

Evaluation of the Antioxidant Potential of Calix[4]Pyrrole ,Resorcin[4]Arene, and Pyrogallol[4]Arene Via DPPH Assay

Samer Salem Alhamami ¹, Maan Alnuaim ², Leaqa Abduredha Raheem ³
^{1,2,3} Department of pharmaceutical chemistry, college of pharmacy, university of Basrah,
Basrah, Iraq

Email: samer.salem.ph83@gmail.com ¹, Maanabdulrazzaqswaid@gmail.com ²,
Leaqa.raheem@uobasrah.edu.iq ³

Abstract. Background: Oxidative stress, caused by an imbalance between free radical production and antioxidant defenses, contributes to various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Antioxidants mitigate these effects by neutralizing free radicals, preventing cellular damage. The DPPH assay is a widely used method for evaluating antioxidant activity, measuring the ability of compounds to reduce the stable DPPH radical and decrease absorbance at 517 nm. Calix[4]pyrrole, Resorcin[4]arene, and Pyrogallol[4]arene derivatives are macrocyclic compounds with structural features that influence their antioxidant properties. objective: This study aims to assess the antioxidant activity of Calix[4]pyrrole derivatives, Resorcin[4]arene derivatives, and Pyrogallol[4]arene derivatives using the DPPH radical scavenging assay to determine their potential for biomedical applications. Material and Methods: The DPPH assay was conducted by preparing a 0.4 mg/mL DPPH solution and measuring the reduction in absorbance at 517 nm after interaction with the tested compounds. The scavenging activity (%) was calculated using the standard equation. Results: : Pyrogallol[4]arene derivative (Cpd5) exhibited the highest antioxidant activity (65.81% ± 0.12), followed by Resorcin[4]arene derivative (Cpd4) with 59.79% ± 0.25. In contrast, Calix[4]pyrrole derivatives displayed lower antioxidant activity, with Cpd1 and Cpd6 showing the least efficiency at 7.38% ± 0.09 and 5.76% ± 0.09, respectively. Conclusion: The findings suggest that the antioxidant activity is significantly influenced by the presence and arrangement of hydroxyl groups. Compounds with a higher number of hydroxyl groups demonstrated superior radical scavenging potential, making them promising candidates for further pharmacological studies.

Highlights:

1. Oxidative stress leads to diseases; antioxidants counteract free radicals.
2. Evaluate antioxidant activity of macrocyclic compounds using DPPH assay.
3. Hydroxyl-rich compounds showed highest activity, promising for pharmacological studies.

Keywords: DPPH assay, antioxidant activity, Calix[4]pyrrole, Resorcin[4]arene, Pyrogallol[4]arene, free radicals

Introduction

Oxidative stress, caused by the imbalance between free radicals and antioxidants, is linked to numerous diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions[1]. The development of potent antioxidant compounds has

garnered significant attention in biomedical research [2]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to evaluate the free radical scavenging capacity of chemical compounds [3].

Calix[4]pyrrole, Resorcin[4]arene, and Pyrogallol[4]arene derivatives are macrocyclic compounds known for their unique structural properties and potential biological activities [4]. Calix[4]pyrroles are composed of four pyrrole units linked by methylene bridges, and their antioxidant properties are influenced by functional groups attached to the ring [5]. Resorcin[4]arene derivatives, on the other hand, contain resorcinol-based cyclic frameworks with hydroxyl groups that contribute to radical scavenging activity [6]. Pyrogallol[4]arene derivatives are similar but have a higher number of hydroxyl groups, which may enhance their antioxidant potential [7].

This study focuses on evaluating the antioxidant potential of these derivatives and aims to determine the correlation between their structural features and radical scavenging activity using the DPPH assay.

Methods

Studying the in vitro antioxidant activity of the calix[4]pyrrole derivatives (Cpd1, Cpd2, Cpd3, Cpd6, Cpd7), resorcin[4]arene derivatives (Cpd4) and pyrogallol[4]arene derivatives (Cpd5) are done at the Baghdad's phi center for nanoscience, department of biology. DPPH, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a common abbreviation for an organic chemical compound that is a dark-colored crystalline powder composed of stable free radical molecules. It has two major applications in laboratory research: As a monitor of chemical reactions involving radicals, most notably as a common antioxidant assay, and as a standard for the position and intensity of electron paramagnetic resonance signals. Because of its strong absorption band centered at about 517 nm, DPPH has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 517 nm or in the EPR signal of the DPPH [8].

