Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cr abp

Journal homepage: ht com/journals/msabp/

In-vitro comparative stud

(RESEARCH ARTICLE)

Check for updates

In-vitro comparative study of solv oxidant properties of leaf extracts of *Anthocleista djalonensis*

Chika C Abba [1, *], Chineze B Nsofor [1], Chioma C Udeozor [1], Joseph E Orjiakor [1], Uchechi K Ajah [1], Nnamdi M Adione [1], Onyeka C Obidiegwu [1] and Peter M Eze [2]

[1] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences. Nnamdi Azikiwe University, Awka 420110, Anambra State, Nigeria.*

[2] *Department of Environmental Health sciences, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus Anambra State, Nigeria.*

Magna Scientia Advanced Biology and Pharmacy, 2024, 11(02), 072–079

Publication history: Received on 18 February 2024; revised on 03 April 2024; accepted on 06 April 2024

Article DOI: https://doi.org/10.30574/msabp.2024.11.2.0020

Abstract

This study demonstrated that the extraction solvents play an important role in the extraction of important bioactive compounds with antioxidant properties from the leaves of *Anthocleista djalonensis*. *Anthocleista djalonensis*, a plant native to tropical Africa, is believed to have antioxidant properties due to its bioactive composition. The powdered leaves were macerated using local gin, hot distilled water, and analytical ethanol to extract bioactive compounds. The in vitro antioxidant activity of these extracts was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays, nitric oxide scavenging assay, and hydrogen peroxide scavenging assay. Ascorbic acid was used as a reference. The DPPH and hydrogen peroxide scavenging tests showed concentration-dependent responses. Distilled water had the lowest IC_{50} value of 0.079 mg/ml and showed the most antioxidant potential in DPPH. Local gin demonstrated significant hydrogen peroxide scavenging ability with an IC_{50} value of 0.12 mg/ml, surpassing the standard drug. The nitric oxide scavenging assay showed a concentration-dependent increase in both ethanol and local gin extracts. Ethanol and local gin extracts showed maximum antioxidant activity, with ethanol extract showing 22.84% inhibition and local gin extract showing a higher activity of 46.29% inhibition. However, ascorbic acid showed the highest inhibition of nitric oxide production at 57.10%. The study highlights the importance of solvent selection in herbal preparation for optimal health benefits regarding antioxidant activity.

Keywords: *Anthocleista djalonensis*; Antioxidant activity; DPPH; Hydrogen peroxide; Nitric oxide

1. Introduction

Anthocleista djalonensis (*A. djalonensis*), commonly known as the cabbage tree, belongs to the Gentianaceae family, is widely found in the tropical rainforests of West Africa [1], and stands out for its acknowledged traditional usage in herbal medicine in Nigeria. Various parts of this plant have been shown in scientific reports to have anti-inflammatory, antidiabetic, analgesic, antifungal, and antimalarial properties [2-4]. Also, the plant has been proven to scavenge free radicals, identifying it as a powerful antioxidant agent [5].

Antioxidants are free radical scavengers that protect the human body from the harmful effects of free radicals [6]. It is vital for preventing cellular damage and countering oxidative stress. Oxidative stress occurs when the body's ability to neutralize free radicals is overwhelmed by their production. These free radicals are products of normal metabolic processes in the human body. They can be generated by external factors such as environmental pollution, radiation, cigarette smoke, automobile exhaust, and pesticides [7, 8], which can trigger a series of harmful reactions in the body,

* Corresponding author: Abba Christiana Chika

resulting in chronic conditions, immune system decline, and a number of other life-threatening diseases such as cardiovascular disorders, neurodegenerative conditions, Parkinson's disease, Alzheimer's disease, pulmonary diseases, cancer, diabetes, and age-related ailments [8–13].

The body combats oxidative stress with the help of antioxidants. These antioxidants can be generated within the body or acquired from dietary sources such as fruits, vegetables, green tea, herbs, nuts, mushrooms, and seeds. Studies have shown that consuming these foods can significantly reduce the harmful effects of free radicals [14]. These natural antioxidants are high in phytochemical compounds and can be used instead of synthetic antioxidants to neutralize free radicals and lessen the adverse effects of oxidative stress [15].

