Antioxidant Activity of *Averrhoa bilimbi* Linn. Leaves Extract Using Two Different Types of Solvents

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Abstract. Exposed to the pollution has led to generation of reactive oxygen species (ROS) in human skin. ROS generated cause many skin diseases such as skin-aging, melanogenesis and skin cancer. ROS is a family of free radicals based on oxygen that contains or may generate an unpaired electron. Antioxidant is a molecule that can inhibit the reaction of free radical from ROS by donating its electron. *Averrhoa bilimbi* Linn. (AVBL) is one of the potent natural antioxidant belongs to the group of Oxalidiaceae which can be widely found in Asia including Malaysia. Traditionally, this plant has been used to treat many diseases such as cough, pimple, fever and inflammation. As a result, much attention has been directed towards the studies regarding the potential of this plant in treating disease. The present study was undertaken to assess the antioxidant activity of AVBL leaves extract. The AVBL leaves were extracted using sonicator with ethanol and distilled water as a solvent. The total phenolic (TPC) and flavonoid content (TFC) of this study were determined by using Folin-Ciocalteu reagent and aluminium chloride colometric assay. Antioxidant activity of the plant extract was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing power (FRAP). From the analyses, water extract of AVBL possessed greater extraction yield (11.231%) as compared to ethanolic extract (5.358%). However, ethanolic extract of AVBL leaves revealed higher result of TPC (126.4±0.35 mg/g gallic acid equivalent), TFC (32.80±0.37 mg/g quercetin equivalent), DPPH (0.0019±0.0003 g/mL) and FRAP (41.81±0.45 mg/g gallic acid equivalent). The results of TPC and TFC have strongly positive correlation with antioxidant capacity (r = 1). Thus, it can be concluded that this plant is a potent source of natural antioxidant.

Keyword: Reactive Oxygen Species, *Averrhoa bilimbi* L leaves., Antioxidant activity, Potent antioxidant

Introduction

Air pollution is one of the world’s largest health and environmental problems. Indeed, World Health Organization (WHO) estimates that around 7 million people die every year from exposure to polluted air. Air pollution is harmful to human skin by producing Reactive Oxygen Species (ROS). Several researches reported, ROS generated from air pollution caused skin diseases such as skin aging, skin inflammation and skin cancer [1]. Thus, antioxidant is the best substance to fight against free radicals.

Antioxidant is a molecule that can inhibit the reaction of free radical from ROS by donating its electron. Antioxidant can be divided into natural and synthetic. Natural antioxidant is considered safe, biodegradable, environmentally friendly and low cost because it is derived from plants, fruits and other natural resources. It consists of many beneficial bioactive compounds such as flavonoids, phenol and tannins which help to protect human body against damage by ROS and also act as an antioxidant [2].

*Averrhoa bilimbi* L. (AVBL) is a potent natural antioxidant which possess many beneficial bioactive compounds such as flavonoids, tannins and phenols [3]. Previous researches show the importance of AVBL fruit in various biological activities such as antioxidant activity, catalytic activity, antihyperlipidemic properties, anti-diabetic, antimicrobial and cytotoxic properties [4-8]. Choosing a suitable, environmentally friendly solvent is important to extract maximum amount of target compounds and to obtain the highest biological activity of the extract besides saving earth. Therefore, finding new and safe natural agents with antioxidant activity is the objective of the continued study.

Methodology
This study was conducted starting with extraction of AVBL by sonicator using two types of solvents (ethanol and water). The crude extract obtained was tested with Folin Ciocalteu reagent and Aluminium Chloride Colorimetric Assay to determine the total phenolic and flavonoid content [9-10]. Lastly, antioxidant activity of this plant extract was determined using stable 2,2- diphenyl-1-pirylhydrazyl (DPPH) assay and Ferric Reducing Power (FRAP) [11-12]. All the result obtained was recorded, calculated and analyzed using suitable formula.