The Preparation of DPPH Stain

The preparation of DPPH (2,2-diphenyl-1-picrylhydrazyl) stain for antioxidant activity involves a few key steps. Here's a brief overview of the process. Typically, 40 mg of DPPH powder is dissolved in 100 ml of ethanol to create a 0.4 mg/ml solution, mixed for 5min on magnetic stirrer. Quantitatively transfer the solution to a volumetric flask, the DPPH solution is stored in the fridge, wrapped in foil to protect it from light and reduce degradation [9].

The Preparation of Ascorbic acid

Dissolving 0.5g of Ascorbic acid in 50ml of deionized water and 50ml of ethanol and mixed for 5min on magnetic stirrer [10].

The Preparation of Samples

Each test sample (1, 2, 3, 4, 5, 6, 7) contains 250 microgram of the substance to be analyzed.

- 250 micrograms of each sample is dissolved in 1 mL of the solvent (ethanol) before being added to the DPPH solution.
- Preparing of (+ve): Adding 1ml of both DPPH and Ascorbic acid in the tube.
- Preparing of (-ve): Adding 1ml of DPPH and Ethanol in the tube.
- Preparing of samples (1, 2, 3, 4, 5, 6, 7): adding 1ml of both DPPH and samples (1, 2, 3, 4, 5, 6, 7). in the tube. Then incubate the tubes for 30min at room temperature, after that we measure the optical density (OD) of them [11].

DPPH Assay

DPPH is stable free radicals, which are binding with replacement electrons. DPPH can be mixed with a substrate that can give a hydrogen atom. This may give a rising reduced form with the color change from violet to yellow protected from light before determining their optical density OD at 517nm. The sample with lowest OD shows the highest scavenging activity (%) of DPPH which is calculated using Equation (1) [12].(Figure 1)

$$DPPH\ Scavenging\ activity\ (\%) = \left[\frac{OD_{Control} - OD_{Sample}}{OD_{Control}} \right] \times 100 \quad (1)$$

Where: $OD_{Control}$ is Negative

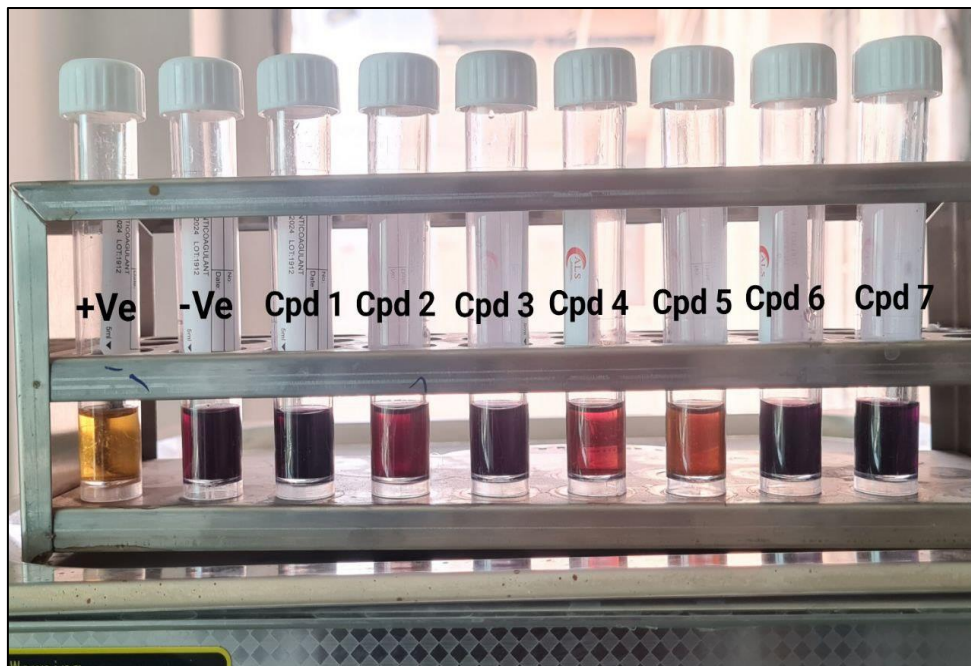


Figure 1: The steps of DPPH Assay.

Statistical analysis

Data were analyzed statically with the Prism tool from Graphpad. The three experiments' mean \pm standard deviation is used to illustrate the data. At $p < 0.05$, indicate a statistically significant difference [13],[14].

Result and Discussion

The antioxidant activity of the tested compounds was evaluated using the DPPH radical scavenging assay, with ascorbic acid (A.C) as the reference standard. The results, presented in (Table 1), indicate a wide variation in scavenging activity among the studied derivatives.

