

ANTIOXIDANT ACTIVITY OF *Vernonia cinerea*, *Peperomia pellucida* AND THE COMBINATION OF *Vernonia cinerea* AND *Peperomia pellucida*

Sarini Ahmad Wakid and Nurul Syafiqah Zaharin

School of Biology, Faculty of Applied Science

Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000, Negeri Sembilan, Malaysia.

Email: sarini@uitm.edu.my

ABSTRACT

The present study was conducted to evaluate the antioxidant activity of *Vernonia cinerea*, *Peperomia pellucida* and combination of *Vernonia cinerea* and *Peperomia pellucida*. These two herbs are plants that often grow at random in different environments but are not commercialized due to the fact that no comprehensive study of the importance of their use. The extract was prepared with methanol respectively. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay were used to study their antioxidant activity. The extracts were compared with commercial antioxidant, butylated hydroxytoluene (BHT). The highest scavenging effect from peel extract was presented by *Vernonia cinerea* with the value of 76.3% scavenging activity ($IC_{50} = 2.909$), followed by the combination of *Vernonia cinerea* and *Peperomia pellucida* (71.21% scavenging activity; $IC_{50} = 5.274$) and *Peperomia pellucida* with value of 68.3% scavenging activity ($IC_{50} = 5.572$). BHT showed the lowest IC_{50} value 1.71 with the scavenging activity 90.0%. Low IC_{50} value will indicate the strong ability of the extracts to act as DPPH scavenger.

Keywords: antioxidant, DPPH, *Vernonia cinerea*, *Peperomia pellucida*

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Introduction

Antioxidants are a wide range of substances or molecules that neutralize free radicals, and can inhibit damage to living cells (Etim *et al.*, 2015). The extracts were tested for their antioxidant activity in scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Ketsuwan *et al.*, 2017). The high antioxidant activity of the plant at low concentration indicates that it could be very useful for the treatment of ailments resulting from oxidative stress (Onocha, 2016). Their antioxidant property has been regarded to protect human beings from oxidative stress and free radical-mediated diseases. Antioxidant in the plants extract can be measured by the capabilities of the plant to have amount of antioxidant necessary to reduce the initial radical concentration by 50% or also called as inhibition concentration value (IC_{50}) (Mn *et al.*, 2014).

Vernonia cinerea also known as Purple Fleabane or Little Ironweed is a non-native species which is the origin is from India (Prabha, 2015). It is belonging to family Asteraceae. It is an annual herb and grown in tropical climate and semi-tropical climate areas as waste land herb and it can achieve its maximum growth to 1 meter height (Kawsar *et al.*, 2011). Ancient folks believe that this plant has the capability to cure fever, abscess, anhidrosis and arthritis. This plant also can use to cure skin problems and

conjunctivitis. This plant had a taste of bitter and pungent in smell. Mostly all of the part of the plants has their own uses including their leaves, stem, root, and seed (Rajamurugan *et al.*, 2011).

Peperomia pellucida also called Silver bush or shiny bush (Onocha, 2016) belongs to the family Piperaceae. It is a herbaceous plant found in many South American and Asian countries. It has power to cure many diseases as it was stated by the local community that the extracts of the plant was useful to treat bone aches and pains. The leaf was also used in headache, fever, eczema, abdominal pains and convulsions treatments (Khan *et al.*, 2008).

Literature review

The use of plants for healing purposes predates recorded history and forms the origin of much of modern medicine (Vickers and York, 2001). Although their application is often viewed with scepticism by the Western medical establishment, they are used in ancient medical traditions such as Ayurveda and traditional Chinese medicine (TCM) pharmaceutical industry (Han *et al.*, 2017). Medicinal herbs cure most of the health ailments when there was no use of modern medicinal instruments and drugs. These herbs have many benefits with their juices and extracts and sometimes the entire plant and their applications were passed on through many generations (Misra, 2013). The combination of different medicinal herbs work better together as compared to a single herb being used for one treatment at a time. These specific combinations are made by considering the different aspects and phases of health needs. Practitioners say that the principles of synergy and buffering apply to combinations of plants and claim that combining herbs improves efficacy and reduce adverse effects (Vickers and York, 2001).

According to Elujoba *et al.* (2005), *Vernonia cinerea* traditionally used in tonsillitis, stomach pain, diarrhea, intermittent fever, eczema, herpes, ringworm and Elephantiasis. The plant is extensively used in indigenous medicine in stomach aches and for cold, asthma and bronchitis.

Hamzah *et al.*(2012) claimed that *Peperomia pellucida* is used locally for hypertension, diabetes and generally as tonic for healthy well-being. This species is employed on abscesses, furuncles, and skin sores, as well eye inflammation (conjunctivitis). Other medicinal properties attributed to *Peperomia pellucida*, varies depending on the region, which are to lower blood cholesterol level (in Northeastern Brazil), against proteinuria, and as a diuretic (in Guyana) (Arrigoni-Blank *et al.*, 2004). Other studies by Onocha (2016) revealed that this plant can be used to treat abdominal pain, abscesses, acne, boils, colic, fatigue, gout headache, renal disorders, rheumatic pain and to treat breast cancer, impotence, measles, mental disorders and small pox.

