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Antioxidant Studies and Phytochemical Screening of the Medicinal Fern *Dicranopteris linearis* Extracts

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Abstract

Methanolic extracts of *Dicranopteris linearis* leaves, stems and roots were evaluated for antioxidant activity using DPPH (2,2-diphenyl-1picrylhydrazyl) radical scavenging assay. Extracts were also investigated for their total phenolic and flavonoid contents, whereas the presence of phytochemical constituents was investigated by phytochemical screening. Obtained results revealed that the root extract exhibited the highest DPPH scavenging activity of 86.14 ± 0.02%, followed by leaves 65.67 ± 0.01% and stems 61.51 ± 0.02% at a concentration of 1 mg/mL compared to butylated hydroxytoluene (BHT) 88.95 ± 0.01% at same concentration. The LC_{50} values for the roots, leaves, stems and BHT were 0.30, 0.65, 0.79 and 0.17 mg/mL respectively. Total flavonoid content of leaves extract possessed the highest of 52.19 mgCEQ/g whereas root and stem extracts were 47.8 ± 0.03 and 30.14 ± 0.02 mgCEQ/g respectively. Total phenolic contents were 266.39 ± 0.04 for leaves, 261.62 ± 0.07 for roots and 194.50 ± 0.03 mgGAE/g for stems. Phytochemical screening of all extracts revealed the presence of flavonoids, phenols, tannins, saponins, steroid, triterpenoid and reducing sugars. Results suggest that *D. linearis* extracts possess antioxidant potential and various phytochemical groups. Further study is needed for the isolation and purification of the bioactive compounds in extracts, which may serve as leads in the development of new pharmaceuticals.

Keywords: *Dicranopteris linearis; Antioxidant Activity; Phytochemical Screening*

Introduction

Oxidative stress causes many disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injuries of many tissues, central nervous system injury, gastritis, cancer and AIDS [1,2], diabetes [3], cataracts [4], muscular degeneration [5], gastrointestinal inflammatory diseases [6], cardiovascular disease [7], neurodegenerative and Alzheimer's diseases [8,9]. Antioxidants are beneficial substances, which significantly delay or prevent the oxidation of free radicals even at low concentrations [10]. Antioxidants are also well known in reduce the risk for chronic diseases including cancer, heart disease and in the aging process. Some of the scientific evidences suggest the antioxidant compound's role as a health-protecting factor [11-13].

On the other hand, *in vivo* studies suggest the suppression of free radicals is efficient to eliminate the oxidative stress related pathophysiology changes in the human. Therefore many researchers have focused on finding novel antioxidants, in particular from natural sources such as edible and medicinal plants to prevent the free radicals related disorders in human. Furthermore, antioxidants from natural sources are thought to be safer than the synthetic compounds, avoiding the probable carcinogenic and harmful effects of the latter [14].

Plants are the potential of natural antioxidants, which are present as their secondary metabolites [15]. Furthermore, it has been reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids and tannins [16]. The presence of flavonoids and phenols in leaves, flowering tissue and woody parts such as stems and barks, shows the importance of these compounds for normal growth in plants and defense against infections and injuries [17].

Pteridophytes, which constitute ferns and fern allies are of great medicinal values. In addition, they are usually not infected by pathogenic microbes. This could be one of the important reasons for the evolutionary success of pteridophytes and the fact that, ferns have survived for more than 350 million years [18]. In the past, the ferns have been widely used in traditional medicine practice. The most common traditional use of ferns is to treat skin problem and wounds, fever, cough, reproductive problems and as insect repellent [19-21]. The ethnobotanical importance has also been investigated and studied by various researchers. However, there are not many reports available on the biological activity of the ferns [22,23]. Although, not much consideration has been given towards the utility of ferns, they do have medicinal values. The pharmacological activities of fern extracts such as antiviral, antipyretic, anti-inflammatory, antibacterial and jaundice have been demonstrated by several studies [24-27]. *Dicranopteris linearis* is one of the popular ferns commonly found in secondary forests and grows well in poor clay soils. In Malaysia, it is known as 'resam' and is traditionally used to decrease body temperature and control fever. In other parts of the world, it is used to treat asthma, woman's sterility, external wound, ulcers, boils and get rid of intestinal worms. A study by Zakaria., *et al*. [28] has revealed that *D. linearis* leaves possess antinociceptive, anti-inflammatory and antipyretic effects. Another study demonstrated that *D. linearis* leaves possess antioxidant and anticancer activities but showing no cytotoxic effect against normal cells [29].

The present study was undertaken to investigate the antioxidant activity in different parts of *D. linearis* extract.