Traditional medicinal practices often utilize various solvents, including locally brewed gin (distilled from the fermented sap of the Raphia palm tree) and water, to extract therapeutic phytocompounds from plants. These solvents are used based on historical usage within communities in herbal preparation. To scientifically justify the traditional use of these solvents as extraction solvents, a comparative study was carried out to understand the most effective solvent for extracting phytochemicals from *Anthocleista djalonensis*, rich in antioxidants. Ethanol was included in this study since it contains similar alcoholic properties to local gin but with a standardized composition, providing a basis for comparative analysis. This research aims to conduct an in vitro comparative study of solvents' effect on the antioxidant properties of leaf extracts of *Anthocleista djalonensis.*

2. Materials and methods

2.1. Plant collection

The leaves of *Anthocleista djalonensis* were collected directly from its natural habitat within Nnamdi Azikiwe University's main campus, Agu-Awka, Anambra state, on 15th April 2023. The plants were grown in natural conditions and were not fertilized. Identification and authentication of the plant specimen were done by a taxonomist in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, with a voucher number PCG/474/9/057 and a sample deposit at the Herbarium for future references.

2.2. Preparation of the crude extracts

The leaves of *Anthocleista djalonensis* were rinsed twice with tap water to remove dust before detaching from the twigs. They were air-dried at room temperature, away from direct sunlight. Once dried, the leaves were ground mechanically into a fine powder. The powdered leaves were stored in an airtight container to preserve them for extraction. For the extraction process, the cold maceration technique was employed using ethanol, local gin, and distilled water solvents. About 150 g of the dried powdered leaves were weighed into three separate extraction tanks. Each tank containing the weighed powdered leaves was soaked in 2000 ml of solvents - ethanol, local gin, and hot distilled water, respectively. The mixtures in each tank were then covered with their respective lids and agitated vigorously for 24 hours at room temperature. The extracts were filtered through a muslin cloth and subsequently through a Whatman No 1 filter paper (Whatman®, England). The filtrate obtained from the ethanol extract was transferred to a round bottom flask and concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) under reduced pressure. For the distilled water and local gin extracts, lyophilization was performed. These extracts were first frozen in a deep freezer overnight and then freeze-dried using a lyophilizer to remove any water content. The resulting concentrated extract was transferred into an amber glass bottle and stored at 4 °C until needed for antioxidant activity testing. The percentage yield of each extract was calculated using the formula:

$$\text{Percentage yield} = \frac{\textit{weight of dried extract}}{\textit{weight of pulverized leave used in maceration}} \times 100$$

2.3. In vitro antioxidant evaluation

2.3.1. DPPH radical scavenging assay

The free radical-scavenging ability of *A. djalonensis* leaf extracts in ethanol, local gin, and distilled water was examined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method described by Brand - Williams et al. [16] with some modifications. About 1 ml of DPPH-methanol solution (0.2 mM) was mixed with 5 ml of different concentrations (1.0 – 9.0 mg/ml) of *A. djalonensis* extracts. The mixture was shaken and allowed to stand at room temperature for 30 minutes; the absorbance at 517 nm was measured using a UV-visible spectrophotometer. Ascorbic acid was used as the standard drug. The percentage of free radical scavenging was calculated using the equation:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of test Sample}}{\text{Absorbance of Control}} \times 100$$

The IC_{50} of the different extracts was interpolated from their absorbance concentration curve.

2.3.2. Hydrogen Peroxide Scavenging Assay

The ability of *A. djalonensis* leaf extracts to scavenge hydrogen peroxide was tested using a slightly modified approach published by Randall Ruch et al.[17]. A solution of 4 mM hydrogen peroxide (H_2O_2) was prepared in phosphate buffer (0.1 M pH 7.4). Plant extracts (5 ml) prepared at various concentrations (1.0 – 9.0 mg/ml) were mixed with 1 ml of 4 mM H_2O_2 solution prepared in phosphate buffer. After 10 minutes, the absorbance of the solutions was measured at 230 nm against a blank containing phosphate buffer without hydrogen peroxide and extract. The experiment was carried out in triplicates. The result obtained was compared with standard ascorbic acid. The percentage scavenging activity of the extracts and standard were calculated using the equation:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The IC_{50} of the different extracts was interpolated from their absorbance concentration curve.