Table (1): The antioxidant activity (%) values of ascorbic acid and compounds (1-7) against DPPH have been expressed as mean of activity $\% \pm$ standard deviation.

| \ | Cpd1 | Cpd2 | Cpd3 | Cpd4 | Cpd5 | Cpd6 | Cpd7 | |
|---|------------------|-----------------|------------------|------------------|------------------|------------------|-----------------|-----------------|
| | 89.70 \pm 0.11 | 7.38 \pm 0.09 | 47.11 \pm 0.62 | 19.01 \pm 0.19 | 59.79 \pm 0.25 | 65.81 \pm 0.12 | 5.76 \pm 0.09 | 6.05 \pm 0.05 |

Discussion

The presence of hydroxyl (-OH) groups significantly influenced antioxidant activity. Pyrogallol[4]arene (Cpd5) exhibited the highest scavenging activity ($65.81\% \pm 0.12$) due to its high number of hydroxyl groups (16 positions) [15],[16]. Calix[4]pyrrole derivatives exhibited low antioxidant activity due to steric hindrance and the limited availability of hydroxyl groups [17],[18]. Although none of the tested compounds surpassed the activity of ascorbic acid ($89.70\% \pm 0.11$), Pyrogallol[4]arene (Cpd5) and Resorcin[4]arene (Cpd4) displayed noteworthy antioxidant potential, suggesting their potential application in biomedical fields [19],[20]. (Figure 2)

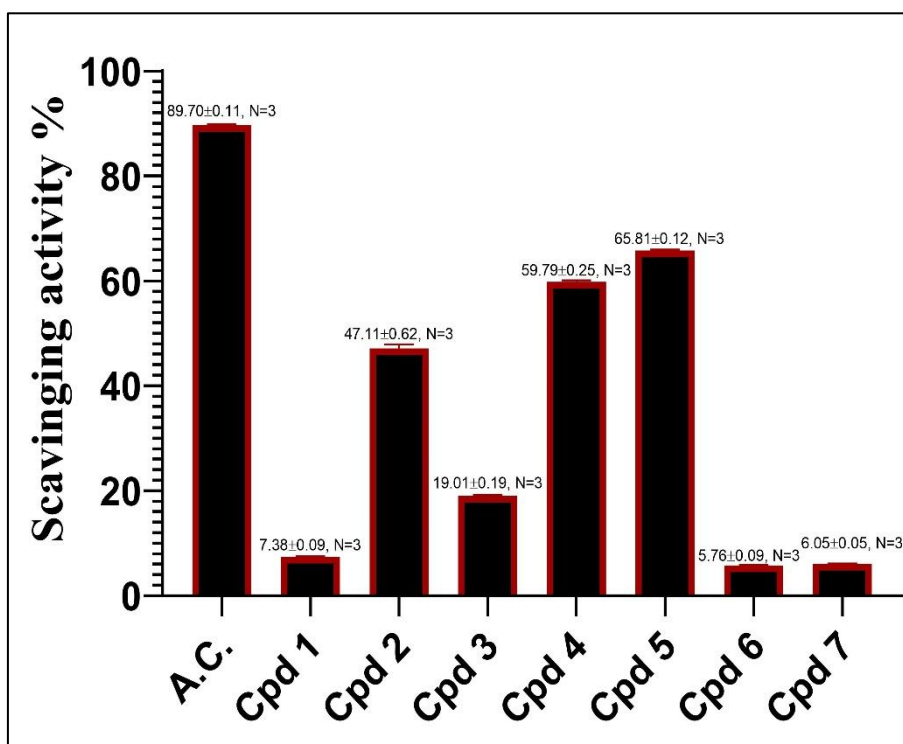


Figure 2: DPPH Assay of samples (Cpd1,Cpd 2,Cpd 3,Cpd 4,Cpd 5,Cpd 6, Cpd7).

Conclusions

This study evaluated the antioxidant activity of Calix[4]pyrrole, Resorcin[4]arene, and Pyrogallol[4]arene derivatives using the DPPH assay. The results indicated that:

Pyrogallol[4]arene(Cpd5) exhibited the highest antioxidant activity, followed by Resorcin[4]arene(Cpd4). Calix[4]pyrrole derivatives displayed lower scavenging potential

due to structural constraints and fewer hydroxyl groups. The presence and arrangement of hydroxyl groups play a crucial role in free radical scavenging activity.

Future studies should focus on modifying these compounds to enhance their antioxidant efficacy for potential pharmaceutical and biomedical applications.

Financial support and sponsorship

Nil.

Conflict of interest

There are no conflicts of interest.