Material and Methods

Plant collection

The fresh plant material was obtained from Bukit Jambul area in Penang, Malaysia, during April 2011. A voucher specimen (No 11297) has been deposited at the Herbarium of the School of Biological Sciences, University Sains Malaysia. The plant parts were separately rinsed with tap water, air-dried for 1 - 2 weeks at room temperature (27 ± 3°C) and then dried in the oven at 60°C for one to two days. The dried parts were ground to a fine powder and stored in clean air-tight bottles.

Preparation of the plant extracts

The extraction process was carried out by maceration using 100g of the plant materials, soaked in 500 mL methanol for 14 days with daily agitation. The extracts were collected and filtered twice using Whatman No.1 filter paper. The filtrate was then concentrated by rotary evaporation under reduced pressure at 40°C and further dried in the oven at 60°C. The resulting extract in the form of paste was stored until further use. The percentage yields of the crude extracts were 18.4%, 7.4% and 6.28% for leaves, stems and roots respectively.

DPPH (2, 2-diphyenyl-1-picrylhydrazyl) assay

Quantitative measurement of radical scavenging activity was carried out according to the method described by Blois [30] and Bondet., *et al* [31]. The test was conducted in triplicate. Fifty µL of various concentrations of the extract solutions ranging from 2 - 0.03 mg/mL was added to 5 mL of 0.004% DPPH in 80% methanol. The mixtures were vortex mixed and left in the dark for 30 minutes at room temperature before reading the absorbance at 517 nm. Methanol was used as the blank and therefore the base line (A_0) contained methanol instead of sample extract. Butylated hydroxytoluene (BHT) was the positive control, used for comparison. The percentage radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{(A0-A1)}{A_0} \times 100$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the solution involving the sample (root extract). The IC_{50} (concentration of the extract scavenge 50% of DPPH radical) was calculated graphically using a calibration curve in the linear range.

Determination of total phenolic content

Total phenolic content was evaluated with Folin-Ciocateu's phenol reagent [32]. Extracts of 200 µL at concentration of 1 mg/mL were mixed with 500 µL 10% Folin-Ciocalteu reagent, 500 µL of distilled water and 800 µL of 7.5% saturated aqueous Na_2CO_3. The mixtures were mixed well and allowed to stand in the dark for 30 minutes before the absorbance were measured at 765 nm using a spectrophotometer. Distilled water was used as the blank and various concentrations of gallic acid ranging from 0 - 250 mg/L were used to produce a standard calibration curve. Samples were analyzed in three replications. Total phenolic content of the root extract was calculated using the following formula:

$$\text{Total phenolic content (TPC)} = \frac{\text{GAE (mg/g) x V (ml)}}{\text{W (g)}}$$

where GAE is gallic acid equivalence (concentration of gallic acid was established from the calibration curve Y= 0.0102X + 0.0311, R^2 =0.9934), V is the volume of used extract and W is the weight of the pure extract.

Determination of total flavonoid content

The total flavonoid content of *D. linearis* was determined by using aluminum chloride colorimetric method [33]. Extracts of 250 µL at concentration of 1 mg/mL were added to 1 mL of distilled water and mixed with 75 µL sodium nitrite solution (5% w/w $NaNO_2$). After 6 minutes of incubation, 75 µL of aluminium trichloride solution (10% $AlCl_3$) was added and allowed to stand for 6 minutes. Subsequently 1 mL sodium hydroxide solution (4% NaOH) was added to the mixture and immediately followed by the addition of water to bring the final volume to 2.5 mL. After mixing well, the mixture was made to stand for 15 minutes at room temperature. Absorbance of the pinkish colour solution was measured at 510 nm. Different concentrations of catechol ranging from 0 - 0.1 mg/mL were used as a standard compound for the quantification of total flavonoid.

Results were expressed as mg of catechol equivalence per gram of dry weight of extract (mgCE/g). All experiments were performed in triplicates.

$$\text{Total flavonoid content (TFC)} = \frac{\text{CEQ (mg/g) x V (ml)}}{\text{W (g)}}$$

where CEQ is the catechol equivalence (concentration of catechin solution was established from the calibration curve Y=12.714X + 0.0648, R^2 =0.9901), V is the volume of used extract and W is the weight of the pure extract.

Phytochemical screening

Standard procedures for phytochemical screening [34-36] were adopted to screen the methanolic extracts of *D. linearis* for the presence of alkaloids, amino acids, flavonoids, phenolic compounds, reducing sugars, saponins, steroids and triterpenoids and tannins.

