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Antioxidant and Phytochemical Profile of Different Varieties of Mother-In-Law's Tongue (*Dracaena trifasciata* (Prain) Mabb.)

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ABSTRACT

Dracaena trifasciata commonly called mother-in-law's tongue is widely cultivated plant in Indonesia and has been traditionally used for the treatment of various diseases. This study aimed to investigate the antioxidant activity and phytochemical profile of ethanol extracts of four different varieties of *Dracaena trifasciata.* Four varieties of *D. trifasciata* leaves, including gold flame, moonshine Brazilian, banded nelsoni, and mediocipta cobra varieties were extracted by refluxing with 96% and 70% ethanol. Phytochemical constituents of the leaves were determined according to standard procedures. The antioxidant activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazil (DPPH), Cupric Ion Reducing Antioxidant Capacity (CUPRAC), and Ferric Reducing Antioxidant Power (FRAP) assays. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined using colorimetric methods. Flavonoid compounds in selected extracts were identified by high-performance liquid chromatography (HPLC). Among the four varieties, *D. trifasciata* var. mediopicta cobra extract had the highest antioxidant activity. The 96% ethanol extract of *D. trifasciata* var. moonshine Brazilian exhibited the highest TPC (138.534 ± 5.217 mg GAE/g), while the highest TFC (368.957 ± 19.461 mg QE/g) was found in the 96% ethanol extract of *D. trifasciata* var. gold flame. The TPC and TFC were strongly correlated with the antioxidant activity. Rutin and kaempferol were found in varying concentrations in the 96% and 70% ethanol extracts of *D. trifasciata* var. mediopicta cobra leaves. These findings suggest that all four varieties of *D. trifasciata* are potential sources of natural antioxidants

Keywords: Antioxidant, *Dracaena trifasciata*, Cupric Ion Reducing Antioxidant Capacity, Ferric Reducing Antioxidant Power, 2,2-diphenyl-1-picrylhydrazyl

Introduction

Free radicals are naturally generated during normal cellular metabolic processes. They are atoms or molecules that contain one or more unpaired electrons in their outer orbitals, allowing them to exist independently. The presence of an unpaired electron makes free radicals highly unstable, short-lived, and extremely reactive. In order to stabilize themselves, they readily capture electrons from nearby molecules.[1] When two free radicals interact, their unpaired electrons may pair up to form a covalent bond leading to a stable molecule. In contrast, most biological molecules are not radicals. Therefore, when a free radical interacts with a non-radical molecule, a new radical is generated, which may trigger a chain reaction. As a result, the affected molecule becomes a free radical in turn, initiating a chain reaction that can ultimately cause damage to living cells.[2] Free radicals are frequently referred to as nitrogen free radicals which are reactive nitrogen species (RNS) and reactive oxygen species (ROS).[3] Every cell in the body undergoes metabolic processes that generate free radicals, which include the formation of ROS. The body needs low levels of ROS, due to its beneficial elements for the process of apoptosis, phagocytosis, and Immunity.

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Excessive production of ROS has a detrimental effect on thebody due to the damage caused to macromolecular structures, such as the lipid bilayer of cell membrane, proteins, and DNA.[4] Under normal conditions in living cells, there is a balance between the production of ROS and their degradation. This equilibrium provides a stable state of ROS levels, where a system is in dynamic redox equilibrium. However, due to certain processes, this balance can be disrupted, leading to an increase in ROS, leading to redox imbalance known as oxidative stress.[5] This oxidative stress triggers the development of cardiovascular diseases (atherosclerosis, ischemic heart disease, hypertension, cardiomyopathy, cardio hypertrophy, congestive heart failure), cancer, type 2 diabetes mellitus, cataract, arthritis, autoimmune diseases, and neurodegenerative diseases.[6-9] Antioxidants are compounds that neutralize free radicals.[10] In the human body, a balance exists between antioxidants and free radicals, and the ability of antioxidants to capture and neutralize free radicals leads to the reduction of their adverse impact on the body.[11,12]

Exogenous antioxidants are categorized into two types according to their source, including synthetic and natural antioxidants. Examples of commonly used synthetic antioxidants are Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA). Previous research found that exposure to BHA affects hormonal homeostasis, leading to obesity and may also cause carcinogenic effects. Some BHT metabolites are reported to induce DNA cleavage by producing superoxide radicals, which are teratogenic and carcinogenic.[13] The potential health risks associated with consuming synthetic antioxidants have driven researchers to investigate the potential of natural antioxidants derived from natural sources, such as *Dracaena trifasciata*.

Dracaena trifasciata, commonly known as the mother-in-law's tongue, was previously classified as *Sansevieria trifasciata*. This plant belongs to the subfamily Nolinoideae within the Asparagaceae family and is placed in the order Asparagales.[14-16] Mother-in-law's tongue originates from Africa, Arabia, India, and Indonesia. In Indonesia, the plant has

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been grown for a long time and is widely distributed across various regions. In its natural habitat, it is a wild plant, but over time, it has been extensively cultivated as an ornamental plant.[17] The plant possesses important medicinal value due to it rich content of phytochemicals, including saponins, cardenolins, polyphenols, flavonoids, steroids, alkaloids, and tannins.[18] In traditional medicine, the roots and leaves of *D. trifasciata* are used for the treatment of coughs, asthma, abdominal pain, diarrhea, hypertension, wounds, etc. Several investigations have shown that *D. trifasciata* has numerous pharmacological activities, such as analgesic, antipyretic, antioxidant, antibacterial, antidiabetic, and anti-alopecia activities.[19] Presently, there is no research that compares the antioxidant activity of *D. trifasciata* of different varieties. This study therefore aimed to evaluate the antioxidant activity of the leaves of four different *D. trifasciata* varieties, including *D. trifasciata* var. gold flame, *D. trifasciata* var. moonshine Brazilian, *D. trifasciata* var. banded nelsoni, and *D. trifasciata* var. mediopicta cobra. Furthermore, this study also determined the total flavonoid and total phenolic contents of the ethanol extracts of the leaves of mother-in-law's tongue and correlated these with their antioxidant activity.

