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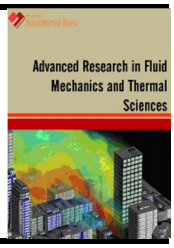

Antioxidant Assay of *Alstonia Angustifolia* Ethanolic Leaf Extract

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ABSTRACT

In current study, the ability of the ethanolic extract of *Alstonia angustifolia* in scavenging free radicals was assessed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and hydrogen peroxide (H_2O_2) radical scavenging assay. The results suggested that the ethanolic extract of *A. angustifolia* leaves has a notable antioxidant activity. In FRAP assay, it showed that the extract have higher total antioxidant activity with FRAP value is 1868.33 µM/g Fe (ii) dry mass ± 0.15 than the control, quercetin with FRAP value is 1336.9 µM/g Fe (II) dry mass ± 0.12 and ascorbic acid with FRAP value is 1720 µM/g Fe (II) dry mass ± 0.02. For DPPH assay, the IC_{50} value of the extract is 384.77 while the IC_{50} value of standards of ascorbic acid and quercetin are 18.07 µg/ml and 39.60 µg/ml, respectively. For H_2O_2 scavenging assay, the IC_{50} value for the extract was discovered to be 186.77 µg/ml compared to standard ascorbic acid 466.56 µg/ml. Thus, the study suggests that *A. angustifolia* ethanolic leaf extract has a good origin of natural antioxidants and might be beneficial in impeding the oxidative stress progression thus averting diseases that related to free radicals.

1. Introduction

Free radicals have a major contribution in many disease conditions and the role has been well renowned. Reactive derivatives of oxygen that exist inside the human body which generally called as reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical (•OH) and superoxide radical (O_2•) are continuously produced from contact with foreign chemicals in an ambient environment and/or due to a number of local metabolic processes that utilizes redox enzymes. Normally, the existence of antioxidants in the body helps in diminishing the ROS produced in humans and there is a balance between the ROS produced and the antioxidants available. However, this balance is interrupted by the overproduction of the ROS or scanty

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antioxidant defense which favoring the ROS increase and end up in oxidative stress [14]. Oxidative stress is correlated with pathogenesis process of several diseases such as diabetes, neurodegenerative diseases, atherosclerosis, cancer, and inflammatory diseases, peptic ulcer, as well as aging processes.

Most commonly known reservoir of natural antioxidants are plants, which includes polyphenols, ascorbate, tocopherols, and terpenoids [3]. To provide the body with a constant supply of antioxidants through dietary supplements is essential although the body has effective defense mechanisms that protect it against oxidative stress because the ability of these defensive mechanisms decreases as one ages [4]. Several biomolecules such as proteins, lipoproteins, lipids and DNA are easily damaged due to the actions of ROS [12]. A research work have discovered that the consumption of antioxidants, such as vitamin C, lowering the chances of cardiovascular diseases, cancers [16] and neurodegenerative diseases [18].

Alstonia angustifolia is usually found in Peninsular Malaysia and known as Pokok Pulai locally and belongs to genus Alstonia and Apocyanacease family. In Peninsular Malaysia, this plant are predominantly found in secondary and primary forests from sea level to about 3000 m altitude, as well as in swampy areas and usually employed in traditional medicine, for instance, for the cure of dysentery and malaria [10,13].

2. Materials and Method

A. Plant Sample and Extracts Preparation

Raw *A. angustifolia* leaves were procured from the Rimba Ilmu, Institute of Biological Sciences, University of Malaya, Kuala Lumpur. The fresh samples were cleaned with distilled water and undergone the drying process in drying oven (FED 53, BINDER, USA) at 50°C for five days followed by grinding process using an electrical blender (Panasonic, Japan). 100 grams of the powder were soaked in 500 ml of 90% ethanol in a conical flask for 72 hours on the shaker at room temperature (27°C). After that, the mixture was filtered using fine muslin cloth and later by Whatman™ No. 1 qualitative filter paper followed by distillation process in Eyela rotary evaporator (Sigma-Aldrich, USA).

B. Ferric Reducing Ability (FRAP) Test

The solutions used in this assay were: 300 mM acetate buffer (3.1 g sodium acetate trihydrate and 16 ml acetic acid) pH 3.6, 20 mm ferric chloride ($FeCl_3$) solution, and 10 mm TPTZ (2,4, 6-tripydridyl-s-triazine) solution in 40 mM hydrochloric acid (HCl). 25 ml acetate buffer, 2.5 ml $FeCl_3$, and 2.5 ml TPTZ were mixed together to prepare the working solution. Prior to the experiment, the temperature of the solution was elevated to 37°C. Under dark conditions, the leaf extract and control solutions (10µl) were mixed with 300 µl of the FRAP working solution for 30 minutes. Absorbance of the coloured ferrous tripyridyltriazine complex was recorded at 593 nm.

C. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Test

1 mg of the leaf extract was dissolved in 1 ml dimethyl sulfoxide (DMSO) to produce the final concentration, 1 mg/ml. The standards used in this test were ascorbic acid and quercetin hydrate at the same concentration. For DPPH reagent (light sensitive), the concentration used was 2.5 mg/ml. The DMSO solution was added to each 96-well plate followed by the addition of leaf extract on first row wells. Once suspended, the mixture of leaf extract was diluted from first row to second row

and the dilution process was continued until the last row. The DPPH reagent was added to each well in dark environment. Then, the plate was wrapped in aluminum foil and incubated on the shaker at room temperature for 20 minutes. The absorbance was recorded at 517 nm.

D. Hydrogen Peroxide (H_2O_2) Scavenging Test

The capacity of the extract in scavenging hydrogen peroxide radicals were measured by the revised method of Dehpour [6]. 40 Mm solution of hydrogen peroxide were prepared in 0.1 M phosphate buffer (pH 7.4). Different concentration of extract (50 µg/ml – 400 µg/ml) was mixed with hydrogen peroxide solution and incubated for 10 minutes. Then, the absorbance was recorded at 560 nm.

3. Results

A. Ferric Reducing Ability (FRAP) Test

Total antioxidant activity in ethanolic leaf extract of *A. angustifolia* was determined by FRAP assay. The FRAP value was obtained by plotting the graph of standard curve of $FeSO_4$ at concentrations between 200 and 1000 µM. The standard curve was found linear within this concentrations range as shown in Figure 1. The antioxidant capacity in FRAP value is presented in Figure 2.

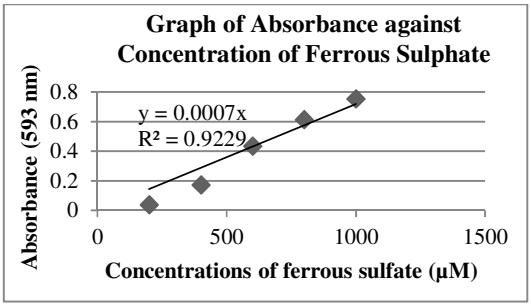

Fig. 1. Standard curve of FRAP assay using ferrous sulphate at concentration between 200 and 1000 M. The value is represents as the mean ± S.E.M.

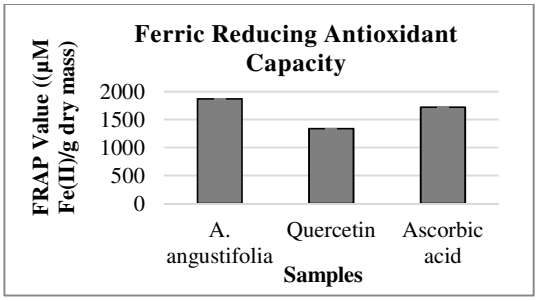

Fig. 2. Total antioxidant activity of A. angustifolia leaf extract and standard controls (quercetin and ascorbic acid) as determined by FRAP assay. The concentration used for all samples was 1 mg/ml dissolved in DMSO. The data are expressed as the mean ± S.E.M.

In this evaluation, the antioxidant activity is evaluated based on the capacity to reduce ferric (III) iron to ferrous (II) iron. The results are indicated as µM ferrous iron equivalents per g of dry weight of plant material. The result demonstrates that the leaf extracts possess higher antioxidant capacity in comparable to the standards used which is quercetin and ascorbic acid. FRAP value of the leaf extract is 1868.33 µM/g Fe(II) dry mass ± 0.15 while the FRAP value of the quercetin and ascorbic acid is 1336.9 µM/g Fe(II) dry mass ± 0.12 and 1720 µM/g Fe(II) dry mass ± 0.02, respectively.

A. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Test

DPPH radical scavenging activity of ethanolic extract of *A. angustifolia* was set against quercetin and ascorbic acid. It was noticed that the plant extract produced slightly lower scavenging activity than those standards. As showed in Figure 3, dose response curve was utilized to determine the half maximal inhibitory concentration (IC_{50}) value for each sample. The IC_{50} of the extract is 384.77 µg/ml while the value for quercetin and ascorbic acid are 39.60 µg/ml and 18.07 µg/ml respectively (Table 1).

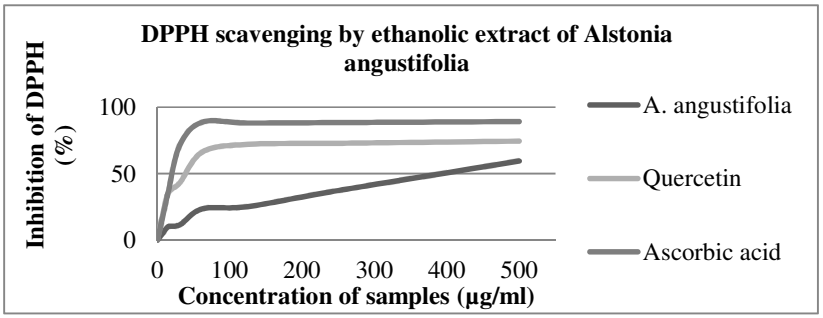

Fig. 3. DPPH radical scavenging activities of A. angustifolia leaf extract compared to standard controls (quercetin and ascorbic acid). The values are represents as the mean ± S.E.M.