2.3.3. Nitric Oxide Scavenging Assay

Nitric oxide scavenging assay was carried out using sodium nitroprusside following the method of Sreejayan and Rao, 1997 [18] with slight modifications. A volume of 2 ml of 5 mM sodium nitroprusside solution was dissolved in 0.5 ml phosphate buffer saline (pH 7.4) and mixed with 5 ml of each plant extract at different concentrations (1.0 – 9.0 mg/ml). The mixture was incubated at 25 °C for 120 minutes. From the incubated mixture, 0.5 ml of the solution was taken out and added into 1 ml Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v)) and further incubated at room temperature for 30 minutes after which absorbance was measured at 550 nm using UV-Visible spectrophotometer. The amount of nitric oxide radical was calculated using the equation:

$$\text{Nitric oxide scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance before the reaction and A_1 is the absorbance after the reaction has taken place.

3. Results and discussion

3.1. Percentage yield of extracts

The leaves of *Anthocleista djalonensis* yielded a total of 30.8g (20.5%) of dried local gin crude extract, 25.6g (17.1%) of dried hot distilled water crude extract and 14.1g (9.4%) of dried ethanol crude extract as shown in **Figure 1.** The Local gin extract yielded more than ethanol and water extract.

3.2. DPPH Free Radical Scavenging Assay

Anthocleista djalonensis leaf extracts' ability to reduce 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to assess their free radical scavenging activities. DPPH is a purple-coloured stable free radical that absorbs light at 517nm. It decolorizes in the presence of antioxidants [19], which indicates that DPPH has been reduced to 2, 2- diphenyl-1-picrylhydrazine and a decrease in absorbance.

As indicated in **figure 2**, the ethanol, water, and local gin extracts had the highest activity at 98.30%, 89.03%, and 73.37%, respectively, whereas ascorbic acid showed 95.61% inhibition. The scavenging activities of the water and ethanol extracts increased as the concentration increased, demonstrating a clear concentration-response relationship. The local gin extract exhibited relatively lower scavenging activity, even at higher concentrations, suggesting that it may be less effective in combating oxidative stress compared to the water and ethanol extracts and standard ascorbic acid, which consistently exhibited strong scavenging activity across all concentrations.

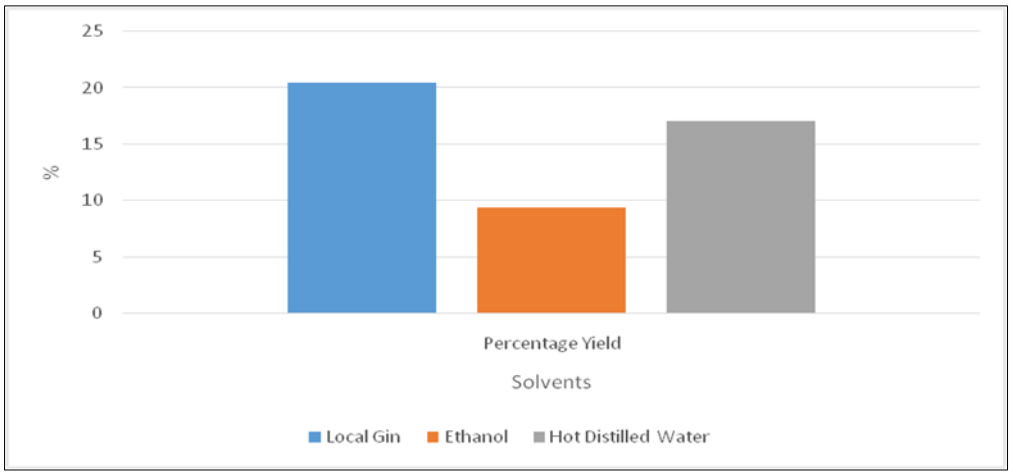

Figure 1 Percentage Yield of *Anthocleista djalonensis* Crude Leaf Extracts

The IC_{50} values can help determine the antioxidant efficacy of the extracts. The concentration at which 50% of the DPPH radicals are scavenged is known as the IC_{50}. A lower IC_{50} value indicates a more potent antioxidant activity. Among the extracts and the standard ascorbic acid, the water extract had the lowest IC_{50} value (0.079 mg/ml), indicating that it was the most effective DPPH radical scavenger with an IC_{50} value of 1.12 mg/ml, ethanol extract demonstrated significant antioxidant activity, though slightly less potent than water extract. In contrast, local gin extract exhibited a higher IC_{50} value of 2.73 mg/ml, indicating lower antioxidant efficiency when compared to the water and ethanol extracts.