Materials and Methods

Chemicals and reagents

The materials used in this research included methanol, distilled water, ethanol, cupric chloride, TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl 1-picrylhydrazyl), neocuproine, Folin-Ciocalteu reagent, ascorbic acid, gallic acid, ammonium acetate, sodium carbonate, aluminum (III) chloride, sodium acetate, quercetin, rutin, and kaempferol.

Collection and identification of plant materials

Fresh leaves of four varieties of mother-in-law's tongue (*D. trifasciata*) were collected from a plant cultivation site in Lembang, West Bandung district, West Java, Indonesia (6°49'37.7"S 107°38'06.7"E) in November 2023. The plant was identified at the Bandungense Herbarium, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia, where the voucher number 2749/2023 was assigned. The varieties collected were gold flame (GB), moonshine Brazilian (MB), banded nelsoni (BM), and mediocipta cobra (MC) varieties.

Phytochemical screening

Phytochemical analysis was performed to determine secondary metabolites including alkaloids, phenols, flavonoids, steroids/triterpenoids, tannins, saponins, and quinones in the leaves of the different varieties of *D. trifasciata*. The screening procedures of the secondary metabolites group were carried out according to the guidelines described in previous research.[20,21]

Extraction of plant materials

The leaves of each variety of *D. trifasciata* were washed, chopped, and dried in the oven at 40°C, and then ground into powder form. The powdered leaves (50 g each) were extracted by refluxing in 96% and 70% ethanol in two separate extractions for each sample. The extraction for each solvent was repeated three times to obtain the optimum yield. The extract was decanted, then filtered, and the resulting filtrate (liquid extract) was concentrated in a rotary evaporator to obtain the concentrated extract. Eight extracts consisting of four 96% ethanol extracts (gold flame variety GB1, moonshine Brazilian MB1, banded nelsoni BM1, and mediocipta cobra MC1) and four 70% ethanol extracts (gold flame variety GB2, moonshine Brazilian MB2, banded nelsoni BM2, and mediocipta cobra MC2) were obtained. The specific gravity of each of the extracts were determined.

Determination of antioxidant activity

The antioxidant activity of the ethanol leaf extracts of each variety of *D. trifasciata* was determined using three different assay methods, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, cupric ion reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP) assays.

DPPH radical scavenging assay

A stock solution was made by mixing 20 mg of ascorbic acid into 100 mL of methanol, which was diluted to produce seven different concentrations (15 µL, 20 µL ,25 µL, 27.5 µL, 30 µL, 35 µL and 40 µL). DPPH solution was prepared by dissolving 2.5 mg DPPH in 50 mL methanol. Each concentration of the ascorbic acid stock was mixed with methanol to achieve a volume of 125 µL. Subsequently, 750 µL of DPPH solution was combined, and the resulting mixture was incubated in a dark room at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using a UV-Vis spectrophotometer (Thermo Scientific Genesys 50, USA). The absorbance obtained was used to calculate the percentage of scavenging activity for each concentration.[22,23] The sample was prepared by dissolving the plant extract in methanol and then filtered. The DPPH radical scavenging activity of the sample was assessed following the same procedure used for the ascorbic acid standard. The measurements were carried out in six replicates for each extract. The percentage of scavenging activity of each concentration was obtained. Antioxidant activity of the sample was determined by applying the percentage of scavenging activity to the ascorbic acid regression equation. Antioxidant activity was quantified as ascorbic acid equivalent, specifically as mg ascorbic acid equivalent antioxidant capacity (AEAC) per gram of sample.[24]

CUPRAC assay

In the determination of antioxidant activity using the CUPRAC method, ascorbic acid served as the reference standard. A stock solution was prepared by mixing 20 mg of ascorbic acid in 100 mL of methanol, which was diluted to produce seven different concentrations (15 µL, 20 µL, 25 µL, 27.5 µL, 30 µL, 35 µL, and 40 µL), then ammonium acetate buffer (250 µL) was added. Subsequently, 750 µL of CUPRAC solution (100 µg/mL) was added, and the resulting mixture was incubated in a dark room at room temperature for 30 minutes. The absorbance was measured at 450 nm using a UV-Vis spectrophotometer. The absorbance obtained was used to calculate the percentage of reducing activity for each concentration.[25] The extract solution was prepared and dissolved in methanol, filtered and the filtrate was used as the sample. The CUPRAC of the sample was assessed following the same procedure used for the ascorbic acid standard. The measurements were carried out in six replicates for each extract, and the antioxidant activity of the sample was evaluated using the calibration curve of ascorbic acid. Antioxidant activity was expressed as mg AEAC per gram of sample.[26]

FRAP assay

The determination of antioxidant activity by the FRAP method was carried out using ascorbic acid as the reference standard. FRAP solution (467.5 µg/mL) was prepared. A stock solution was prepared by mixing 20 mg of ascorbic acid in 100 mL of methanol, which was diluted to produce four different concentrations. (12.5 µL, 22.5 µL, 25 µL, and 30 µL), then made up to 500 µL with distilled water. Subsequently, 500 µL of CUPRAC solution was added, and the resulting mixture was incubated in a dark room at room temperature for 30 minutes. The absorbance was measured at 595 nm using a UV-Vis spectrophotometer. The absorbance obtained was used to calculate the percentage of reducing activity for each concentration.[25]

