

Antioxidant Activity of Different Plant Part Extracts of *Dracaena umbratica* Ridl

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Abstract: Medicinal plants contain phytochemicals which show various significant biological and pharmacological activities. In this study, the hexane, dichloromethane and methanol extracts from different parts (leaves, rhizomes and roots) of a local medicinal plant, *Dracaena umbratica*, Ridl (Agavaceae) were evaluated for antioxidative activity using ferric thiocyanate (FTC) method and also DPPH (1, 1 – diphenyl – 2 – picrylhydrazyl) radical scavenging assay. The results showed that all the extracts exhibited weak radical scavenging activity and differ significantly from the standard antioxidants which are vitamin C and butylated hydroxytoluene (BHT). However results from the ferric thiocyanate (FTC) method showed that all the extracts except that of hexane and dichloromethane extracts of the rhizome of this plant possess high antioxidative property which was comparable to the standard reference, BHT. The antioxidative activity of these extracts did not differ significantly ($P < 0.05$) from BHT. These results suggest that this plant species may have low content of radical scavenging compounds but can still be developed as potential antioxidant.

Key words: *Dracaena umbratica* • Radical scavenging • Antioxidant • DPPH • Ferric thiocyanate (FTC)

INTRODUCTION

Medicinal plants provide a variety of medicinal values, such as antioxidant, anti inflammatory, anticancer and antifungal. Antioxidants are a group of substances which can delay oxidative processes while often being oxidized themselves. The presence of excess oxygen radicals can cause damage in human cells and thus lead to several diseases such as heart disease, arteriosclerosis, diabetes, cancer and neurodegenerative diseases (Parkinson's and Alzheimer's disease). Plants have many phytochemicals which are potential sources of natural antioxidants, e.g. vitamin C, E, carotenoids, flavonoids, tannins and phenolic acids [1-3].

Dracaenas are a group of foliage plants that come from the family of Agavaceae (Liliaceae). Most of the *Dracaena* species are distributed in the tropic and subtropic dry climate regions throughout the world [4]. The many varieties of *Dracaena* species are either serve as ornamental or are known to possess medicinal values. The many species and varieties of *Dracaena* includes *D. cinnabari*, =*D. draco*, *D. deremensis*, =*D. fragrans*, *D. marginata*, *D. augustifolia* and *D. loureiri*. Burkill [4] listed a number of *Dracaena* species that can be found in

the Peninsular of Malaysian and that are known to possess medicinal value. Among them are *D. congesta* (jenjuang, senjuang) and *D. porteri* (also known as Elephant-medicine) which were traditionally used to treat rheumatism and ulcer.

A number of researches have been carried out on *Dracaena* to study the phytochemistry of the various species. Ionikawa *et al.* [5] had carried biological evaluation on methanol extract from the stem wood of *D. loureiri*. The extract was found to exhibit significant capacity to inhibit [³H] -estradiol binding to estrogen receptor. Masaoud *et al.* [6-8] investigated the chemical components of *D. cinnabari*. In their work, they were able to isolate and identify a number of flavonoids, the new biflavonoid cinnbarone as well as a series of sterols and triterpenoids in the resin and roots of *D. cinnabari*. Vesela *et al.* [9] succeeded in isolating dracophane, a novel structural derivative of metacyclopheane from the resin of *D. cinnabari* Balf. The structure of the compound was identified by spectroscopic methods to be 3,12,21-trihydroxy-1,10,19-tris(4-hydroxyphenyl)-5,14,23-trimethoxy [3.3.3] metacyclopheane. Machala *et al.* [10] tested a series of homoisoflavonoids and chalcones isolated from *D. cinnabari* Balf for their potential to

inhibit cytochrome P4501A(CYP1A) enzymes and Fe-enhanced in vitro peroxidation of microsomal lipids in mouse liver. The chalcones show poor antioxidative property but one of the homoisoflavonoids, 7,8-methylenedioxy-3(4-hydroxybenzyl) chrome exhibited strong antioxidant activity comparable to that of the strongest flavanol antioxidant, quercetin. Gonzalez *et al.* [11] isolated three new compounds namely, draconins A-C along with 17 other known compounds from the stem bark of *D. draco*. Several of the isolated compounds showed potent cytotoxic activities measured on the human leukemia cell line HL-60. Zheng *et al.* [12] had isolated new spirostanols (dracaenogenins A and B) from the red resin of *D. cochinchinensis* while steroidal saponins were detected in the fresh stem [13].