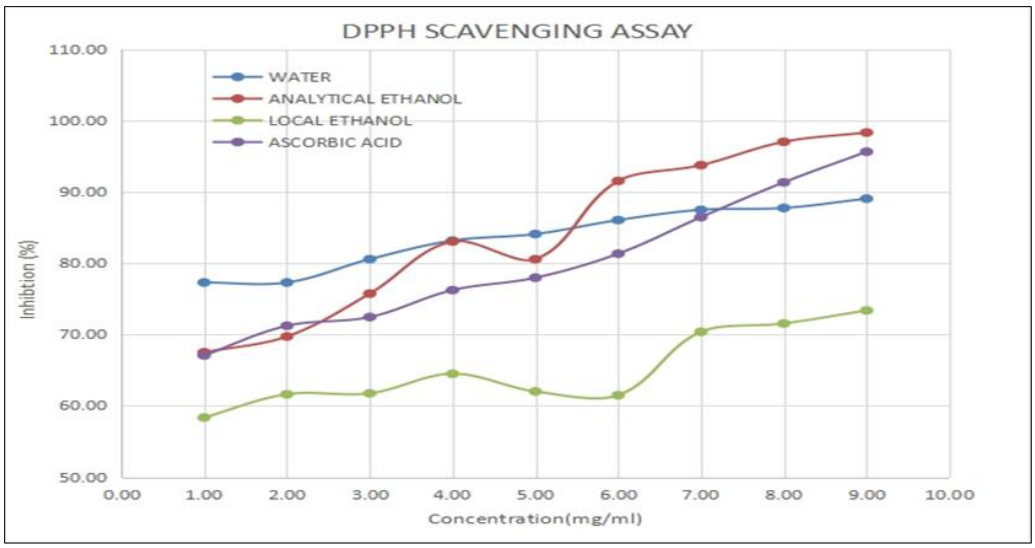

Figure 2 Percentage inhibition on DPPH radical by *Anthocleista djalonensis* leaves extracts compared to ascorbic acid

DPPH assay has been widely used to study the ability of natural antioxidants to scavenge free radicals [20]. The deep purple colour is caused by free radicals, each of which has an odd/ unpaired electron [21]. When an antioxidant compound interacts with the DPPH radical, it donates an electron, neutralizing the radical and discolouring, allowing for measurement by changes in absorbance [22]. The purple colour changes to yellow in the experimental observations, indicating that the odd/unpaired electron of DPPH radical is paired with hydrogen from a free radical scavenging antioxidant to form the reduced 2, 2- diphenyl-1-picrylhydrazine (DPPH-H) at 517 nm.

3.3. Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide is a reactive oxygen species (ROS) that is produced naturally in the human body as a result of several metabolic activities, such as respiration and immunological responses. Hydrogen peroxide acts as a signalling molecule in a variety of biological activities, including cell proliferation, immunological responses, and wound healing, at low concentrations. When hydrogen peroxide levels become high or uncontrolled, it produces hydroxyl radicals within cells, causing lipid peroxidation, DNA damage, and cell death [23]. A. djalonensis leaf extracts were shown to have a high

ability to scavenge hydrogen peroxide radicals, thereby stopping the chain reaction that causes cell damage. Figure 3 proved that the extracts increased their ability to scavenge hydrogen peroxide radicals in a concentration-dependent manner. The water extract had the highest percentage of scavenging activity (98.16%) when compared to the local gin extracts (96.93%), ethanol extracts (88.01%), and the standard drug Ascorbic acid with inhibition of 95.60% at a concentration of 9.0 mg/ml. In contrast to ascorbic acid, with an IC_{50} of 0.31 mg/ml, the IC_{50} values for water, ethanol, and local gin were 0.63 mg/ml, 0.50 mg/ml, and 0.12 mg/ml, respectively. The local gin extract had the lowest IC_{50} value, suggesting that it is the most effective at scavenging hydrogen peroxide.

