e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.12, 2022
 UGC CARE Listed (Group -I) Journal

In Vitro Antioxidant Activities of *Anaphyllum wightii* Schott Rhizome Extracts

Blessy R[1]*, Hanna Jeeja Alexander[1], Anami Augustus Arul A[2], Catherine Sheeja V[1], Ani Besant S[1]

[1] Research Scholar, Department of Botany, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India

[2] Assistant Professor, Department of Botany, Holy Cross College (Autonomous), Nagercoil, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

*Corresponding Author: blessyrichard1994@gmail.com

Abstract

Antioxidant activities of *Anaphyllum wightii* Schott rhizome was estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and nitric oxide (NO) scavenging activity at different concentrations. The results are compared with the standard ascorbic acid. The scavenging effect increases with the concentration of standard and samples. Overall observation shows, among all the tested solvents ethyl acetate shows significant inhibition percentage followed by aqueous, methanol and ethanoic extracts. DPPH radical scavenging activity of ethyl acetate extract possess promising free radicle scavenging percentage 52.19 at lower concentration that is 10 µg/ml. The inhibition percentage of ethyl acetate and aqueous extract is 63.31%, 63.52% at 100 µg/ml concentration. For DPPH assay, Ascorbic acid at a concentration of 10 µg/mL exhibited 60.32% of inhibition and for 100 µg/mL 98.39%. NO scavenging activity shows significant free radicle inhibition percentage 48.04 at lower concentration that is 10 µg/ml. The inhibition percentage of ethyl acetate extract exhibit 71.50 % scavenging activity at 100 µg/ml concentration and the standard ascorbic acid shows 89.94% of inhibition in NO scavenging assay. The present study revealed that the *Anaphyllum wightii* rhizome has significant radical scavenging activity that may be useful for their medicinal functions.

Keywords: *Anaphyllum wightii* Schott, Antioxidant activity, DPPH radical scavenging activity, nitric oxide scavenging activity

Introduction

Oxidative stress can affect essential molecules in human cells, including DNA and proteins, which are responsible for many processes in the body (Luo *et al*., 2020). In the human body, uncontrolled oxidation is commonly caused by highly reactive molecules known as free radicals (Souza-Monteiro *et al*., 2019). Free radicals' role in damaging cells and tissues occupies the most critical position in the body's metabolism. Free radical reactions occur due to the oxidation reaction of stable compounds becoming unstable and reactive. The reactive species in the body can react with other compounds and cause tissue damage which will lead to diseases such as cancer, Alzheimer's disease, cardiac reperfusion, and abnormalities. Free radicals such as peroxide, hydroperoxide or lipid peroxyl can oxidize nucleic acids, proteins, lipids, and DNA, which can lead to degenerative diseases (Sarma *et al*., 2010).

Antioxidants are chemical compounds that can neutralize free radical agents. These compounds work by donating electrons to form a stable form, thereby inhibiting the oxidative mechanism that is lead to degenerative disease. Antioxidant compounds can include natural and synthetic compounds. In addition to having side effects, synthetic antioxidants can be carcinogenic agents (Sarma *et al*., 2010).

An antioxidant is a substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011). Oxidative stress can be prevented through the use of natural antioxidants, whether in the form of raw extracts or their chemical constituents (Zengin *et al*., 2011). A significant number of medicinal plants contain compounds that have antioxidant properties, such as phenolic compounds, which possess strong antioxidant properties and may help to protect cells from free radical damage (Verma and Kumar, 2011).

Increasing safety concerns regarding synthetic antioxidants have prompted interest in cheaper and safer antioxidants, especially from plants (Zheng and Wang, 2001). Medicines derived from plant products are generally safer than their synthetic counterparts, even though the toxicity profile of most medicinal plants has not been thoroughly evaluated (Oluyemi *et al*., 2007). Our investigation focused on evaluating the antioxidant activity of *Anaphyllum wightii* rhizome using different extracts that could provide natural antioxidants.

Materials and methods

Plant material

Anaphyllumn wightii Schott were collected from Mangamalai tribal settlement of Kanyakumari district in the month of May-June, 2018. The authenticity was determined by comparing its morphological characteristics to those found in the literature, and it was authenticated at the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. The collected plants are preserved as per the standard procedure (Jain and Rao, 1977).

Extraction

Rhizomes of the plant were thoroughly washed with running water and dried at room temperature, then ground into a coarse powder. The coarse powder of rhizome (10 g) was extracted successively with 100 ml of methanol, ethanol, ethyl acetate and aqueous solutions through a Soxhlet apparatus for 24 hrs. Extracts obtained were kept refrigerated until they were used.