Results

Figure 1 represents the percentages of DPPH radical scavenging activities. The scavenging effects of roots, leaves and stem extracts were 86.14 ± 0.02%, 65.67 ± 0.01% and 61.51 ± 0.02% respectively at concentration of 1 mg/mL, whilst the scavenging effect of BHT was 88.95 ± 0.01%.

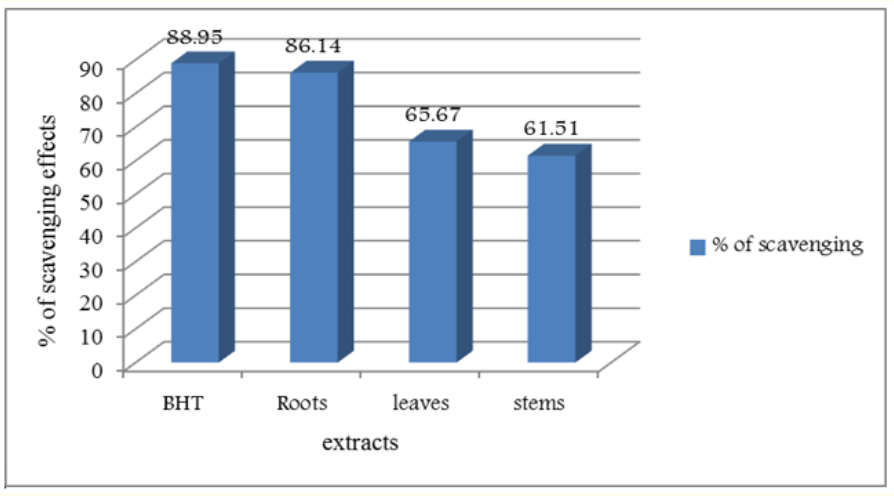

Figure 1: *DPPH scavenging (%) of extracts of D.linearis and the standard BHT at 1 mg/ml.*

The IC_{50} of the extract, as the amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%, was calculated using the calibration curve in the linear range (Figures 2 and 3). The curves of extract concentrations versus corresponding scavenging effects gave the IC_{50} values of 0.31, 0.65, 0.79 and 0.17 mg/mL for roots, leaves, stem extracts and BHT respectively. In generally comparison with BHT, *D. linearis* root extract possessed relatively good antioxidant activity. Furthermore, methanolic extract of roots had the highest antioxidant activity with the lowest IC_{50} compared to stems and leaves extracts.

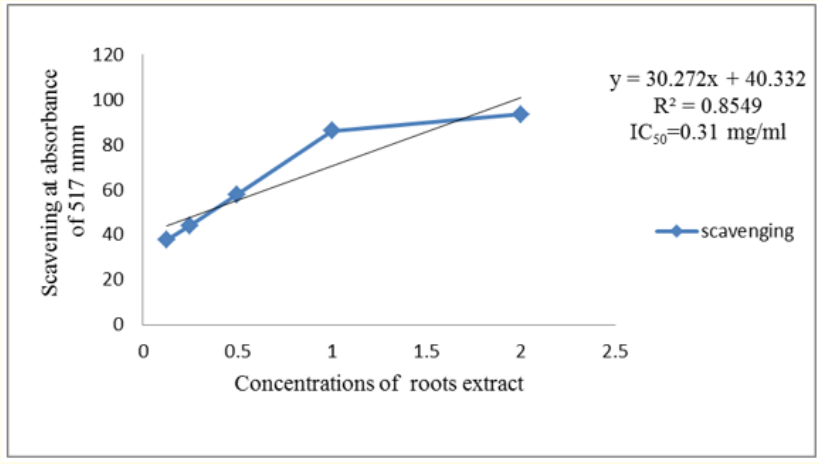

Figure 2: *IC_{50} values of root extract of D.linearis.*

Total phenolic content (TPC) of the extracts of *D. linearis* parts were found as 266.39 ± 0.04, 261.62 ± 0.17 and 194.50 ± 0.03 mgGAE/g for leaves, roots and stems respectively. The total flavonoid contents (TFC) were 52.19 ± 0.04, 47.39 ± 0.08 and 30.14 ± 0.02 mgCEQ/g for leaves, roots and stems extracts respectively.