D. umbratica is a low, little-branched shrub found in the Malay Peninsula very plentifully in the lowland forests. A decoction of the roots is given for rheumatism [4]. To our knowledge and also based on the literature review of previous studies done on *Dracaena* species, there is no report on the scientific study on *D. umbratica*. Therefore the current research is carried out with the objectives of extracting the different parts (leaves, roots and rhizome) of *D. umbratica* plant using organic solvents; hexane, dichloromethane and methanol and assessing the antioxidative of all of these extracts using DPPH radical scavenging and ferric thiocyanate (FTC) methods.

MATERIALS AND METHODS

Plant Material: The plant materials (*D. umbratica*) were collected from the area of Ulu Pauh in Perlis, Malaysia. A voucher specimen was earlier prepared and identified by a botanist in Universiti Putra Malaysia.

Chemicals: Linoleic acid and DPPH were purchased from Sigma Chemical Co. (USA). All reagents and solvents used were of analytical grade.

Extraction of the Plant Material: The fresh leaves of *D. umbratica* (6 kg) were washed using tap water and air-dried at room temperature for about 1 week. After 1 week, the leaves were then dried in the oven which was set at temperature 45°C overnight. The dried leaves were then chopped into small pieces and ground using blender to form powder. The powder leaves was successively extracted with solvents of increasing polarity which were hexane, dichloromethane and methanol. The extraction was done by soaking the leaves sample in each

solvent for 4 days. At the end of the fourth day, the sample was filtered and for each solvent, the soaking was repeated twice using fresh volume each time. The filtrates of the same solvent obtained from the soaking were then combined and dried under reduced pressure using rotatory evaporator. The yield of the dried extracts obtained was 33.1 g of hexane extract, 34.2 g of dichloromethane extract and 30.0 g of methanol extract. The rhizomes and the roots of *D. umbratica* were also air-dried and cut into small pieces. Both the rhizomes and the roots were successively extracted with hexane, dichloromethane and methanol. The solvent was evaporated off from the extracts under reduced pressure using rotatory evaporator. The yields of dried extracts for the rhizome were 0.229 g of hexane extract, 0.316 g of dichloromethane extract and 2.6 g of methanol extract. The yields obtained from the roots were 0.139 g of hexane extract, 0.04 g of dichloromethane extract and 2.3 g of the methanol extract.

Evaluation of Antioxidant Activity

Preparation of Sample Extracts: The sample extracts (from leaves, rhizomes and roots of *D. umbratica*) were dried in the oven at 40°C - 50°C for about 5 days. The samples were then weighed separately and dissolved in methanol to prepare stock solutions of the extracts with concentration of 10 mg/ml. The hexane and some of the dichloromethane extracts from different parts of *D. umbratica* were not that readily soluble in methanol. These samples solutions were placed in the ultrasonic bath for 5-10 minutes to facilitate the samples dissolve in methanol.