DPPH Radical Scavenging Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was measured using the Wong (2006) method. Various concentrations (20, 40, 60, 80 and 100 µg/ml) of rhizome extracts prepared by different solvents were taken in a number of vials containing 3 ml of 0.1 mM methanolic solution of DPPH. The test tubes were shaken gently and set aside for 30 minutes at room temperature in dark. Optical density of samples was read at 517 nm against blank. Ascorbic acid was used as the standard control. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of Control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Nitric Oxide Scavenging Assay

The nitric oxide radical scavenging activity was done using the method of Alderson *et al*., (2001). 1 ml of sodium nitroprusside (10mM) in phosphate buffered saline was mixed with the 1 ml of different concentration of extract (20, 40, 60, 80 and 100 µg/ml) and incubated at 25°C for 180 minutes. To the incubation solution, 1 ml of Griess reagent (prepared by mixing equal

volume of 1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added and the absorbance was read at 546nm Ascorbic acid was used as a positive control treated in the same way with Griess reagent. The percentage inhibition was calculated using the formula**:**

$$\textbf{Percentage inhibition (\%)} = \frac{\textbf{Absorbance of Control - Absorbance of test}}{\textbf{Absorbance of control}} \times 100$$

Results and Discussion

Antioxidant activities of *Anaphyllum wightii* rhizome were estimated by DPPH radical scavenging activity and nitric oxide scavenging activity using different solvents like methanol, ethanol, ethyl acetate and aqueous in varying concentrations like 10, 20, 40, 80 and 100 (µg/ml) and results are compared to the standard Ascorbic acid. The results are represented in the Figure 1 and Figure 2. There was a dose dependent increase in the percentage of antioxidant activity for all the tested concentrations. Overall observation shows, among all the tested solvents ethyl acetate shows significant inhibition percentage followed by aqueous, methanol and ethanoic extracts.

An antioxidant is a compound that neutralizes free radicals by donating an electron to the free radicals. Antioxidant compounds that are able to lose electrons to reactive radicals will convert them into a more stable and passive state. This will terminate the radical chain reaction Ganu *et al*. (2010). According to Ijaz *et al*. (2017) natural antioxidant compounds include flavonoids, polyfunctional organic acids, phenolic acids, lignans, terpenes, tocopherols, phospholipids, and others. Research has extensively examined the biological activities of phenols, which are highly effective antioxidants and free radical scavengers. Plant-derived antioxidants have no side effects, while synthetic antioxidants have been found to be genotoxic (Rohman *et al*., 2010).

The effect of different solvent extracts of *Anaphyllum wightii* rhizome and standard ascorbic acid on DPPH radical scavenging activity were compared. The scavenging effect increases with the concentration of standard and samples. DPPH radical scavenging activity of ethyl acetate extract possess promising free radicle scavenging percentage 52.19 at lower

concentration that is 10 µg/ml. The inhibition percentage of ethyl acetate and aqueous extract is 63.31%, 63.52% at 100 µg/ml concentration. For DPPH assay, Ascorbic acid at a concentration of 10 µg/mL exhibited inhibition of 60.32% and for 100 µg/mL 98.39%. From Figure 1. it is observed that both extracts show significant DPPH radical scavenging property.

Guchu *et al*., (2020) documented that methanolic extracts of *Caesalpinia volkensii, Vernonia lasiopus*, and *Acacia hockii* showed concentration-dependent DPPH scavenging capacity. Ganie *et al*., 2011 reported that a dose-dependent inhibition of DPPH activity was observed in an ethyl acetate extract of *Podophyllum hexandrum*, and the scavenging activities of the extract increased with increasing concentration. In agreement with earlier reports, all analyzed extracts showed free radical scavenging activity on a dose-dependent manner

Hydrogen-donating abilities of antioxidants are thought to be responsible for their effect on DPPH (Baumann *et al*., 1979). According to Adedapo *et al*. (2008), the DPPH radical scavenging abilities of the extracts at 0.1 mg/ml were less than those of ascorbic acid (100%) and BHT (98.3). However, the study proved that the extracts have proton-donating abilities that could serve as free radical inhibitors or scavengers, possibly serving as primary antioxidants.

Nitric oxide has an active role in the initiation or progression of many diseases such as inflammation, cancer and other pathological conditions (Nabavi *et al*., 2010). Natural products with nitric oxide scavenging ability may be useful in preventing inflammation and cancer diseases (Alinezhad, *et al*., 2012).