Table 1
Inhibition activity of DPPH radical scavenging for A. angustifolia leaf extract and IC50 value compared with standard controls (quercetin and ascorbic acid). The value is represents as the mean ± S.E.M.

Sample	DPPH inhibition (%)	IC_{50} (µg/mL)
A. angustifolia	59.56 ± 0.006	384.77
Quercetin	74.54 ± 0.03	39.60
Ascorbic Acid	89.31 ± 0.006	18.07

B. Hydrogen Peroxide (H_2O_2) Scavenging Test

The capacity of *A. angustifolia* ethanolic leaf extract in scavenging H_2O_2 was measured spectrophotometrically. In Figure 4, it showed that the scavenging was in a dose-dependent manner and was set against the standard, ascorbic acid. The ethanol extract demonstrated a robust scavenging ability in contrast to the ascorbic acid at higher concentration. At lower concentration, the effect of ascorbic acid is higher than the sample while in slightly higher concentration (400 µg/ml), the effect of the ethanolic sample is greater than the ascorbic acid. The IC_{50} value for the extract was found to be 186.77µg/ml against ascorbic acid, 471.36µg/ml as showed in Table 2.

Table 2
Inhibition activity of H2O2 radical scavenging for A. angustifolia leaf extract and IC50 value compared with standard controls (quercetin and ascorbic acid). The value is represents as the mean ± S.E.M.

Sample	H_2O_2 inhibition (%)	IC_{50} (µg/mL)
A. angustifolia	46.88 ± 0.006	186.77
Ascorbic Acid	51.28 ± 0.006	466.56

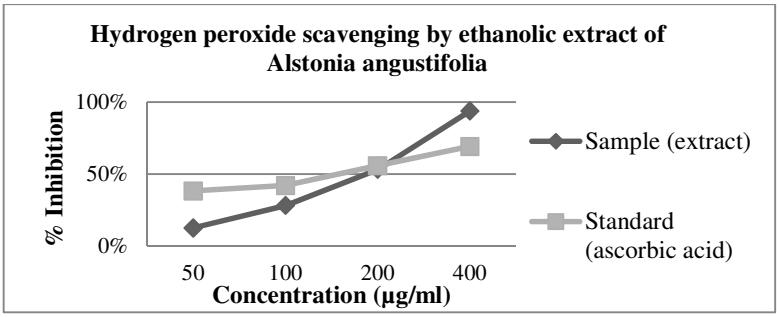

Fig. 4. Hydrogen peroxide scavenging activities of A. angustifolia leaf extract compared to standard controls (quercetin and ascorbic acid). The values are represents as the mean ± S.E.M.

4. Discussion

An antioxidant is a compound that able to neutralize radicals usually by donating an electron to the free radicals. Antioxidant compounds that able to lose electrons to the reactive radicals will convert them into more stable and passive condition thus aborting the radical chain reaction [9,8].

In FRAP assay, reducing power of a compound was evaluated by direct electron donation in the reduction of ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}) [7]. Through this assay, it is indicated that the extract of *A. angustifolia* leaf have higher reducing power to react with ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex and yielded a final product of ferrous tripyridyl triazine (Fe^{2+}-TPTZ) as compared to the controls (quercetin and ascorbic acid). Basically, the presences of compounds that possess the reducing properties apply their effect by donating a hydrogen atom that results in interruption of the free radical chain [15]. In FRAP assay, the antioxidants presence in the sample act as a reductant in a redox-linked colorimetric reaction [5]. The FRAP test is usually performed for determination of total antioxidant status in samples.

The DPPH radical scavenging assay is considered as the common method as it is very simple and gives satisfactory results for the antioxidant activity in the sample. Principally, the discoloration of DPPH is stechiometrically associated with the number of electrons acquired which resulted in the existence of a free radical scavenger [19]. The antioxidants effect on DPPH is suggested owing to their capacity to donate hydrogen [11]. The analysis demonstrated that the extracts have the capacity to donate proton which results in the ability to act as free radical scavengers. This indicates that the plant extract may act as primary antioxidants.