Methods

Preparation of melon peel extracts

Vernonia cinerea and *Peperomia pellucida* was obtained from UiTM Kuala Pilah campus area. Each sample were washed and dried in oven at 40°C for three days. A blender is used to mill the samples into a fine powder and stored in air-tight bottles at -4°C. Then, 25g sample of powder were weighed and separately extracted using 250 ml methanol to obtain final concentration of extract 100mg/ml (Kawsar *et al.*, 2011). The suspension was filtered by using Whatman filter paper and was evaporated by using rotary evaporator. The extracts were kept at 4°C.

DPPH free radical scavenging assay

The antioxidant activity of the extracts was determined by DPPH free radical scavenging assay as describes by Wakid *et al.*, (2014) with some modifications. 25 µl of 8 mg/ml DPPH solution was added into each sample that has different concentration making the total volume of the mixture equal to 1 ml. The reaction mixture was incubated at room temperature and allowed to react for 30 minutes. The optical density was measured at 520nm using UV-Vis spectrophotometer. BHT (butylated hydroxytoluene) was used as a positive control. The capability of each extracts to scavenge the DPPH radical was calculated by using equation: Scavenging activity (%) = $1 - [\text{Absorbance of sample at 520nm} / \text{Absorbance of control at 520nm}] \times 100$. IC₅₀ value was determined from the plotted graph of scavenging activity versus the concentration of extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

Results and Discussion

The free radical scavenging activity by using DPPH assay is a convenient method to identify the antioxidant activity of *Vernonia cinerea*, *Peperomia pellucida*, and the combination of *Vernonia cinerea* and *Peperomia pellucida*. The radical scavenging activity will increase according to the concentrations of the sample. It is due to when the concentrations is high, there are more molecule of the extracts to reduce the DPPH and vice versa. The properties of DPPH itself that is stable and have delocalisation of electron will give the deep violet colour and when another substance mixed with DPPH, it will turn into the pale yellow colour. Antioxidant activity of *Vernonia cinerea* is higher compare to *Peperomia pellucida* and the combination of these two plants.

DPPH free radical scavenging assay

The capabilities of DPPH as the scavenger of free radicals are widely used in the experimental activities due to its convenience and easily obtainable. DPPH is actually a very stable molecule, which undergo delocalisation of electrons and if this molecule is disrupted by the substance that can donate hydrogen electron, the molecule itself will be reduced. According to (Kedare & Singh, 2011), the delocalisation also gives rise to the deep violet colour, and on mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour.

The DPPH free radical scavenging activity and IC₅₀ value listed in Table 1.

Table 1 Scavenging activity (%) and IC₅₀ value of *Vernonia cinerea*, *Peperomia pellucida*, combination of *Vernonia cinerea* and *Peperomia pellucida* extracts and BHT

Samples	Scavenging activity (%)	IC ₅₀ Value (mg/ml)
<i>Vernonia cinerea</i>	76.3	2.909
<i>Peperomia pellucida</i>	68.31	5.572
Combination of <i>Vernonia cinerea</i> and <i>Peperomia pellucida</i>	71.21	5.274
Butylated hydroxytoluene (BHT)	90.0	1.71

In DPPH assay as presented in the **Table 1**, it can be concluded that the scavenging activity of each extracts were moderately good. The highest percentage of DPPH inhibition of the extracts were

recorded at their highest concentration that was at 100 mg/ml concentration with *Vernonia cinerea* (76.3%), combination of *Vernonia cinerea* and *Peperomia pellucida* (71.21%) and *Peperomia pellucida* (68.3%) respectively. Meanwhile, the DPPH inhibition presented by butylated hydroxytoluene (positive control) were 90.0% scavenging inhibition.

IC₅₀ can be defined as the maximum concentration value when half of the free radicals are scavenged or inhibited by the samples. The comparison of the mean concentration for 50% free radical scavenging activity (IC₅₀) of plant extracts and BHT also shown in Table 1. The lowest IC₅₀ of *Vernonia cinerea* is 2.909 mg/ml. However, those radical scavenging activities in the samples are not exceeding the commercial synthetic antioxidant, BHT with the value of 1.71mg/ml. Low IC₅₀ value indicates strong ability of the extracts to act as DPPH scavenger.

Conclusion

The results in the present study showed that the extracts of *Vernonia cinerea* and *Peperomia pellucida* species showed scavenging effect towards DPPH free radical. *Vernonia cinerea* exhibits highest antioxidant activity compared to *Peperomia pellucida* and combined samples. More research is needed to establish the nutritional value of these extracts especially in the fields of biochemical analysis that can contribute to human health.

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