In nitric oxide scavenging activity, among all the tested solvents ethyl acetate shows good scavenging activity followed by aqueous, methanol and ethanoic extracts. The NO scavenging activity of ethyl acetate extract shows significant free radicle inhibition percentage 48.04 at lower 10 µg/ml concentration. Ascorbic acid at a concentration of 10 µg/mL exhibited a percentage inhibition of 53.63%. The inhibition percentage of ethyl acetate extract exhibit 71.50 % scavenging activity at 100 µg/ml concentration and the standard ascorbic acid shows 89.94% of inhibition in NO scavenging assay. Reduced scavenging activity was observed in ethanol extract 3.35% at 10 µg/ml concentration and 20.11% at 100 µg/ml concentration. Many studies have shown that phenolic compounds are the main antioxidant components of medicinal plants.

According to Turkoglu *et al*. (2007) since antioxidants donate protons to the nitrite radical, the absorbance is reduced. The decrease in absorbance was used to determine the extent of nitrite radical scavenging.

Figure 1: Antioxidant activity of *Anaphyllum wightii* Schott. rhizome using different solvent extracts- DPPH radical scavenging activity

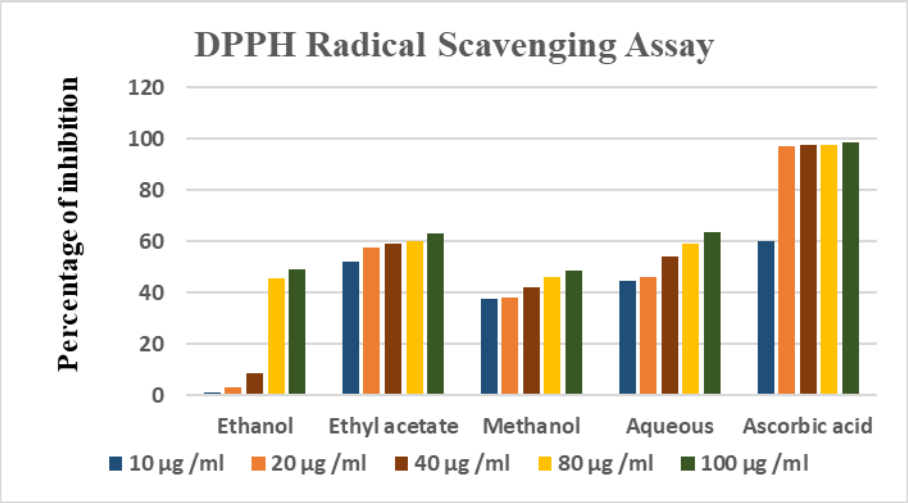
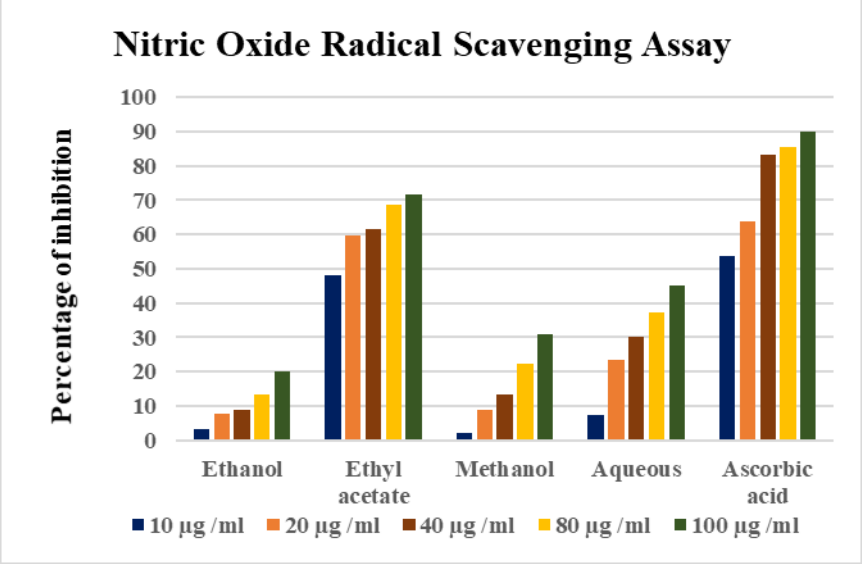


Figure 2: Antioxidant activity of *Anaphyllum wightii* Schott. rhizome using different extracts- Nitric Oxide radical scavenging activity



Conclusion

Plants possess therapeutic chemicals that have been known for their healing properties since the dawn of mankind. Effective research into medicinal plants will provide safe alternative therapies for the next generation. The present study revealed that the rhizome of *Anaphyllum wightii*, has significant antioxidant properties, which could be used to design novel treatment procedures for disorders caused by free radicals.

