 of 0.8012 µg/mL concentration.

The concentration for *D. trifasciata* begins with 100 µg/mL down to 0.078 µg/mL (Table 7). Data showed that the IC50 was greater than 100 µg/mL, Moreover, it was cited by UP Mammalian Cell Culture Laboratory from Jokhadze et al. procedure and protocol that samples with IC50 values less than 30 µg/mL are considered active.[21] In addition, there is a prominent result particularly on the following: in Trial 1 across 50 to 1.56 (µg/mL) concentration (6.79, 3.15, 1.83, 12.61 and 7.44 µg/mL), in Trial 2 across 100 to 6.25 (µg/mL) concentration (8.71, 16.95, 3.55, and 2.67 µg/mL), and in Trial 3 across 100 to 1.56 (µg/mL) concentration (6.29, 11.30, 15.09, and 17.56 µg/mL).

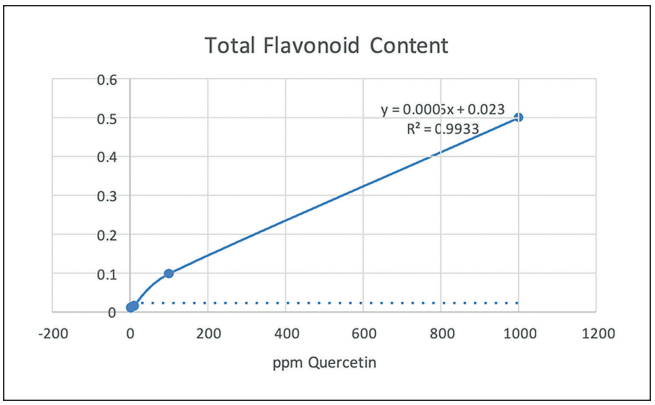


Figure 2. ***Quercetin Standard Curve.*** The *D. trifasciata* plant extract quercetin standard curve shows that as the absorbance reading increases, the amount of quercetin in ppm also increases. Data shows the linear line y=0.0005x + 0.023 with a coefficient R^2 value of 0.9933.

Table 6. MTT Cytotoxicity Assay Results of the Percent Inhibition Computed from the Absorbance Readings of each Concentration of the Positive Control, Doxorubicin, against HCT-116 Cells

Conc (µg/mL)	Trial 1		Trial 2		Trial 3	
12.5	54.4006706	56.4124057	71.2072305	72.1110394	68.1118881	65.5944056
6.25	61.8618619	55.1051051	66.407465	69.8289269	54.9407115	60.3162055
3.125	84.8341232	83.8862559	82.9817159	82.7004219	84.040532	84.6738442
1.5625	64.3652561	62.8804751	80.9815951	81.2883436	72.4585436	75.7750541
0.78125	23.1117825	38.2175227	51.2592593	57.9259259	22.9422067	31.17338
0.390625	-13.180516	41.260745	42.8571429	49.9118166	43.9058172	41.966759
0.1953125	27.0341207	14.6106737	43.08094	49.8694517	32.1148825	24.8041776
0.09765625	11.1847556	7.2079536	42.2389464	34.901223	27.2900763	-0.9541985
IC50	**0.8885 µg/mL**		**0.8012 µg/mL**		**0.8335 µg/mL**	

Table 7. MTT Cytotoxicity Assay Results of the Percent Inhibition of the Different Concentrations of *D. trifasciata* Extract Computed from the Absorbance Readings against HCT-116 Cells