DPPH Radical Scavenging Assay: Radical scavenging activity of plant extracts against stable DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate; Sigma Aldrich) radical was determined spectrometrically. The color changes (from deep violet to light yellow) were measured at 515 nm on a spectrophotometer. Experiments were carried out according to the method of Brand-Williams *et al.* [14] with slight modification. 3.9 ml of recently prepared 60 µM DPPH in methanol was mixed with 0.1 ml sample solution (1mg/mL) in methanol in a vial. The vial was covered and placed (incubated) in the oven at 37°C for 30 minutes. Finally, after 30 minutes, the absorbance was measured at 515 nm. Decrease in the absorbance of DPPH solution indicated an increase in its radical scavenging activity. This activity was expressed as percentage DPPH radical scavenging that was calculated from the following equation:

$$\text{Radical Scavenging activity (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

The DPPH solution without sample solution was used as a control. Vitamin C and BHT were used as the reference standard. All determinations were performed in triplicate.

$$\text{Amount (mg)} = \frac{\text{M.Wt} \times \text{Conc. (mM)} \times \text{Volume (ml)}}{1000}$$

Ferric Thiocyanate (FTC) Method: The method of Kikukazi and Nakatani [15] was slightly modified. A screw-cap vial containing a mixture of 4 mg sample in 4 ml of methanol (final concentration 0.02%), 4.1 ml of 2.5% linoleic acid (Sigma, USA) in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in the dark at 40°C. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely three minutes after addition of 0.1 ml of 0.02 M ferrous chloride in 3.5 % hydrochloric acid to the reaction mixture, the absorbance of the resulting red colour was measured at 500 nm every 24 hours until the day after the absorbance of the control reached maximum value. The control and the standard were subjected to the same procedures as the samples except that for the control, only the solvent was added. Butylated hydroxy toluene (BHT) was used as the standard antioxidant. All determinations on the samples were recorded in triplicate.

Statistical Analysis: All experiments were conducted in triplicate and statistical analysis was done using SPSS software. To determine whether there was any difference between activities of the samples, analysis of variance was performed by the ANOVA procedure. Bonferroni multiple comparisons were used to determine significant differences between the means. Values of $p < 0.05$ were considered as significantly different ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The antioxidant activity of the hexane, dichloromethane and methanol extracts from the leaves, rhizomes and the roots of *D. umbratica* was assessed by using two different methods which are the DPPH radical scavenging assay and the ferric thiocyanate (FTC) method.

DPPH Radical Scavenging Activities of the Various Extracts from Different parts of *D. umbratica*: The 1,1-diphenyl -2- picrylhydrazyl (DPPH) radical is a stable radical at room temperature with a maximum absorption at 517 nm. It produces a violet solution in ethanol. It can readily undergo reduction by an oxidant molecule, giving rise to uncoloured ethanol solution [16]. The mechanism involves the donation of hydrogen ions to DPPH radical by the antioxidant molecule which leads to the reduction of DPPH radical into a stable molecule.

The DPPH radical scavenging experiments using photometric measurements were carried out according to the method of Brand-William *et al.* [14] with slight modification. 3.9 ml of recently prepared 60 µM DPPH in methanol was mixed with 0.1 ml of sample solution in methanol. Finally, after 30 minutes, the absorbance was measured at 517 nm. Decrease in absorbance of DPPH solution indicated an increase in its radical scavenging activity. This activity was expressed as percentage DPPH radical scavenging that was calculated from the following equation:

$$\text{Radical Scavenging activity (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

The DPPH solution without sample solution was used as a control. The synthetic antioxidant, butylated hydroxytoluene (BHT) and the natural antioxidant, vitamin C were used as the reference standards. The DPPH assay was run three times altogether, each time with triplicates for each sample. The measured radical scavenging activity was analyzed statistically. The result of the DPPH radical scavenging activity (%) of the various extracts from the leaves, rhizomes and roots of *D. umbratica* is shown in Figure 1 below.

The highest radical scavenging activity (96%) is shown by the reference standard which is vitamin C followed by BHT (78%). All the nine extracts *D. umbratica* however showed a significantly weak radical scavenging activity compared to the reference standards ($p < 0.05$), which are the vitamin C and BHT. The weak radical scavenging activity could indicate that all of these extracts contained a lower amount or weak radical scavenging compounds.

Of all the extracts, the dichloromethane extracts of the leaves showed the highest percentage of radical scavenging activity (21.3%) while the methanol extract of the root showed the weakest activity (2.9%).