Ethanolic extract of *A. Angustifolia* demonstrated a significant in vitro hydrogen peroxide scavenging activity. Hydrogen peroxide is a weak oxidizing agent and a notable type of reactive oxygen species that able to penetrate biological membranes. Hydrogen peroxide can sometimes be

cytotoxic by producing hydroxyl radicals in the cells by reacting with Fe^{2+} and possibly Cu^{2+}, but it is not that reactive by itself [1]. It is able to deactivates some enzymes immediately, generally by oxidation of essential thiol (-SH) groups. A significant indicator of a compound that possesses the potential antioxidant activity may be their reducing power. The capability of the plant extracts to scavenge hydrogen peroxide might be due to the existence of the phenolic compound which donate electron to the hydrogen peroxide thus reducing it to water [17].

5. Conclusion

Current study demonstrated that the ethanolic extract of *Alstonia angustifolia* leaf possess antioxidant activity, which might be beneficial in prevention of various oxidative stress- related diseases.

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References

[1] Kumaran, A., and R. Joel Karunakaran. "In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India." LWT-Food Science and Technology 40, no. 2 (2007): 344-352.
[2] Luximon-Ramma, Amitabye, Theeshan Bahorun, Mohammed A. Soobrattee, and Okezie I. Aruoma. "Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Cassia fistula." Journal of Agricultural and Food Chemistry 50, no. 18 (2002): 5042-5047.
[3] Dimitrios, Boskou. "Sources of natural phenolic antioxidants." Trends in Food Science & Technology 17, no. 9 (2006): 505-512.
[4] B. Halliwell and J. M. C. Gutteridge, “Free Radicals in Biology and Medicine,” Oxford University Press, UK, 60–67, 1999.
[5] Guo, Changjiang, Jijun Yang, Jingyu Wei, Yunfeng Li, Jing Xu, and Yugang Jiang. "Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay." Nutrition Research 23, no. 12 (2003): 1719-1726.
[6] Ngonda, Frank. "In-vitro Anti-oxidant Activity and Free Radical Scavenging Potential of roots of Malawian Trichodesma zeylanicumm (burm. f.)." Asian Journal of Biomedical and Pharmaceutical Sciences 3, no. 20 (2013): 21.
[7] Yen, Gow-Chin, and Hui-Yin Chen. "Antioxidant activity of various tea extracts in relation to their antimutagenicity." Journal of Agricultural and Food Chemistry 43, no. 1 (1995): 27-32.
[8] Ganu, G. P., S. S. Jadhav, and A. D. Deshpande. "Antioxidant and antihyperglycemic potential of methanolic extract of bark of mimusops elengi l. In mice." International journal of phytomedicine 2, no. 2 (2010).
[9] Gülçın, İlhami, Münir Oktay, Ekrem Kıreçcı, and Ö. İrfan Küfrevıoğlu. "Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts." Food chemistry 83, no. 3 (2003): 371-382.
[10] Bulkill, I. H. "A dictionary of economic products of the Malay Peninsular." Ministry of Agriculture and cooperatives, Kulau Lumpur, Malaysia (1966).
[11] Baumann, J., G. Wurm, and F. Von Bruchhausen. "Prostaglandin synthetase inhibition by flavonoids and phenolic compounds in relation to their O2--scavenging properties (author's transl)." Archiv der Pharmazie 313, no. 4 (1980): 330.
[12] Farber, John L. "Mechanisms of cell injury by activated oxygen species." Environmental health perspectives 102, no. Suppl 10 (1994): 17.
[13] Perry, Lily M., and Judith Metzger. Medicinal plants of east and southeast Asia: attributed properties and uses. MIT press, 1980.
[14] Ebrahimzadeh, Mohammad Ali, Fereshteh Pourmorad, and Samira Hafezi. "Antioxidant activities of Iranian corn silk." Turkish Journal of biology 32, no. 1 (2008): 43-49.

[15] Duh, Pin-Der, Pin-Chan Du, and Gow-Chin Yen. "Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage." Food and Chemical Toxicology 37, no. 11 (1999): 1055-1061.
[16] Kris-Etherton, Penny M., Kari D. Hecker, Andrea Bonanome, Stacie M. Coval, Amy E. Binkoski, Kirsten F. Hilpert, Amy E. Griel, and Terry D. Etherton. "Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer." The American journal of medicine 113, no. 9 (2002): 71-88.
[17] Banerjee, Saurabh K., and C. G. Bonde. "Total phenolic content and antioxidant activity of extracts of Bridelia Retusa Spreng Bark: Impact of dielectric constant and geographical location." Journal of Medicinal Plants Research 5, no. 5 (2011): 817-822.
[18] Di Matteo, Vincenzo, and Ennio Esposito. "Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis." CURRENT DRUG TARGETS-CNS AND NEUROLOGICAL DISORDERS- 2, no. 2 (2003): 95-108.
[19] Silva, E. M., J. N. S. Souza, H. Rogez, Jean-François Rees, and Yvan Larondelle. "Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region." Food Chemistry 101, no. 3 (2007): 1012-1018.