Conc (µg/mL)	Trial 1		Trial 2		Trial 3	
100	-20.368818	-13.830679	8.71530019	3.55067786	11.7482518	6.29370629
50	-34.234234	12.6126126	16.9517885	-12.44168	-53.359684	11.3043478
25	-123.85466	-55.292259	-3.3755274	2.67229255	-30.968968	-29.829006
12.5	-40.757238	-120.78693	-9.9693252	-16.257669	-140.8075	-128.55083
6.25	6.79758308	-168.12689	3.55555556	-37.62963	-29.422067	-92.994746
3.125	3.15186246	7.44985673	-15.343915	-25.396825	22.8531856	15.0969529
1.5625	1.83727034	-1.6622922	-16.623151	-15.4047	18.6327078	17.5603217
0.78125	-18.641259	-21.789561	-16.274694	-27.939793	-8.5877863	-17.175573
IC50	**Greater than 100 µg/mL**		**Greater than 100 µg/mL**		**Greater than 100 µg/mL**	

MTT Cytotoxicity IC50 revealed that the plant sample *D. trifasciata* exhibits minimal inhibitory activity in all of the trials performed in the different concentrations compared with the standard drug Doxorubicin. Though the results of IC50 were shown to be greater than 100 µg/mL, the above-mentioned results obtained were associated to the study conducted by El-Hawary S.[14] that *S. trifasciata* Prain. (a monophylogeny of *D. trifasciata*, same taxon that share a common recent ancestor) showed low cytotoxic activity against HepG-2 with IC50 = 81 ± 18.8 µg/ml and no activity against CACO2 and A-549 cancer cell line. *S. trifasciata* and *D. trifasciata* have been suggested to be a candidate for cytotoxicity and cancer related assay, the researcher's major findings through the in-vitro study using different cell line particularly the HCT-116 as compared to CACO2, indicated that it can be a source of drug molecule against colorectal cancer.[24]

Antiproliferative Potential

The antiproliferative activity is a compound's ability to halt the proliferation of cells. This involves preventing the cells from rapidly multiplying. Furthermore, proliferation literally refers to the rapid growth of anything, and in the case of cells, cell proliferation alludes to cancer. While cytotoxicity refers to harming cells to the point of death. Assume 100 cells are incubated in a plate without a medicine, and after a period of time, the cells have multiplied to 200. If the number of cells in the plate is between 100 and 200 after adding a medicine, this indicates growth inhibition, which is a measure of antiproliferative activity. Cytotoxicity, on the other hand, is defined as a reduction in the number of cells in the plate below 100.[14] In this study, the antiproliferative activity was measured using MTT cytotoxic assay.

The antiproliferative property of *D. trifasciata* and the negative control (DMSO) in Trial 1, revealed that *D. trifasciata* exhibited antiproliferative property as compared to the negative control (Table 8A). On the other hand, Trials 2 and 3 demonstrated no significant differences on the antiproliferative property of *D. trifasciata* and the negative control (DMSO) in different concentrations as indicated by the computed F-ratio of 2.69392 and 3.29054 which both obtained the *p*-value of 0.111176 and 0.079694, respectively. Data depicted that *D. trifasciata* did not even reach the minimum absorbance level needed to show antiproliferative properties similar to the negative control.

The standard drug (Doxorubicin) as compared to the different concentrations of *D. trifasciata*, showed that it exhibits greater antiproliferative property (Table 8B) specifically in Trial 2 where the F-ratio of 247.82505 proved its utmost effect to cause inhibition against HCT-116 – human colorectal carcinoma when it was associated with the different concentrations of *D. trifasciata*.

The different concentrations of the standard drug (Doxorubicin) exhibit maximum antiproliferative property against HCT-116 cells (Table 8C) when compared with the negative control (DMSO). It exhibits greater antiproliferative property specifically in Trial 2 where the F-ratio of 127.56301. Furthermore, the negative control demonstrated no significant differences on the antiproliferative property as compared to standards.

Statistical Treatment

The ANOVA F-test revealed in Trial 1, a computed F-value of 26.50137 being greater than the *p*-value of <0.000015 at 0.05 level of significance with 1 degree of freedom between treatments and 30 degrees of freedom within treatments, in Trial 2 a computed F-value of 167.34404 being greater than the *p*-value of <0.00001 at 0.05 level of significance with 1 degree of freedom between treatments and 30 degrees of freedom within treatments. Lastly, Trial 3 computed the F-value of 27.42424 being greater than the *p*-value of <0.000012 at 0.05 level of significance with 1 degree of freedom between treatments and 30 degrees of freedom within treatments. Data proved that the positive control Doxorubicin exhibits higher inhibitory activity against HCT-116 human colorectal carcinoma specifically observed at 3.125 µg/mL concentration (Table 9).