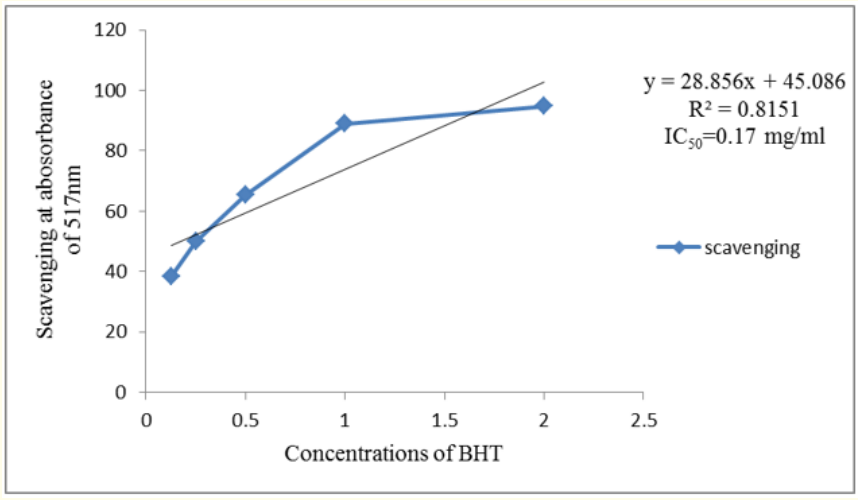

Figure 3: *IC_{50} values of commercial antioxidant BHT.*

Following various phytochemical screening assays in order to determine certain compounds, the present study also revealed the presence of important active secondary metabolites in the methanolic extracts of the different parts of *D. linearis.* The fundamental scientific bases for the use of *D. linearis* as a potential therapeutic plant could then be investigated by defining the presence of the constituents. All obtained results were mostly based on the colour changes of the reaction mixtures. The results summarized in table 1 revealed the presence of six major groups which were flavonoids, phenols, saponins, tannins, phenols, reducing sugars and steroids and triterpenoids, which are generally known to be biologically active.

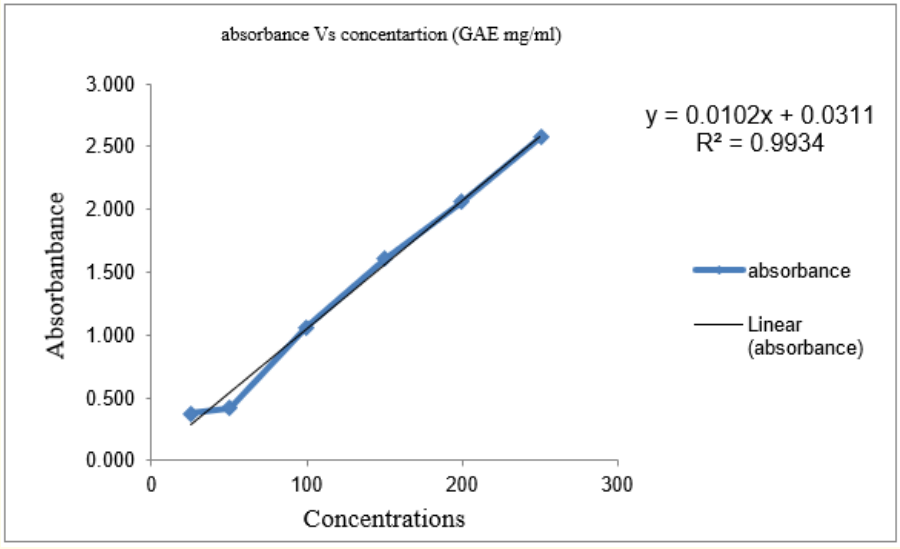

Figure 4: *Standard curve for determination of gallic acid equivalents for total phenolic content.*

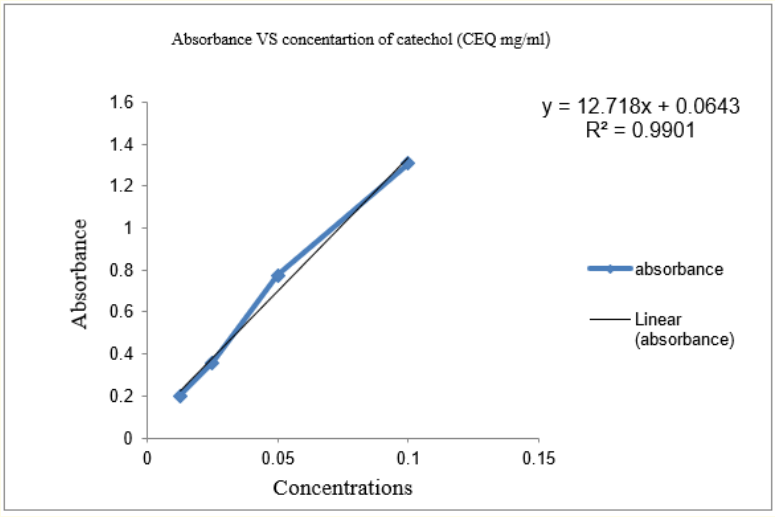

Figure 5: *Standard curve for determination of catechol equivalents for total flavonoid content.*