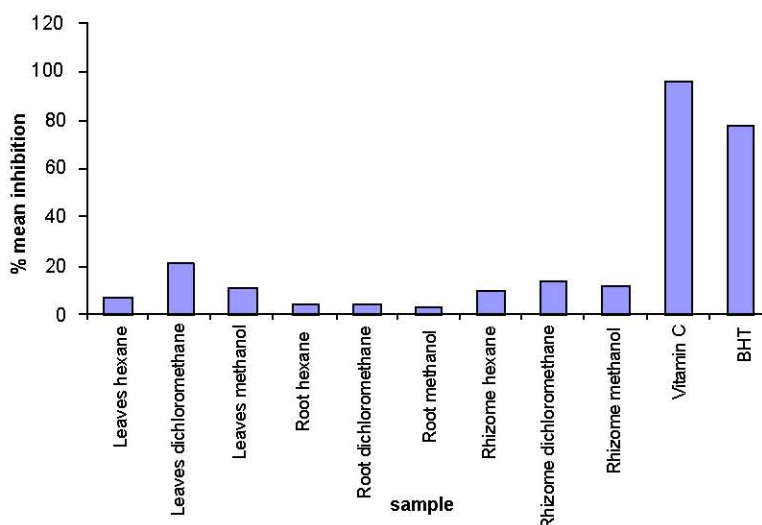


Fig. 1: Radical scavenging activity (% mean inhibition) of various extracts from the rhizome, root and leaf of *D. umbratica*

Mechanism involves in the reduction of DPPH radical is based on the scavenging activity. The availability of the hydroxyl group, the structure (both planar and spatial) of the compound is important for its capacity of donating hydrogen ions. Compounds that are able to donate hydrogen are derived from the shikimate pathways, as for example, flavonoids [17, 16].

Radical scavenging activity is usually associated to the presence of phenolic compounds amongst which includes the phenolic acids, flavanoids, tannins, coumarins, lignans, quinones, stilbenes and curcuminoids. The variation of antioxidant /radical scavenging activity of medicinal plants is due to types of natural phenolic compounds and their varying levels and also the different molecular structural features of those compounds [18,19]. Structural radical scavenging activity relationships of phenolic compounds had been investigated by several researchers [18, 20, 21]. Cai *et al.* [18] had investigated structure-radical scavenging activity phenolic compounds from traditional Chinese medicinal plants. Different categories of tested phenolic showed significant mean differences in radical scavenging activities using DPPH and ABTS[•]. Tannins demonstrated the strongest activity, while most quinones, isoflavones and lignans showed the weakest activity. Sequence of activity: Tannins > flavanols > chalcones > stilbenes and curcuminoids > hydroxy cinnamic acid > flavones > hydroxy benzoic acids > quinones > flavanones > isoflavones. The differences in radical scavenging activity were attributed to structural differences in hydroxylation,

glycosylation and methoxylation. The orthodihydroxy groups were the most important structural feature of high activity for all tested phenolic compounds. The flavonoids without any hydroxyl group have no radical scavenging capacity. Plants showing low radical scavenging activity but able to prevent oxidation of lipid are very rich in compound of the acetate pathway, like the terpenoids and fatty acids, which are unable to scavenge DPPH radical, but are able to avoid oxidative damage of cell membrane [16, 22].

Plants extracts that scavenge the DPPH radical and protect against oxidative damage are probably rich in both compounds, encompassing both pathways [18].