The extract solution was prepared and dissolved using methanol, and filtered and the filtrate was used as the sample. FRAP activity of the sample was performed using the same procedure as the ascorbic acid standard. The measurements were done in six replicates for each extract. Antioxidant activity of the sample was evaluated by entering the percentage of reducing activity into the regression equation of ascorbic acid calibration curve. Antioxidant activity was expressed as milligram ascorbic acid equivalence per gram of sample (mg AEAC/g sample).[27]

Determination of total phenolic content (TPC)

Gallic acid served as the standard for determining the TPC. A stock solution of gallic acid was prepared with a concentration of 1,000 µg/mL, and subsequently diluted to achieve various concentrations ranging from 60 to 130 µg/mL. Subsequently, 50 µL from each concentration of the gallic acid solution was added to 400 µL of 1 M sodium carbonate and 500 µL of 10% Folin-Ciocalteu reagent in an

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Eppendorf tube. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Then, the absorbance values were used to construct a gallic acid calibration curve. The blank used was methanol with the addition of Folin-Ciocalteu reagent and sodium carbonate.[28]
The extract solution was prepared and dissolved using methanol, filtered, and the filtrate was treated in the same way as the gallic acid standard. The measurements were performed in six replicates for each extract. The TPC of each extract was quantified by applying the gallic acid calibration curve and reported as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g).[28]

Determination of total flavonoid content
The total flavonoid content of *D. trifasciata* leaf extracts was determined according to the method described by Pourmorad *et al*. (2006)[28] with quercetin as the reference standard. A stock solution of quercetin was prepared at a concentration of 1,000 µg/mL, and subsequently diluted to obtain concentrations ranging from 40 to 110 µg/mL. To erom each concentration of quercetin solution (100 µL) was added 560 µL of distilled water; 20 µL of 1 M sodium acetate; 20 µL of 10% $AlCl_3$; and 300 µL of methanol. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 415 nm using a UV-Vis spectrophotometer. The absorbance values were used to construct a quercetin calibration curve.[28]
The extract solution was prepared by dissolving the plant material in methanol. The solution was then filtered, and the filtrate was used as the sample. Subsequent analysis of the samples followed the same procedure as that used for the quercetin standard. The measurements were performed in six replicates for each extract. The total flavonoid contents were estimated from the linear regression equation of the quercetin calibration curve, and results were expressed as mg of quercetin equivalent (QE) per gram of extract (mg QE/g).[29]

Identification and quantification of flavonoid compounds
Identification and quantification of flavonoid compounds were conducted using high-performance liquid chromatography (HPLC). The stationary phase was LiChrospher® 100 RP-C18 (5 µm) column. The mobile phase was methanol and distilled water at a flow rate of 1 mL/min (Pump CTO-20A, Shimadzu, Japan). The injection volume was 20 µL and the temperature of the column was 30°C. The detector was UV/Vis SPD-20A at 360 nm (Shimadzu, Japan). The standards (rutin and kaempferol) were 50 µg/mL, and the extract was 100,000 µg/mL and 150,000 µg/mL.

Statistical analysis
Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, as well as independent t-test using SPSS 22 for Windows. Each sample measurement was conducted in six replicates and results were presented as mean ± standard deviation (SD). The correlation between TPC and TFC with antioxidant activity and the correlation between antioxidant activity testing methods were determined by Pearson's correlation coefficient.

Results and Discussion

Phytochemical constituents of D. trifaciata leaves
The phytochemical constituents of *D. trifaciata* leaves are presented in Table 1. The results showed that the leaves of the four varieties of *D. trifaciata* leaves contained alkaloids, flavonoids, phenols, saponins, and steroids/triterpenoids. The result is similar to that reported by Berame *et al*. (2017)[30] who confirmed the presence of alkaloids and tannins in *D. trifasciata* leaves, as well the work of Kumar *et al*. (2022)[31] in which alkaloids, flavonoids, and steroids were found in *D. trifaciata* leaves.

Table 1: Phytochemical constituents of different varieties of *Dracaena trifasciata* leaves

Secondary Metabolite	Result			
	GB	MB	BM	MC
Flavonoids	+	+	+	+
Phenol	+	+	+	+
Tannins	-	-	-	-
Quinone	-	-	-	-
Saponins	+	+	+	+
Steroid/triterpenoid	+	+	+	+
Alkaloids	+	+	+	+

Key: '+' = detected, and '-' = not detected.
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

Extraction outcome
The specific gravity of the different extracts of *D. trifaciata* leaves are shown in Table 2. During the extraction process, compounds are extracted based on the similarity of their polarity with the extracting solvent. The dielectric constant of solvents indicates their degree of polarity; the greater the dielectric constant, the greater the polarity of the solvent. The high polarity of ethanol is useful for the extraction of a wide range of compounds, both polar and semipolar compounds. Ethanol is effective in extracting sterols, flavonoids, phenolic compounds, and alkaloids.[32] In this study, two concentrations of ethanol (70% and 96%) were used for the extraction of four varieties of *D. trifaciata* leaves. Since 70% ethanol is more polar than 96% ethanol, it extracts a greater proportion of polar compounds, and a smaller proportion of semi-polar and non-polar compounds compared to 96% ethanol. The use of different ethanol concentrations (96% ethanol and 70% ethanol) provides a range of solvent polarities, thereby influencing the TPC and TFC. This approach aims to obtain a diverse range of non-polar, semi-polar, and polar compounds.

Antioxidant activity of four varieties of D. trifasciata
Antioxidants have gained significant scientific attention in recent times due to their being related to the prevention of various degenerative diseases. Since the antioxidant activity cannot be measured directly, assessment methods evaluates the effects of antioxidants in controlling the extent of oxidation. The antioxidant capacity has been extensively studied *in vitro*, and different methods have been used to assess its activity.[33] The antioxidant activity of the different varieties of *D. trifasciata* leaf extracts are shown in Tables 3 - 5.