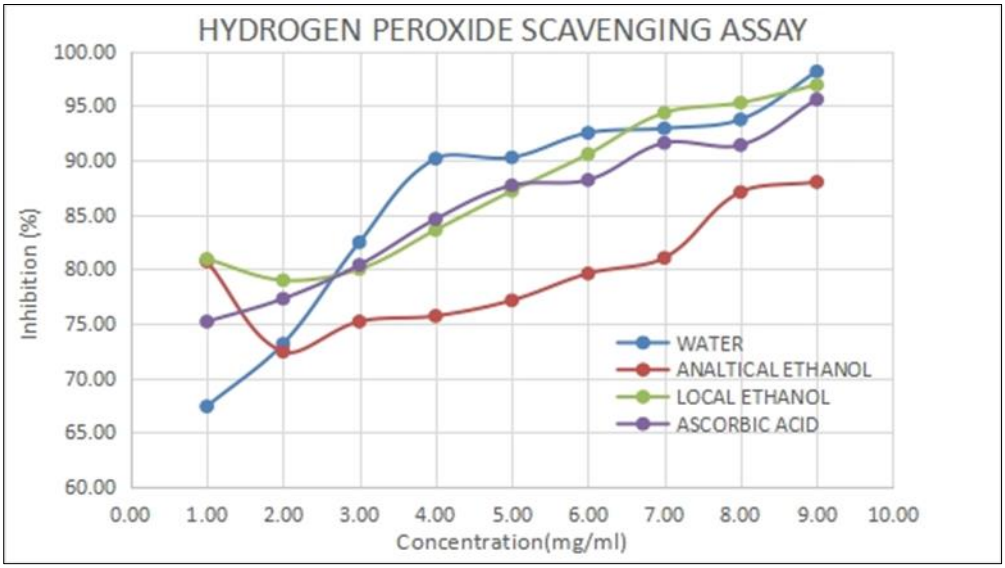

Figure 3 Percentage inhibition on hydrogen peroxide radical by *Anthocleista djalonensis* leaf extracts compared to ascorbic acid

3.4. Nitric Oxide Scavenging Activity

Nitric oxide (NO) is a crucial signalling molecule in the human body, involved in various physiological processes like smooth muscle relaxation, blood pressure control, neuronal signalling, platelet inhibition, and cell toxicity regulation [24-27]. Nitric oxide and reactive nitrogen species (RNS) are formed during their reaction with oxygen or with superoxide, which are very reactive molecules, leading to the generation of harmful radicals [28]. At low concentrations, NO is necessary for its beneficial functions in the body in maintaining overall health and homeostasis [39]. However, during inflammatory responses, infections, or pathological conditions, its production can surge, leading to detrimental effects due to its conversion into reactive nitrogen species (RNS), including free radicals. Nitric oxide scavenging assay suggests that certain compounds known as nitric oxide scavengers or inhibitors can mitigate the harmful effects of excess nitric oxide by reducing its concentration. The leaf extracts from *Anthocleista djalonenesis* showed the ability to scavenge nitric oxide radicals, as seen in Figure 3 of the results. It was observed that the nitric oxide scavenging activity varied with the different concentrations of the leaf extracts used, indicating that its ability to neutralize nitric oxide radicals is dependent on the concentration of the extracts. Figure 3 illustrates the results of NO radical scavenging by *Anthocleista djalonensis* leaf extracts. The leaf extracts of *Anthocleista djalonenesis* exhibited nitric oxide scavenging activity at various concentrations, and this suggests that the effectiveness of these extracts in neutralizing nitric oxide radicals is influenced by the concentration at which they are used. Among the extracts studied, the water extract displayed the highest nitric oxide scavenging activity, with a maximum inhibition of 34.81% at a concentration of 8.00 mg/ml.