Phytochemicals	Leaves	Stems	Roots	Observation
Flavonois and Glycoside	+++	++	++	Pink, tomato, or red colours
Phenol	+	+	+	Bluish colour
Saponin	+	+	+	Persistent frothing
Tannin	++	+	+	Black precipitate
Reducing sugar	++	+++	+++	Brick red colour
Steroid and Triterpenoid	++	+++	++	Reddish violet ring
Alkaloid	_	_	_	Yellowish, orange brown, or reddish brown
Amino acid	_	_	_	Purple colour

Table 1: *Phytochemical screening of methanolic extracts of the different parts of D. linearis.*
+++: High (the strongest colour); ++: Moderate (mild colour); +: Slight (the weakest colour); -: Absent.

Discussion

The antioxidant activities in various plant parts are mainly contributed by their active compounds. The quantitative measurement by the decrease in absorbance was obtained as the DPPH radical receives an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule [37]. Basically, a higher radical scavenging is associated with a lower IC_{50}. Thus, methanolic extract of *D. linearis* roots possessed the highest antioxidant activity compared to stems and leaves extracts. Previous evaluation studies focused on antioxidant activity of *D. linearis* leaves extracts also reported high activities compared to the other tested plants [38,39].

Phenolic constituent is one of the major groups of compounds acting as the primary antioxidant or free terminator [40]. On the other hand, it has been recognized that flavonoids also show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of the flavonoids are via scavenging or chelating process [41]. Flavonoids have been correlated with the possible role of preventing several chronic diseases including oxidative stress [42]. In the recent years, the interests in phenolic

compounds specifically flavonoids and tannins, have increased because of their broad spectrum of chemical and diverse biological properties, which involve the antioxidant effect [19]. Leaves, roots and stems extracts of *D. linearis* in this study were considered to have high content of phenols, which may be due to many factors to partially justify the exceptionally high TPC in this fern. For example, the phenolic compounds were created by the plants in response to environmental stress [43], since phenolics are assumed to provide protection against UV-B damage cells by protecting DNA from dimerization and breakage [44,45]. Therefore the environment of high-mountain areas which are exposed to number of stress factors for instance low air temperature, decreased partial O_2 pressure, increased UV radiation and inappropriate water system have normally increased the accumulation of antioxidants such as flavonoids [45].

The different extracts were negatively correlated with their DPPH IC_{50} values. A strong negative correlation was found between the IC_{50} values of the extracts and their phenolic contents (C= -0.978) which was non-significant ($p > 0.05$), indicating that the TPC in the extract decreased with increasing IC_{50} values and vice versa. A non-significant ($p > 0.05$) and moderate negative correlation was found between the IC_{50} values of the extracts and their flavonoid content (C = -0.566). This indicates that the flavonoids (subgroups of phenolic compounds), had a lesser antioxidant activity than those of phenolic compounds. Thus, the antioxidant activity of *D. linearis* might be due to phenolic compounds rather than flavonoids. It has been mentioned previously that leafy part of plants contains various types of bioactive constituents [45].

In this study, the roots remain as the most active in free radical activity, which may be due to the antioxidant activity being exerted by another bioactive compound(s) which would not be present in the leaves or stems, or present at higher amount in the roots than leaves and stems. Furthermore, this finding may justify and support the traditional uses of the fern for the treatment of various diseases related to oxidative stress, in the same time providing and suggesting a new natural antioxidant from *D. linearis*.

Most of the phytochemicals classified as secondary metabolities are being produced mainly by different parts of the plant. Often their function in plant is unkown but some phytochemicals are believed to have structural defence mechanism against plant pathogens [37]. Plants generally also contain a wide variety of free radical scavenging molecules such as flavonoids, carotenoids, alkaloids, tannins, saponins and terpenoids which possess good antioxidant activities [46]. Often their function in plant is unkown but some phytochemicals are believed to have structural defence mechanism against plant pathogens [47]. Furthermore, they have potential application in human health care [48].

Conclusion

In the present study, the evaluation of antioxidant activity of the leaves, stems and roots of *D. linearis* was carried out spectrophotometrically. The findings concluded that all the plant parts possessed good radical scavenging potential and had high phenolic and flavonoid contents. Furthermore, phytochemical screening of these extracts revealed the presence of many constituents including phenolics and flavonoids. In conclusion, *D. linearis* can be considered for use as a natural antioxidant source and may positively serves as an alternative to synthetic antioxidants. Further evaluations and analysis, however, should be conducted to investigate the antioxidant activity *in vivo* and to isolate and purify the bioactive compounds responsible for the activity.

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