References

1. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. (2008) Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complement Altern Med. 8(53), 1-8.
2. Alderson WK, Copper CE, Knowel RG. (2001) Nitric oxide synthesis, structure, function and inhibition. J. Biol. Chem.. 593-615.
3. Alinezhad H, Baharfar R, Zare M, Azimi R, Nabavi SF, Nabavi SM. (2012) Biological activities of ethyl acetate extract of different parts of *Hyssopus angustifolius*. Pharm. Biol. 50(8), 1062-1066.
4. Baumann J, Wurn G, Bruchlausen FV. (1979) Prostaglandin synthetase inhibiting O2 radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche Pharmakologische Gesellschaft Abstracts of the 20th spring meeting, Naunyn-Schmiedebergs Abstract No: R27 cited in Arch Pharmacol 307: R1 –R77.
5. Ganie SA, Haq E, Masood A, Hamid A, Zargar MA. (2011) Antioxidant and protective effect of ethyl acetate extract of *Podophyllum hexandrum* rhizome on carbon tetrachloride induced rat liver injury. Evid.-based Complement. Altern. Med. 2011, 1-12.
6. Ganu GP, Jadhav SS, Deshpande AD. (2010) Antioxidant and antihyperglycemic potential of methanolic extract of bark of *Mimusops elengi* L. in mice. Int. J. Phytomedicine. 2(2). 116-123.
7. Guchu BM, Machocho AKO, Mwihia SK, Ngugi MP. (2020) In vitro antioxidant activities of methanolic extracts of *Caesalpinia volkensii* Harms.,*Vernonia lasiopus* O. Hoffm., and *Acacia hockii* De Wild. Evid.-based Complement. Altern. Med. 2020, pp 1-10.

8. Ijaz F, Iqbal Z, Rahman IU, Ali N, Qadir G. (2017) The role of plants in human welfare., J. tradit. med. clin. naturopat. 6(2), 1-4
9. Jain SK, Rao RR. (1977) A Handbook of Field and Herbarium Methods. Today and Tomorrow Printers and Publishers, New Delhi.
10. Luo J, Mills K, Cessie SL, Noordam R, Heemst, DV. (2020) Ageing, age-related diseases and oxidative stress: what to do next?. Ageing Res. Rev. 57, 100982.
11. Nabavi SF, Ebrahimzadeh MA, Nabavi SM, Eslami B. (2010) Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. Grasas y aceites. 61(3), 244-250.
12. Oluyemi KA, Okwuonu UC, Baxter DG, Oyesola T. (2007) Toxic effects of methanolic extract of *Aspilia africana* Leaf on the Estrous Cycle and Uterine Tissues of Wistar Rats. Int. J. Morphol. 25(3), 609-614.
13. Rohman A, Riyanto S, Yuniarti, Saputra WR, Utami R. (2010) Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). Int Food Res. J. 17(1), 97-106.
14. Sarma AD, Mallick AR, Ghosh AK. (2010) Free radicals and their role in different clinical conditions: an overview. Int. J. Pharm. Sci. Res. 1(3), 185-192.
15. Souza-Monteiro JR, Arrifano GP, Queiroz AID, Mello BS, Custódio CS, Macêdo DS, Hamoy M, Paraense RS, Bittencourt LO, Lima RR, Burbano RR (2019) Antidepressant and antiaging effects of Açaí (*Euterpe oleracea* Mart.) in Mice. Oxid. Med. Cell. Longev. 1-16
16. Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. (2007) Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill, Food Chem. 101(1), 267-273.
17. Verma SK, Kumar A, (2011) Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian J. Pharm. Clin. Res. 4(1), 1-4.
18. Wong SP, Lai PL, Jen HW. (2006) Antioxidant activities of aqueous extracts of selected plants. Food Chem. 99, 775-83.
19. Yamagishi SI, Matsui T. (2011) Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy-Friend or foe?. Pharmacol. Res. 64(3), 187-194.

20. Zengin G, Aktumsek A, Guler GO, Cakmak YS, Yildiztugay E. (2011) Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of *Centaurea urvillei* DC. subsp. hayekiana Wagenitz. Rec. Nat. Prod., 5(2), pp. 123-132.
21. Zheng W, Wang SY. (2001) Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49(11), 5165-5170.