Furthermore, both ethanol and local gin extracts showed concentration-dependent increases in nitric oxide scavenging activity, with maximal activities of 22.84% and 46.29% at the highest concentration of 9.00 mg/ml, respectively. Higher concentrations of these extracts may be more efficient in reducing excess nitric oxide. In comparison to the leaf extracts, ascorbic acid, used as the reference, inhibited nitric oxide production the most, with 57.10% inhibition at a concentration of 9.00 mg/ml. The IC_{50} values for the distilled water, absolute ethanol, and local gin were 18.13mg/ml, 17.93mg/ml and 9.35 mg/ml, respectively. The standard (Ascorbic acid) had an IC_{50} value of 7.17mg/ml, indicating that the local gin extract of Anthocleista djalonensis leaf demonstrated the most potent nitric oxide scavenging activity among the studied solvents but was still less efficient than ascorbic acid. Based on the observed differences in nitric oxide scavenging activity among the extracts, it appears that Anthocleitsa djalonensis leaves may not be as effective as

the standard drug used in neutralizing nitric oxide radicals. This difference could be attributed to the specific compounds present in the extracts, which may not be as effective at neutralizing nitric oxide radicals. The effectiveness of antioxidant activity depends on the types of compounds present and the radical or reactive species exposed to the extract. The extracts may show more significant nitric oxide scavenging activity at higher concentrations than those tested in the study. Antioxidants often have preferences for certain types of radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as nitric oxide. An antioxidant that is effective at scavenging certain radicals might possess a different efficacy against nitric oxide [30].

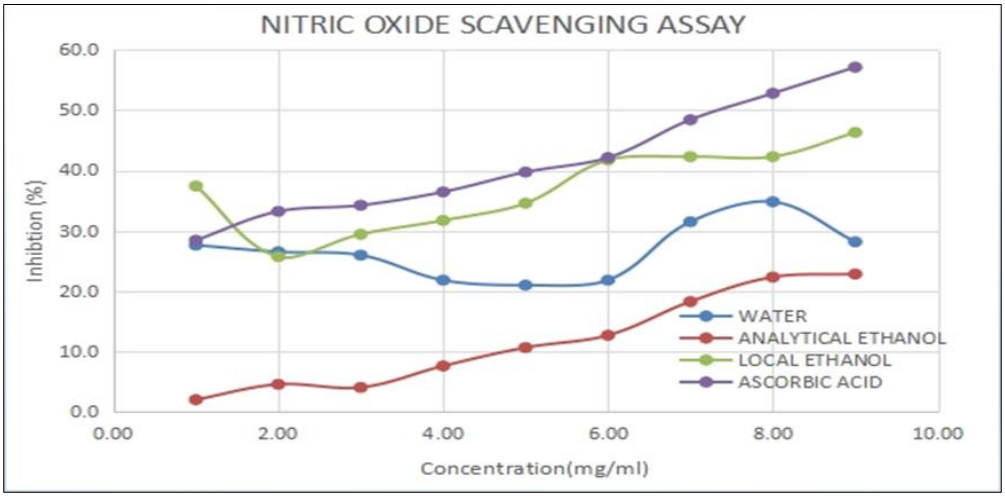

Figure 4 Results of nitric oxide scavenging activities of *Anthocleista djalonensis* leaf extracts compared to ascorbic acid

4. Conclusion

Based on the obtained results, it can be concluded that the crude extracts of *A. djalonensis* leaf in ethanol, local gin, and distilled water have appreciable antioxidant activities and contain antioxidant-associated phytochemicals. The varying antioxidant activities observed across different solvent extracts can be explained by the variation of bioactive compounds extracted by the solvents. Different solvents have varying polarities, which affect their ability to extract specific bioactive compounds. Therefore, the choice of solvent used for extraction can significantly impact the type and amount of bioactive compounds present in the extract and, consequently, its antioxidant activity. The DPPH free scavenging assay showed the highest activity of the crude extracts in the following order: ethanol > distilled water > local gin. On the other hand, in the hydrogen peroxide scavenging activity, the order of potency was seen as distilled water > local gin > ethanol. Furthermore, the local gin extract demonstrated the most potent nitric oxide scavenging activity among the extracts. Further studies that aim at isolating and characterizing the pure bioactive compounds of *Anthocleista djalonensis* are highly recommended. The identification of these compounds will help in understanding their mode of action and provide insights into the mechanism of their antioxidant activity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Ezirim CY, Abarikwu SO, Uwakwe AA. Effects of Anthocleista djalonensis root extracts on reproductive hormones and testicular marker enzymes in adult male Wistar rats. Andrologia. 2019 Dec;51(11):e13442.

[2] Anyanwu GO, Onyeneke CE, Rauf K. Medicinal plants of the genus Anthocleista—A review of their ethnobotany, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2015 Dec 4;175:648-67.