The Antioxidant Activity of the Extract Measured Using Ferric Thiocyanate (FTC) Method: Lipid peroxidation has been defined as the biological damage caused by free radical that formed under oxidative stress. The inhibitions of activities against lipid peroxidation in linoleic acid can be evaluated by FTC method. According to this method the amount of peroxide formed in the primary stage of linoleic acid oxidation were measured by the reaction with ferrous chloride. The peroxides will oxidize ferrous (Fe^{2+}) to ferric (Fe^{3+}) ion. The ferric ion will then react with ammonium thiocyanate to produce red colored complex, ferric thiocyanate which has a maximum absorbance at 500 nm. The thicker the color, the higher will be the absorbance. High absorbance values is an indication of high concentration of peroxides formed during the linoleic oxidation, thus indicates low levels

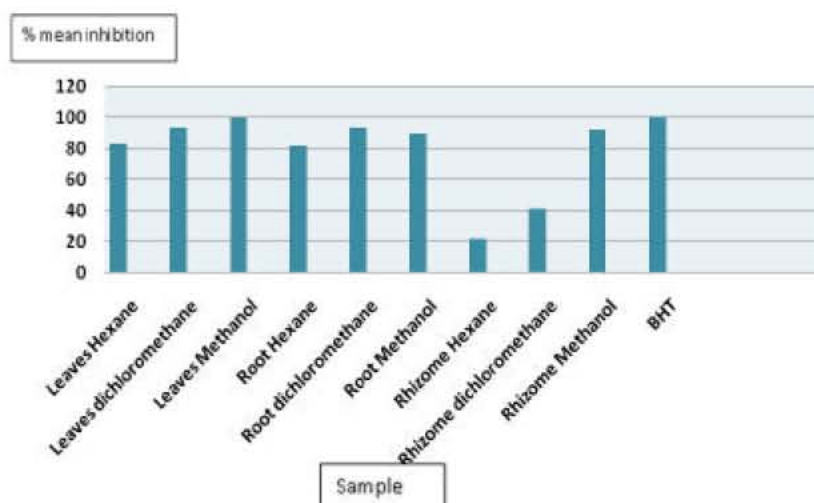


Fig. 2: Antioxidant activity (% mean inhibition) of the various extracts from the roots, rhizomes and leaves of *D. umbratica*

of antioxidative activities [23, 1]. In performing the FTC assay/method, the synthetic antioxidant butylated hydroxytoluene (BHT) was used as the reference standards and assigned as 100 % inhibition. The FTC reagents without the sample were used as the control. The inhibition of lipid peroxidation as a percentage was calculated according to the following equation and the absorbance values were those obtained on the day when the absorbance of control reached maximum value:

$$\% \text{Inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance} - \text{reference (EHT) absorbance}} \times 100$$

Several sets of experiments, each with triplicate were carried out to measure the antioxidant activity of the extracts using Ferric thiocyanate (FTC) method and statistical analysis using ANOVA was performed on the results.

Figure 2 showed the result on the antioxidant activity of the various extracts from the rhizomes, root and leaves of *D. umbratica*. The percentage mean inhibition referred to the total antioxidant activity of the samples (the extracts). From Figure 2 and the statistical analysis, all of these extracts except that of hexane and dichloromethane extracts of the rhizome, showed no significant difference ($p > 0.05$) in term of their antioxidative activity from the standard reference, butylated hydroxytoluene (BHT). In other word, the antioxidant activity of these extracts is comparable to the standard antioxidant, BHT. This result proved to be

very interesting, because the same extracts had been shown to have weak radical scavenging activity from the DPPH radical scavenging assay that we had conducted earlier.

Nevertheless, all these extracts except that for the dichloromethane extract of the rhizome seemed to contain some active components which were able to protect the lipid cell membrane from oxidative damage and the mechanism of their antioxidant property is not that of radical scavenging. The antioxidant activities of antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging [24].

CONCLUSION

All the extracts from the leaves, rhizomes and roots of *D. umbratica* showed high antioxidative property based on the assay using ferric thiocyanate method. The radical scavenging property of this plant however is quite weak indicating the absence or the presence of low content of radical scavenging compounds such as the phenolic compounds and flavonoids.

As this plant possesses strong antioxidant property and had never been reported in any scientific literature before, so it is worthwhile to explore into the active components contained in the various parts of the extracts of this plant such as dichloromethane and the methanol extracts of its leaves and roots in the near future.

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