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The antioxidant activity according to the three assay methods; DPPH, CUPRAC, and FRAP were presented as milligram of ascorbic acid equivalent antioxidant capacity per gram of sample (mg AEAC/g sample). The greater the value of mg AEAC/g sample, the greater the antioxidant activity possessed by the sample. Antioxidant activity assays using the DPPH and CUPRAC methods showed that the MC1 extract had the highest antioxidant activity (11.870 ± 0.309 mg AEAC/g and 76.808 ± 1.537 mg AEAC/g, respectively) compared to the other extracts. Furthermore, antioxidant testing with the FRAP method showed that the MC2 extract had highest antioxidant activity (18.379 ± 2.204 mg AEAC/g) compared to other extracts. Many factors can affect the antioxidant activity of a plant extract, such as the composition of the extract and the method of assessing antioxidant activity. Natural antioxidants are multifunctional, thereby requiring reliable antioxidant testing methods.

One of the most commonly used methods of assessing antioxidant activity of compounds is the DPPH radical scavenging activity. The DPPH free radical possesses unpaired electrons, exhibits a purple colour with maximum absorbance at a wavelength of 517 nm. The purple colour of DPPH changes quickly to yellow when the electrons are paired. When DPPH solution is mixed with an antioxidant molecule, which can donate a hydrogen atom, a reduced form of DPPH will be formed with a loss of purple colour, and the decrease in colour intensity is linked to the quantity of DPPH electrons that abstract hydrogen atoms.

Table 2: Specific gravity of extracts of four varieties of *Dracaena trifasciata* leaves

Extract	Specific gravity 1% extract (g/mL)
GB1	0.967 ± 0.022
MB1	1.011 ± 0.060
BM1	0.891 ± 0.035
MC1	0.898 ± 0.003
GB2	1.048 ± 0.026
MB2	1.101 ± 0.026
BM2	0.976 ± 0.003
MC2	0.984 ± 0.004

GB1: Gold flame 96% ethanol extract, MB1: Moonshine Brazilian 96% ethanol extract, BM1: Banded nelson 96% ethanol extract, MC1: mediocipta cobra 96% ethanol extract, GB2: Gold flame 70% ethanol extract, MB2: Moonshine Brazilian 70% ethanol extract, BM2: Banded nelson 70% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

Table 3: Antioxidant activity of four varieties of *Dracaena trifasciata* leaves using DPPH assay

Sample	96% ethanol extract	70% ethanol extract
GB	10.988 ± 0.377^{ay}	10.113 ± 0.216^{ay}
MB	7.957 ± 0.748^{by}	10.557 ± 0.513^{bz}
BM	11.114 ± 0.475^{cy}	6.531 ± 0.249^{cz}
MC	11.870 ± 0.309^{dy}	10.577 ± 0.406^{bz}

Values represent mean ± standard deviation (SD). a – d: Different lower-case letters in the same column indicate significant difference ($p < 0.05$). y-z: Different lower-case letters in the same row indicate significant difference ($p < 0.05$).
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

Table 4: Antioxidant activity of four varieties of *Dracaena trifasciata* leaves using CUPRAC assay

Sample	96% ethanol extract	70% ethanol extract
GB	35.457 ± 0.478^{ay}	13.476 ± 0.624^{az}
MB	32.938 ± 0.519^{by}	11.246 ± 0.377^{bz}
BM	24.611 ± 0.529^{cy}	8.727 ± 0.894^{cz}
MC	76.808 ± 1.537^{dy}	13.289 ± 0.679^{az}

Values represent mean ± standard deviation (SD). a – d: Different lower-case letters in the same column indicate significant difference ($p < 0.05$). y-z: Different lower-case letters in the same row indicate significant difference ($p < 0.05$).
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

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Table 5: Antioxidant activity of four varieties of *Dracaena trifasciata* leaves using FRAP assay

Sample	96% ethanol extract	70% ethanol extract
GB	14.816 ± 1.654^{ay}	6.464 ± 0.806^{az}
MB	14.588 ± 1.022^{ay}	11.331 ± 0.488^{bz}
BM	14.837 ± 2.372^{ay}	12.933 ± 1.066^{cz}
MC	10.645 ± 1.553^{by}	18.379 ± 2.204^{dz}

Values represent mean ± standard deviation (SD). a – d: Different lower-case letters in the same column indicate significant difference ($p < 0.05$). y-z: Different lower-case letters in the same row indicate significant difference ($p < 0.05$).
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

Therefore, the reduction in colour intensity indicates an increase in free radical scavenging, and antioxidant ability. The DPPH method is straightforward and rapid, requiring fewer reagents compared to other methods. Moreover, this method is recognized for its practicality, accuracy, and reliability.[34,35] The CUPRAC method for measuring antioxidant capacity offers advantages over other methods, particularly in its ability to rapidly oxidize thiol-type antioxidants. In addition, CUPRAC reagents are more stable and accessible than other chromogenic reagents such as ABTS, and DPPH. This method measures antioxidant activity based on the reduction reaction of Cu^{2+}-neocuproine to Cu^{+} - neocuproine by a sample which functions as an antioxidant. Neocuproine has the ability to generate a chromophore in conjunction with Cu(I), producing a yellow colour. This method is easy and applicable in conventional laboratories using standard colorimeters without requiring advanced equipment and qualified operators.[36,37] The FRAP method is cheap, easy to prepare, and quite simple and fast. This method assesses the total antioxidant content of a substance by measuring the capability of antioxidant compounds to convert Fe^{3+}-TPTZ to Fe^{2+}-TPTZ, correlating antioxidant potency of a substance with its reducing capacity.[38] The FRAP method is particularly applicable for evaluating antioxidants in plant materials.[39] The FRAP reagent is composed of tripyridyltriazine (TPTZ) and iron (III) chloride. The assay is conducted at pH 3.6 (acidic pH conditions) to achieve iron solubility and promote electron transfer. Decreasing the pH elevates the redox potential, decreases the ionization potential, and enhances electron transfer, which is the dominant reaction mechanism.[40] At acidic pH, ferric-tripyridyltriazine (Fe^{3+}-TPTZ) is converted to the ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) form which displays a vibrant blue colour having an absorption maximum at λ 593 nm. An increase in absorbance indicates greater reduction capability in the reaction mixture. Therefore, performing more than one assay methods for antioxidant activity is necessary.[41] Variations in test methods on the same sample can be observed when using different analytical tools.[42] For example, the 96% ethanol extract of *S. trifasciata* was reported to have very strong antioxidant activity in the DPPH assay,[43] whereas, a moderate activity have been reported for antioxidant activity of the extract assessed through inhibition of lipid peroxidation (LP) using the thiobarbituric acid (TBA) assay.[44] The observed variations in antioxidant activity among the different varieties may be attributed to several factors, including inherent genetic differences and environmental influences such as climate, temperature, and cultivation practices.