[3] Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. Journal of ethnopharmacology. 2006 Mar 8;104(1-2):164-7.

[4] Bassey AS, Okokon JE, Etim EI, Umoh FU, Bassey E. Evaluation of the in vivo antimalarial activity of ethanolic leaf and stembark extracts of Anthocleistadjalonensis. Indian journal of pharmacology. 2009 Nov 1;41(6):258-61.

[5] Monon KO, COULIBALY OS, KOUANGBE AM, OUATTARA K, COULIBALY A. Evaluation of the antioxidant and antibacterial activity of aqueous and ethanoic extracts of two medicinal plants used to treat diarrhea in Côte d'Ivoire. GSC Biological and Pharmaceutical Sciences. 2022;21(3):014-20.

[6] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 2007 Jan 1;39(1):44-84.

[7] Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford university press, USA; 2015.

[8] Ebadi M. Antioxidants and free radicals in health and disease: An introduction to reactive oxygen species, oxidative injury, neuronal cell death and therapy in neurodegenerative diseases. Arizona: Prominent Press. 2001.

[9] Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Radical Biology and Medicine. 2002 Jun 1;32(11):1102-15.

[10] Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. Cardiovascular research. 2002 Aug 1;55(2):239-49.

[11] Wright Jr E, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. International journal of clinical practice. 2006 Mar;60(3):308-14.

[12] Fasano M, Bergamasco B, Lopiano L. Modifications of the iron–neuromelanin system in Parkinson's disease. Journal of neurochemistry. 2006 Feb;96(4):909-16.

[13] Meydani M. Nutrition interventions in aging and age-associated disease. Annals of the New York Academy of Sciences. 2001 Apr;928(1):226-35.

[14] Traber MG, Shils ME, Shike M, Ross AC, Caballero B, Cousins R. Modern nutrition in health and disease. 10th edition. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 396–411.

[15] Patel VR, Patel PR, Kajal SS. Antioxidant activity of some selected medicinal plants in western region of India. Advances in Biological research. 2010;4(1):23-6.

[16] Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995 Jan 1;28(1):25-30.

[17] Ruch RJ, Crist KA, Klaunig JE. Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. Toxicology and applied pharmacology. 1989 Sep 15;100(3):451-64.

[18] Sreejagan P, Rao MVR. Antioxidant Activity of Flavonoids. Free radical biology and medicine. 1997;23(2):215–224.

[19] Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free radical scavenging activity. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2007 Jul;21(7):615-21.

[20] Jao CL, Ko WC. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Fisheries science. 2002;68(2):430-5.

[21] Gülçin İ. Antioxidant activity of eugenol: A structure–activity relationship study. Journal of medicinal food. 2011 Sep 1;14(9):975-85.

[22] Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant Kandeliacandel. International journal of molecular sciences. 2010 Oct 21;11(10):4080-93.

[23] Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. Journal of the American college of Nutrition. 2000 Jun 1;19(sup3):312S-9S.

[24] Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proceedings of the National Academy of Sciences. 1990 Jan;87(2):682-5.

[25] Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proceedings of the National Academy of Sciences. 1989 May;86(9):3375-8.

[26] Tsai PJ, Tsai TH, Yu CH, Ho SC. Evaluation of NO-suppressing activity of several Mediterranean culinary spices. Food and chemical Toxicology. 2007 Mar 1;45(3):440-7.

[27] Wink DA, Kasprzak KS, Maragos CM, Elespuri RK, Misra M, Dunams TM, Cebula TA, Koch WH, Andrews AW, Allen JS, Keefer LK. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science. 1991;254:1001–1003.

[28] Tsai P-J, Tsai T-H, Yu C-H, Ho S-C. Evaluation of NO suppressing activity of several Mediterranean culinary spices. Food ChemToxicol. 2007;45(3):440-447.

[29] Bhaskar H, Balakrishnan N. In vitro antioxidant property of laticiferous plant species from western ghatsTamilnadu, India. International journal of health research. 2009;2(2).

[30] Oliveira-Alves SC, Andrade F, Sousa J, Bento-Silva A, Duarte B, Caçador I, Salazar M, Mecha E, Serra AT, Bronze MR. Soilless cultivated halophyte plants: volatile, nutritional, phytochemical, and biological differences. Antioxidants. 2023 May 26;12(6):1161.