Results and discussions

Extraction

Table 1 summarize the extraction results of AVBL using ethanol and distilled water. From the table, it can be concluded that the use of different solvents resulted in the variation of colour, odour and also extraction yield. The variation is due to the nature and amount of secondary metabolites extracted. The use of distilled water as a solvent give highest extraction yield compared to ethanol which is 11.231% to 5.358%. This result illustrated that the extraction yield increase with increase in polarity of the solvent. During extraction, compounds other than phenolics such as proteins and carbohydrates may have been extracted and contributed to higher yield. This may be attributable to the higher solubility of proteins and carbohydrates in water than in ethanol. The results of this study are in agreement with the extraction yields of some medicinal plant [13-15].

| Characteristic       | Type of Solvent          |
|----------------------|--------------------------|
| Polarity of solvent  | Ethanol (AVBE) 4.3       |
|                      | Distilled Water (AVBW) 10.2 |
| Colour               | Green                    |
|                      | Orange to Brown          |
| Nature               | Gel Type                 |
|                      | Gel Type                 |
| Odour                | Nature                   |
|                      | Nature                   |
| Extraction Yield (%) | 5.358%                   |
|                      | 11.231%                  |

Table 1. The Characteristic of AVBL Extract According to Their Solvent.

Phenolic, Flavonoids and Antioxidant Activity

Results on the phenolic and flavonoid contents of AVBL leaves extracts obtained using different solvents are presented in Table 2. From the results, AVBL extracted with ethanol shows higher phenolic and flavonoids content which is 126.4±0.35 and 65.94±2.18 compared to 32.80±0.37 and 13.84±0.11. Apart from that, AVBE also shows better performance of antioxidant activity using DPPH and FRAP. The antioxidant performance of DPPH was calculated using IC50. IC50 value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC50 value, the higher the antioxidant activity of the sample [16]. The observed IC50 value showed that AVBE has smaller value of IC50 compared to AVBW (0.0019±0.0003 than 0.0039±0.001). Thus, indicated that AVBE is a strong antioxidant. The same trend also can be seen from FRAP result. AVBE resulted in higher result compared to AVBW (41.81±0.45 and 20.85±1.59). The differences in impact of solvents on antioxidant capacity of AVBL extract in the current study can be explained by the variation of bioactive groups extracted by the different solvents. Each bioactive group contributed to the different antioxidant power as these groups were found to have differing correlation with antioxidant capacity. The higher value of TPC, TFC, DPPH and FRAP of AVBE shows that ethanol is a good solvent to extract phenolic compound from AVBL. Ethanol as a solvent is a polar molecule with OH group. It has high electronegativity which allow hydrogen bonding to take place with other molecules. OH group in ethanol attract polar molecules and C2H5- attract non-polar substances. Thus, ethanol can dissolve in both polar and non-polar compounds.
Table 2. Results of Total phenolic, flavonoids and antioxidant activity of two types of solvents.

| Type of Solvent      | Total Phenolic Contenta | Total Flavonoid Contentb | Antioxidant Activity | DPPHc (IC50) | FRAPa |
|----------------------|-------------------------|---------------------------|----------------------|---------------|-------|
| Ethanol (AVBE)       | 126.4±0.35              | 32.80±0.37                | 0.0019±0.0003        | 41.81±0.45    |       |
| Distilled Water (AVBW)| 65.94±2.18              | 13.84±0.11                | 0.0039±0.001         | 20.85±1.59    |       |

*aExpressed as mg gallic acid/g of dry material
bExpressed as mg quercetin/g of dry plant material
cExpressed as g/mL

Figure 1. Comparison of total phenolic content of two different solvents.

Figure 2. Comparison of total flavonoid content of two different solvents.

Figure 3. Scavenging activities of the ethanolic extract and water extract of AVBL leaves.

Figure 4. Comparison of total FRAP value of AVBL of two different solvents.

Conclusion
The findings showed a strong correlation between the activity of antioxidants and the extract's phenolic content, which may be the key contributor to the plant's antioxidant activity. The current study showed that, ethanolic extract of AVBL shows higher result of total phenolic and flavonoids content compared to water extract. It also shows higher performance of antioxidant activity of DPPH and FRAP. From the results, it is confirmed that AVBL leaves extract is a potential source of natural antioxidant.

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