TPC and TFC of four varieties of D. trifasciata leaves

Phenolic compounds are potent antioxidants due to their ability to act as free radical terminators.[45] The free radical scavenging activity of phenolic compounds depends on their molecular weight, the presence of aromatic rings, and the substitution of the OH group.[46] Another important class of phenolic compounds which possess significant antioxidant activity are flavonoids. Flavonoids are recognized for their effectiveness in neutralizing oxidizing molecules, such as singlet oxygen and various free radicals, which are implicated in the development of numerous diseases.[47] Flavonoids are categorized into various types, each exhibiting different polarities influenced by the number and position of the hydroxyl groups. These variations in hydroxyl groups affect the solubility of flavonoids in different solvents. Phenols and flavonoids are bioactive compounds that tend to be polar to semi-polar.[48] This explains why semi-polar to polar solvents are frequently used in the extraction process. The extraction yield generally increases with increasing solvent volume. This enhancement in yield is attributed to improved contact between the solvent and the plant material, facilitating more efficient extraction of the target compounds and mitigating solvent saturation.[49] Therefore, the composition of phenols and flavonoids in samples plays an important role in their antioxidant activities and different structures may result in different levels of antioxidant activities. A sample with a phenolic compound containing more hydroxyl groups or conjugated double bonds shows higher antioxidant capacity. The DPPH assay shows significant antioxidant activity in flavonoids possessing hydroxyl group at C-3′-C-4′, a hydroxyl group at C-3, an oxo group at C-4, and a double bond between C-2 and C-3. Therefore, the position and amount of hydroxyl groups in the structure play an important role in antioxidant activity of a compound.[37]

The determination of TPC is based on the reaction of phenolic compounds with Folin-Ciocalteu reagent, resulting in a yellow hue which turns purple on addition of sodium carbonate (Na_2CO_3) solution. The intensity of the purple colour which is measured spectrophotometrically at 765 nm is directly proportional to the concentration of the phenolic compounds. In this study, TPC of each extract was quantified using a gallic acid calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g). The calibration curve was constructed from six different concentrations of gallic acid, with absorbance values ranging from 0.2 to 0.8. The linear regression equation of the gallic acid calibration curve was $y = 0.0067x - 0.0374$, $R^{2.} = 0.9956$. The results of the TPC of each extract of *D. trifasciata* varieties are shown in Table 6. From the results obtained, the 96% ethanol extracts showed higher TPC compared to the 70% ethanol extracts.

Table 6: Total phenol content of four varieties of *Dracaena trifasciata* leaves

Sample	96% ethanol extract	70% ethanol extract
GB	84.358 ± 4.904^{ay}	41.124 ± 2.871^{az}
MB	138.534 ± 5.217^{by}	51.482 ± 1.815^{bz}
BM	112.65 ± 8.297^{cy}	71.157 ± 1.613^{cz}
MC	93.014 ± 1.849^{dy}	39.588 ± 0.493^{az}

Values represent mean ± standard deviation (SD). a – d: Different lower-case letters in the same column indicate significant difference ($p < 0.05$). y-z: Different lower-case letters in the same row indicate significant difference ($p < 0.05$).
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

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This finding is consistent with earlier research which reported that the TPC of *S. trifasciata* extract decreases with higher water content. This may be attributed to the higher content of non-phenolic compounds, such as terpenes and carbohydrates, in water extracts. Additionally, the potential formation of complexes involving multiple phenolic compounds in ethanol-soluble extracts could also contribute to this observation. The phenolic compounds in ethanol extract may have more hydroxy groups or have a greater molecular weight than the phenolics in aqueous extract. The ratio of solvents to crude drug, extraction time, and other factors affect phenolic content. Therefore, the same crude drug can produce different phenolic content.[50-52] Antioxidants are not only found in polyphenols, but also in a diverse group of amino acids, phenylamines, and tyrosine, including simple phenolic acids, hydroxybenzoic acid, tannins, and flavonoids.[53,54] This may explain why MB1 (96% ethanol extract of Moonshine Brazilian variety) had the highest TPC (138.534 ± 0.037 mg GAE/g) but not the highest antioxidant activity. It is possible that the MB1 extract contained a higher proportion of phenolic compounds with lower antioxidant activity, potentially due to a reduced number of hydroxyl groups in their structures. TFC was evaluated using the aluminium chloride colorimetric method. This method is based on the formation of stable complexes involving the C-4 keto groups and the hydroxyl groups on C-5 or C-3 of flavonoids.[55] Additionally, aluminum chloride can produce an unstable acidic complex with an ortho-hydroxyl group on the A- or B-ring of flavonoid compounds. Quercetin was selected as a reference solution because it is a flavonoid compound that can react with $AlCl_3$ to form such a complex.[56] TFC was estimated from the linear regression equation $y = 0.0052x + 0.0022$, $R^2 = 0.9945$ obtained from quercetin calibration curve, and the result was expressed as milligrams of quercetin equivalent (QE) per gram of extract (mg QE/g). From the results obtained, GB (Gold flame variety) had the highest TFC (368.957 ± 0.052 mg QE/g), with the 96% ethanol extract showing higher TFC than the 70% ethanol extract (Table 7). This result is in contrast with that reported by [52] in which the 76% ethanol extract of *S. trifasciata* var. laurentii had higher TFC than the 96% ethanol extract. This variation may be due to differences in plant varieties and growing environments, which affect the types of flavonoid compounds present in the plant.[52]