It was found out that the MTT Cytotoxicity Assay revealed a significant difference among different concentrations of the standard drug (Doxorubicin) and the *D. trifasciata* against HCT-116 cells. Data showed that the positive control Doxorubicin exhibits higher inhibitory activity which indicates the potency of the standard drug against HCT-116 cell line, specifically observed at 3.125 µg/mL concentration. Still, the snake plant *D. trifasciata* possesses minimal inhibitory activity, specifically observed in Trials 1 and 3 at the same concentration of 3.125 µg/mL.

CONCLUSION

The researcher conducted the study to identify the antiproliferative and cytotoxic potential of the plant extract from *D. trifasciata* leaves. With extensive use of the resources available in the University of Perpetual Help – Dr. Jose G. Tamayo Medical University, DOST (FPRDI, ITDI-STD, CED), DLSU-CIF, UST-ASL, and UP Diliman Mammalian Cell Culture Laboratory, the researcher was able to obtain promising results. The MTT Assay revealed that the snake plant *D. trifasciata* extracts has a minimal antiproliferative activity against HCT 116 cells [Inhibitory concentration (IC50) = greater than 100 µg/mL]. Given the overall IC50 obtained in Trials 1-3, this study showed that *D. trifasciata* extracts did not sufficiently reach the minimum absorbance level needed to show cytotoxic potential. With the remarkable cell viability decrease associated with cytotoxicity increase for positive control, Doxorubicin exhibited higher inhibitory activity which showed the potency of the standard drug against HCT-116 cell line, specifically observed at 3.125 µg/mL concentration. Still, the *D. trifasciata* possesses minimal inhibitory activity, specifically in Trials 1 and 3 at the same concentration of 3.125 µg/mL.

Table 8. Results of the Absorbance Readings of Treated HCT Cells with the Different Variables. Methanolic semi-purified extract of *D. trifasciata* were tested in different variables such as **(A)** *D. trifasciata* and negative control (Dimethyl sulfoxide – DMSO), **(B)** *D. trifasciata* and standard drug (Doxorubicin), and **(C)** Standard drug (Doxorubicin) and negative control (DMSO)

	Source	SS	df	MS	F-ratio	Interpretation
(A) *D. trifasciata* and negative control (Dimethyl sulfoxide – DMSO)						
Trial 1	Between treatments	0.4529	1	0.4529	F = 6.42759	Significant
	Within treatments	2.1139	30	0.0705	*p*-value is <0.016685	*p* <0.05
	Total	2.5668	31			
Trial 2	Between treatments	0.0271	1	0.0271	F = 2.69392	Not significant
	Within treatments	0.3023	30	0.0101	*p*-value is <0.111176	*p* <0.05
	Total	0.3294	31			
Trial 3	Between treatments	0.2406	1	0.2406	F = 3.29054	Not significant
	Within treatments	2.1940	30	0.0731	*p*-value is <0.079694	*p* <0.05
	Total	2.4346	31			
(B) *D. trifasciata* and standard drug (Doxorubicin)						
	Source	**SS**	**df**	**MS**	**F-ratio**	**Interpretation**
Trial 1	Between treatments	2.0620	1	2.0620	F = 24.44965	Significant
	Within treatments	2.5301	30	0.0843	*p*-value is <0.000027	*p* <0.05
	Total	4.5920	31			
Trial 2	Between treatments	1.6933	1	1.6933	F = 247.82505	Significant
	Within treatments	0.2050	30	0.0068	*p*-value <0.00001	*p* <0.05
	Total	1.8982	31			
Trial 3	Between treatments	2.2882	1	2.2882	F = 31.78411	Significant
	Within treatments	2.1598	30	0.0720	*p*-value <0.00001	*p* <0.05
	Total	4.4479	31			
(C) Standard drug (Doxorubicin) and negative control (DMSO)						
	Source	**SS**	**df**	**MS**	**F-ratio**	**Interpretation**
Trial 1	Between treatments	0.5821	1	0.5821	F = 30.22302	Significant
	Within treatments	0.5778	30	0.0193	*p*-value <0.00001	*p* <0.05
	Total	1.1599	31			
Trial 2	Between treatments	1.2916	1	1.2916	F = 127.56301	Significant
	Within treatments	0.3038	30	0.0101	*p*-value <0.00001	*p* <0.05
	Total	1.5954	31			
Trial 3	Between treatments	1.0447	1	1.0447	F = 61.50555	Significant
	Within treatments	0.5096	30	0.0170	*p*-value <0.00001	*p* <0.05
	Total	1.5543	31			

