# 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 pants 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 indicates the strong ability of the extracts to act as DPPH scavenger.

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

*Article history:- Received: 10 October 2019; Accepted: 12 December 2019; Published: 14 March 2020*


## Introduction

Antioxidants are a wide range of substances or molecules that neutralize free radicals, and can inhibits 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 (IC50) (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 hydroxtoulene) was used as a positive control. The capability of each extracts to scavenge the DPPH radical was calculated by using equation: Scavenging activity (%) = 1 – [Absorbance of sample at 520nm / Absorbance of control at 520nm] x 100. $IC_{50}$ 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 $IC_{50}$ value listed in Table 1.

**Table 1 Scavenging activity (%) and $IC_{50}$ value of *Vernonia cinerea*, *Peperomia pellucida*, combination of *Vernonia cinerea* and *Peperomia pellucida* extracts and BHT**

| Samples | Scavening activity (%) | $IC_{50}$ Value (mg/ml) |
|---|---|---|
| *Vernonia cinerea* | 76.3 | 2.909 |
| *Peperomia pellucida* | 68.31 | 5.572 |
| *Combination of Vernonia cinerea and Peperomia pellucida* | 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 ($IC_{50}$) of plant extracts and BHT also shown in Table 1. The lowest $IC_{50}$ 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 $IC_{50}$ 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.

### Acknowledgements

The authors would like to thank UiTM Cawangan Negeri Sembilan for the support.

## References

Arrigoni-Blank, M. D. F., Dmitrieva, E. G., Franzotti, E. M., Antoniolli, A. R., Andrade, M. R., & Marchioro, M. (2004). Anti-inflammatory and analgesic activity of Peperomia pellucida (L.) HBK (Piperaceae). *Journal of Ethnopharmacology*, *91*(2–3), 215–218. https://doi.org/10.1016/j.jep.2003.12.030

Elujoba, A., Odeleye, O., & Ogunyemi, C. (2005). Traditional medicine Development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional , Complementary and Alternative Medicines*, *2*(1), 46–61

Etim, E., Udobre, A., Udoh, A., & Eduoku, E. (2015). Evaluation of the antioxidant property of Vernonia Cinerea ( L .) LESS . ( Asteraceae ) using 2 . 2- Diphenyl-1- Picrylhydrazine ( DPPH ) Assay Method, *4*(6), 10–14.

Hamzah, R. U., Odetola, A. A., Erukainure, O. L., & Oyagbemi, A. A. (2012). Peperomia pellucida in diets modulates hyperglyceamia, oxidative stress and dyslipidemia in diabetic rats. *Journal of Acute Disease*, *1*(2), 135–140. https://doi.org/10.1016/S2221-6189(13)60031-1

Han, Y. K., Kim, S. Y., Ahn, J. Y., & Baek, J. U. (2017). An analysis of the combination frequencies of constituent medicinal herbs in prescriptions for the treatment of bone and joint disorder in Korean medicine : determination of a group of candidate prescriptions for universal use. *Integrative Medicine Research*, 1–10. https://doi.org/10.1016/j.imr.2017.09.001

Kawsar, H., Sikder, A. A., Rana, S., & Nimmi, I. (2011). Studies of Thrombolytic , Antioxidant and Cytotoxic Properties of Two Asteraceous Plants of Bangladesh, *14*(2), 103–106.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Ketsuwan, N., Leelarungrayub, J., Kothan, S., & Singhatong, S. (2017). Antioxidant compounds and activities of the stem, flower, and leaf extracts of the anti-smoking Thai medicinal plant: Vernonia cinerea Less. *Drug Design, Development and Therapy*, *11*, 383–391. https://doi.org/10.2147/DDDT.S126882

Khan, A., Rahman, M., & Islam, S. (2008). Antipyretic activity of Peperomia pellucida leaves in rabbit. *Turkish Journal of Biology*, *32*(1), 37–41.

Misra, L. (2013). Traditional Phytomedicinal Systems, Scientific Validations and Current Popularity as Nutraceuticals. *International Journal of Traditional and Natural Medicines International Journal of Traditional and Natural Medicines Journal Homepage: Www.ModernScientificPress.Com/Journals/IJTNM.Aspx J. Trad. Nat. Med*, *2*(21), 27–75.

Mn, U., Ma, R., Mitra, K., & Akter, R. (2014). Preliminary phytochemical analysis and in vitro antioxidant activities of methanol extract of Argyreia argentea ( Roxb ), *7*(1), 1–7.

Onocha, P. (2016). antioxidant screening of leaf extracts of Peperomia pellucida from Nigeria, (November 2011).

Prabha, L. J. (2015). Therapeutic uses of Vernonia cinerea - A short review. *International Journal of Pharmaceutical and Clinical Research*, *7*(4), 323–325.

Rajamurugan, R., Selvaganabathy, N., Kumaravel, S., Ramamurthy, C. H., Kumar, M. S., & Thirunavukkarasu, C. (2011). Identification , quantification of bioactive constituents , evaluation of antioxidant and in vivo acute toxicity property from the methanol extract of Vernonia cinerea leaf extract Identification , quantification of bioactive constituents , evaluation of , *0209*(October 2017). https://doi.org/10.3109/13880209.2011.604334

Vickers, A., & York, N. (2001). Toolbox, *175*(August), 125–128.

Wakid, S.A., Nor'aishah, H., & Zaini, N.M. (2014). Determination of antioxidant activity for seven types of macroalgae. IPCBEE vol. 65 (2014).