Correlation between the TPC, TFC and antioxidant activity of four varieties of D. trifasciata

Quantitative correlation analyses between TPC and TFC and antioxidant activity, as assessed by FRAP, DPPH, and CUPRAC methods were conducted using SPSS version 22 for Windows with the Pearson correlation method. A positive and significant correlation result indicated that there was a correlation between TPC, TFC and antioxidant activity, meaning antioxidant activity is related to TPC and TFC in the sample. The correlation results are shown in Table 8. According to the result, TPC and TFC showed moderate (r = 0.40-0.69) to very strong (r = 0.90-1.00) correlation with DPPH, CUPRAC, and FRAP in all samples at $p < 0.01$

Table 7: Total flavonoid content of four varieties of *Dracaena trifasciata* leaves

Sample	96% ethanol extract	70% ethanol extract
GB	84.358 ± 4.904^{ay}	41.124 ± 2.871^{az}
MB	138.534 ± 5.217^{by}	51.482 ± 1.815^{bz}
BM	112.65 ± 8.297^{cy}	71.157 ± 1.613^{cz}
MC	93.014 ± 1.849^{dy}	39.588 ± 0.493^{az}

Values represent mean ± standard deviation (SD). a – d: Different lower-case letters in the same column indicate significant difference ($p < 0.05$). y-z: Different lower-case letters in the same row indicate significant difference ($p < 0.05$).
GB: Gold flame variety, MB: Moonshine Brazilian variety, BM: Banded nelson variety, MC: mediocipta cobra variety

Table 8: Correlation of total phenol content (TPC) and total flavonoid content (TFC) with antioxidant activity of *Dracaena trifasciata* varieties

Sample	DPPH		CUPRAC		FRAP	
	TPC	TFC	TPC	TFC	TPC	TFC
GB1	0.894^{***}	0.932^{****}	0.956^{****}	0.960^{****}	0.946^{****}	0.921^{****}
MB1	0.911^{****}	0.599^{**}	0.971^{****}	0.858^{***}	0.953^{****}	0.712^{***}
BM1	0.995^{****}	0.802^{***}	0.800^{***}	0.953^{****}	0.761^{***}	0.919^{****}
MC1	0.967^{****}	0.994^{****}	0.950^{****}	0.896^{***}	0.970^{****}	0.985^{****}
GB2	0.901^{****}	0.942^{****}	0.961^{****}	0.992^{****}	0.955^{****}	0.912^{****}
MB2	0.973^{****}	0.854^{***}	0.962^{****}	0.797^{***}	0.898^{***}	0.810^{***}
BM2	0.967^{****}	0.990^{****}	0.861^{***}	0.912^{****}	0.929^{****}	0.933^{****}
MC2	0.963^{****}	0.911^{****}	0.978^{****}	0.875^{***}	0.892^{***}	0.763^{***}

** = moderate correlation, *** = strong correlation, **** = very strong correlation.
GB1: Gold flame 96% ethanol extract, MB1: Moonshine Brazilian 96% ethanol extract, BM1: Banded nelson 96% ethanol extract, MC1: mediocipta cobra 96% ethanol extract, GB2: Gold flame 70% ethanol extract, MB2: Moonshine Brazilian 70% ethanol extract, BM2: Banded nelson 70% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

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The correlation between DPPH, CUPRAC, and FRAP test methods is expressed in Tables 9, 10, and 11, respectively. The results of the correlation analysis between test methods showed a strong and very strong correlation between the DPPH, FRAP, and CUPRAC test methods with Pearson's correlation coefficient in the range of r = 0.782 – 0.974. The three methods showed different values, but based on Pearson correlation, it can be concluded that the DPPH, FRAP, and CUPRAC methods produced consistent results in determining the antioxidant activity of *D. trifasciata* leaves extract.

Flavonoid compounds identified in selected extracts of D. trifasciata leaves

Flavonoids are group of compounds strongly related to antioxidant activity. In this study, the content of flavonoids was identified and quantified in selected extracts of *D. trifasciata leaves* by HPLC. Based on previous study, kaempferol and rutin were identified in ethanol and methanol extracts of *Sansevieria trifasciata*.[56,57] Therefore, in this study, kaempferol and rutin identification was conducted in selected extracts, namely MC1 (96% ethanol extract) and MC2 (70% ethanol extract) of the leaves of *D. trifasciata* var. mediocipta cobra. These two extracts were selected because the MC1 extract exhibited the highest antioxidant activity according to the CUPRAC and DPPH methods, while the MC2 extract showed the highest antioxidant activity based on the FRAP method. According to the chromatograms in Figures 1, 2, and 3, by comparing the retention time on the chromatogram of the sample it was observed that the two selected extracts (MC1 and MC2) contained components with the same retention time as rutin and kaempferol. Therefore, it can be concluded that both extracts contain rutin and kaempferol. By comparing the area under the curve, rutin and kaempferol contents of the extracts were evaluated (Table 12). The contents of rutin and kaempferol in the MC1 extract were 0.026 ± 0.002 mg/g and 0.020 ± 0.002 mg/g, respectively, while in the MC2 extract they were 0.071 ± 0.002 mg/g and 0.052 ± 0.004 mg/g, respectively.