Table 9. NOVA F-Test Results for the Significant Difference between the Percent Inhibition Exhibited by Different Concentrations of the Standard Drug (Doxorubicin) and the Snake Plant (*D. trifasciata*) against HCT-116 Cells

	Source	SS	df	MS	F-ratio	Interpretation
Trial 1	Between treatments	49666.6373	1	49666.6373	F = 26.50137	Significant
	Within treatments	56223.4763	30	1874.11590	*p*-value is <0.000015	*p* <0.05
	Total	105890.1136	31			
Trial 2	Between treatments	40666.7964	1	40666.7964	F = 167.34404	Significant
	Within treatments	7290.3935	30	243.0131	*p*-value is <0.00001	*p* <0.05
	Total	47957.1899	31			
Trial 3	Between treatments	46311.4254	1	46311.4254	F = 27.42424	Significant
	Within treatments	50661.1188	30	1688.7040	*p*-value is <0.000012	*p* <0.05
	Total	96972.5442	31			

A highest peak and intensity revealed the presence of OH group responsible for the polarity of *D. trifasciata* extract and presence of all the bioactive components as shown in Figure 1. Furthermore, the presence of an -OH stretch is indicative of alcohols or polyhydroxylated metabolites that is found within the major flavonoid classes (which are structures responsible for antioxidant property), that corresponds to the data obtained from Total Flavonoid Content.

It is also visible that the plant extract has a minimal activity even at 1.56 µg/mL (Trial 3) concentration. This implies a positive chance to meet its antiproliferative property with the use of other extraction processes such as fractionation and purification. This extraction process is proven effective in one of the researches on California olive pomace which showed that using macroporous resin for purification significantly increased the total phenolic content and antioxidant activity of the extracts. The resin-purified extracts had 3.7 to 4.7 times higher antioxidant activity compared to the crude extracts, demonstrating the efficacy of fractionation and purification in enhancing the extraction of valuable antioxidants like hydroxytyrosol and oleuropein.

Overall, Dracaena species feature remarkable biological capabilities. The findings revealed the *D. trifasciata* extract exhibited minimum absorbance to show antiproliferative property and cytotoxic potential (at increasing concentration) with comparable effects to the positive control.

Recommendations

It is recommended that more comprehensive and large scale studies be conducted, including: (1) a more detailed isolation of the plant constituents responsible for the antiproliferative and cytotoxic potential, (2) conducting other extraction processes such as fractionation and purification, (3) utilizing other plant parts, related family or in combination study under Asparagaceae genus, that is essential to identify its potential synergistic effect, (4) perform in-vitro assay using other cell line like AA8 Chinese hamster ovarian fibroblast, which is categorized as animal normal cell line. This can only be utilized if the sample turned out to be active against a certain type of cancer cells, and lastly, (5) conduct a local study in the Philippines about genetic variation and/or molecular phylogeny of Dracaena and Sansevieria species through DNA barcoding.

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Statement of Authorship

The author certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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