Table 9: Correlation of antioxidant activity of *Dracaena trifasciata* varieties using DPPH and CUPRAC methods

Antioxidant Parameter	Pearson Correlation Coefficient (r)							
	CUPRAC GB1	CUPRAC MB1	CUPRAC BM1	CUPRAC MC1	CUPRAC GB2	CUPRAC MB2	CUPRAC BM2	CUPRAC MC 2
DPPH GB1	0.954****							
DPPH MB1		0.880***						
DPPH BM1			0.803***					
DPPH MC1				0.886***				
DPPH GB2					0.967****			
DPPH MB2						0.974****		
DPPH BM2							0.904****	
DPPH MC2								0.931****

*** = strong correlation, **** = very strong correlation.
GB1: Gold flame 96% ethanol extract, MB1: Moonshine Brazilian 96% ethanol extract, BM1: Banded nelson 96% ethanol extract, MC1: mediocipta cobra 96% ethanol extract, GB2: Gold flame 70% ethanol extract, MB2: Moonshine Brazilian 70% ethanol extract, BM2: Banded nelson 70% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

Table 10: Correlation of antioxidant activity of *Dracaena trifasciata* varieties using DPPH and FRAP methods

Antioxidant Parameter	Pearson Correlation Coefficient (r)							
	FRAP GB1	FRAP MB1	FRAP BM1	FRAP MC1	FRAP GB2	FRAP MB2	FRAP BM2	FRAP MC2
DPPH GB1	0.954****							
DPPH MB1		0.986****						
DPPH BM1			0.782***					
DPPH MC1				0.978****				
DPPH GB2					0.827***			
DPPH MB2						0.925****		
DPPH BM2							0.972****	
DPPH MC2								0.797***

*** = strong correlation, **** = very strong correlation.
GB1: Gold flame 96% ethanol extract, MB1: Moonshine Brazilian 96% ethanol extract, BM1: Banded nelson 96% ethanol extract, MC1: mediocipta cobra 96% ethanol extract, GB2: Gold flame 70% ethanol extract, MB2: Moonshine Brazilian 70% ethanol extract, BM2: Banded nelson 70% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

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Table 11: Correlation of antioxidant activity of *D. trifasciata* varieties using CUPRAC and FRAP methods

Antioxidant Parameter	Pearson Correlation Coefficient (r)							
	FRAP GB1	FRAP MB1	FRAP BM1	FRAP MC1	FRAP GB2	FRAP MB2	FRAP BM2	FRAP MC2
CUPRAC GB1	0.971****							
CUPRAC MB1		0.936****						
CUPRAC BM1			0.971****					
CUPRAC MC1				0.952****				
CUPRAC GB2					0.876***			
CUPRAC MB2						0.888***		
CUPRAC BM2							0.865***	
CUPRAC MC2								0.905****

*** = strong correlation, **** = very strong correlation.
GB1: Gold flame 96% ethanol extract, MB1: Moonshine Brazilian 96% ethanol extract, BM1: Banded nelson 96% ethanol extract, MC1: mediocipta cobra 96% ethanol extract, GB2: Gold flame 70% ethanol extract, MB2: Moonshine Brazilian 70% ethanol extract, BM2: Banded nelson 70% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

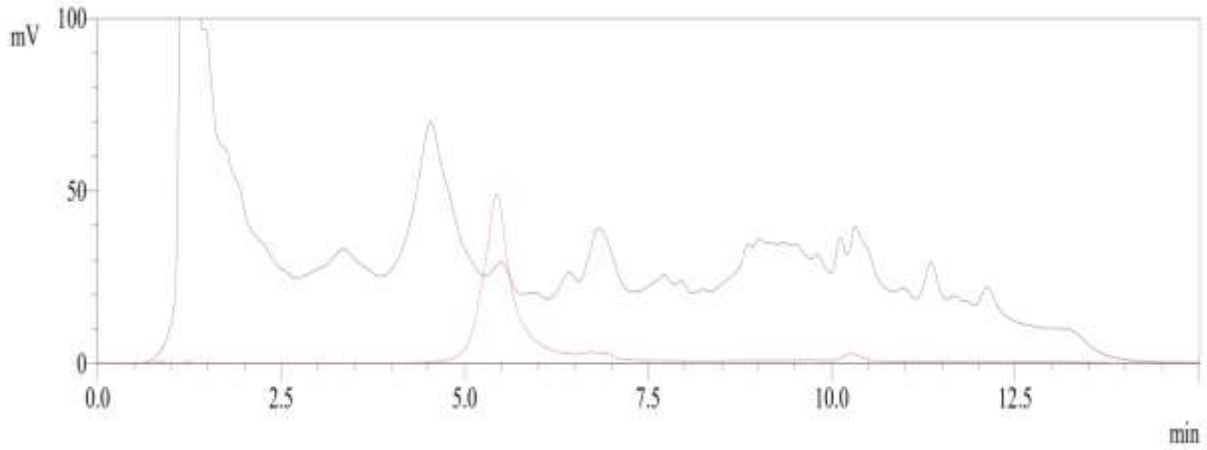

Figure 1: HPLC chromatogram of 96% ethanol extract of *Dracaena trifasciata* var. mediopicta cobra leaves and rutin; black line: extract, red line: rutin

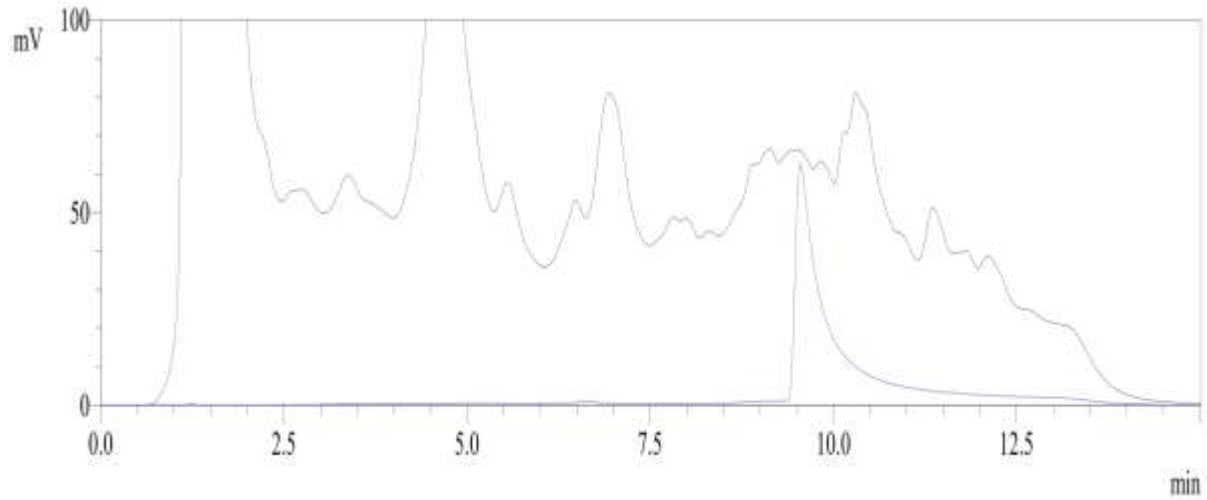

Figure 2: HPLC chromatogram of 96% ethanol extract of *Dracaena trifasciata* var. mediopicta cobra leaves and kaempferol; black line: extract, blue line: kaempferol

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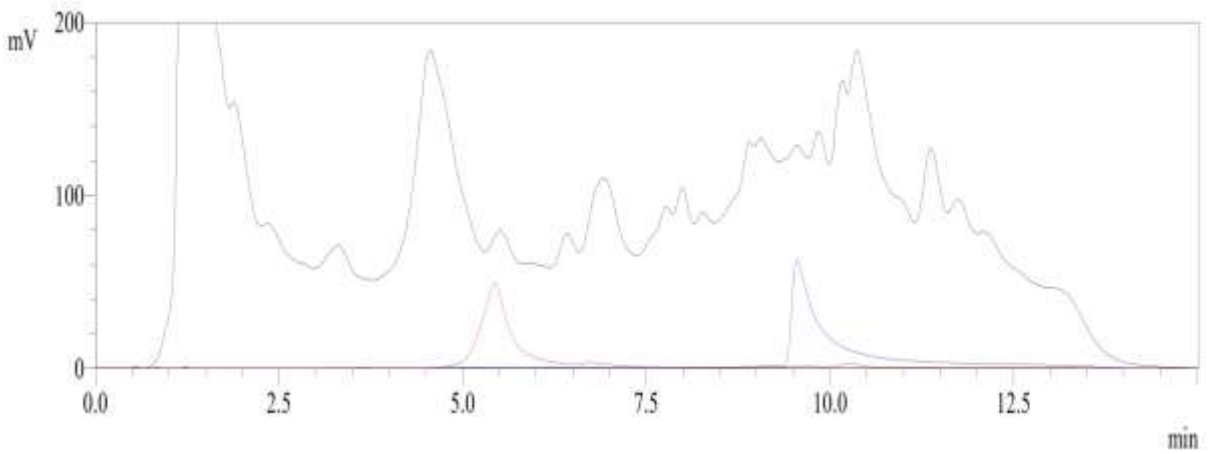

Figure 3: HPLC chromatogram of 70% ethanol extract of *Dracaena trifasciata* var. mediopicta cobra leaves, rutin, and kaempferol; black line: extract, red line: rutin, blue line: kaempferol

Table 12: Flavonoid compounds in *Dracaena trifasciata* var. mediopicta cobra leaves

Sample	Retention time (min)		AUC		Content (mg/g)
	Standard	Sample	Standard	Sample	
MC1	5.436 (rutin)	5.478	1496903	793047.33 ± 60974.01	0.026 ± 0.002
	9.552 (kaempferol)	9.526	2137028	872519.66 ± 93924.09	0.020 ± 0.002
MC2	5.436 (rutin)	5.510	1496903	2129804.33 ± 61336.01	0.071 ± 0.002
	9.552 (kaempferol)	9.554	2137028	2251218 ± 181519.19	0.052 ± 0.004

MC1: mediocipta cobra 96% ethanol extract, MC2: mediocipta cobra 70% ethanol extract.

Conclusion

In conclusion, the 96% ethanol and 70% ethanol leaf extracts of *D. trifasciata* var. mediopicta cobra showed higher antioxidant activity compared to the gold flame, moonshine Brazilian, and banded nelsoni varieties. Furthermore, the use of ethanol solvents with different degrees of polarity significantly influenced the TFC and TPC contents of the extracts. Generally, strong to very strong correlations were observed between TFC and TPC in the leaf extracts of four varieties of *D. trifasciata* and their antioxidant activity. This suggests that flavonoid and phenolic compounds significantly contribute to the antioxidant properties of these extracts. Rutin and kaempferol were found in 96% ethanol and 70% ethanol leaves extracts of *D. trifasciata* var. mediopicta cobra. Based on the findings from this study, all four varieties of *D. trifasciata* have the potential to be developed as sources of natural antioxidants, which can be used in food and nutraceutical products.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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