References

- [1] R. P. Singh, S. Sharad, and S. Kapur, "Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants," *J. Indian Acad. Clin. Med.*, vol. 5, no. 3, pp. 218–225, Jul. 2004.
- [2] Z. Hussain, H. E. Thu, M. W. Amjad, F. Hussain, T. A. Ahmed, and S. Khan, "Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: A review of new trends and future perspectives," *Mater. Sci. Eng. C*, vol. 77, pp. 1316–1326, Aug. 2017.
- [3] J. Xie and K. M. Schaich, "Re-evaluation of the 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity," *J. Agric. Food Chem.*, vol. 62, no. 19, pp. 4251–4260, May 2014.
- [4] M. A. Al-Nuaim, "The physico-chemical properties of supramolecular systems involving pharmaceuticals and anions," Ph.D. dissertation, Univ. Surrey, United Kingdom, 2016.
- [5] A. Y. Rubaye, A. A. Abdulwahid, and H. T. Abdulsahib, "Designing novel calix [4] pyrrole derivative for treatment of herbicides: Thermodynamics of host-guest interactions," *Asian J. Res. Chem.*, vol. 8, no. 6, pp. 413–427, 2015.
- [6] R. Kashapov et al., "Emergence of nanoscale drug carriers through supramolecular self-assembly of RNA with calixarene," *Int. J. Mol. Sci.*, vol. 24, no. 9, p. 7911, Apr. 2023.

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April

ISSN 3063-8186. Published by Universitas Muhamamadiyah Sidoarjo
Copyright © Author(s). This is an open-access article distributed under the terms of
the Creative Commons Attribution License (CC-BY).

<https://doi.org/10.21070/ijhsm.v2i2.107>

- [7] J. Ni, L. Lu, and Y. Liu, "Antiradical and antioxidative activity of azocalix [4] arene derivatives: combined experimental and theoretical study," *Molecules*, vol. 24, no. 3, p. 485, Jan. 2019.
- [8] M. E. Abalaka, A. Mann, and S. O. Adeyemo, "Studies on in-vitro antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spinachristi* L. compared with ascorbic acid," *J. Med. Genet. Genomics*, vol. 3, no. 2, pp. 28–34, Feb. 2011.
- [9] C. Egbuna et al., "Phytochemical test methods: qualitative, quantitative and proximate analysis," in *Phytochemistry*, Apple Academic Press, Dec. 2018, pp. 381–426.
- [10] Y. Y. Shao, "Synthesis and properties of D-erythroascorbic acid, and the vitamin C activities of D-erythroascorbic acid and other derivatives of ascorbic acid in the tobacco hornworm, *Manduca sexta*," Ph.D. dissertation, Kansas State Univ., 1992.
- [11] S. B. Kedare and R. P. Singh, "Genesis and development of DPPH method of antioxidant assay," *J. Food Sci. Technol.*, vol. 48, pp. 412–422, Aug. 2011.
- [12] O. P. Sharma and T. K. Bhat, "DPPH antioxidant assay revisited," *Food Chem.*, vol. 113, no. 4, pp. 1202–1205, Apr. 2009.
- [13] G. Lladós et al., "Vagus nerve dysfunction in the post-COVID-19 condition: a pilot cross-sectional study," *Clin. Microbiol. Infect.*, vol. 30, no. 4, pp. 515–521, Apr. 2024.
- [14] Z. Zhu et al., "Lactate mediates the bone anabolic effect of high-intensity interval training by inducing osteoblast differentiation," *JBJs*, vol. 105, no. 5, pp. 369–379, Mar. 2023.
- [15] H. M. Abosadiya, S. A. Hasbullah, and B. M. Yamin, "Synthesis, crystal structure and antioxidant evaluation of C-4-acetamidophenylcalix [4] pyrogallolarene," in *AIP Conf. Proc.*, vol. 1678, no. 1, AIP Publishing, Sep. 2015.
- [16] K. Sikligar, "The investigation of the self-assemblies of pyrogallol [4] arenes with large metal ions," Ph.D. dissertation, Univ. Missouri–Columbia, 2015.
- [17] S. Ishihara et al., "Porphyrin-based sensor nanoarchitectonics in diverse physical detection modes," *Phys. Chem. Chem. Phys.*, vol. 16, no. 21, pp. 9713–9746, 2014.
- [18] R. Castañeda-Arriaga, A. Pérez-González, M. Reina, J. R. Alvarez-Idaboy, and A. Galano, "Comprehensive investigation of the antioxidant and pro-oxidant effects

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April

ISSN 3063-8186. Published by Universitas Muhamamadiyah Sidoarjo
Copyright © Author(s). This is an open-access article distributed under the terms of
the Creative Commons Attribution License (CC-BY).

<https://doi.org/10.21070/ijhsm.v2i2.107>

- of phenolic compounds: A double-edged sword in the context of oxidative stress?,"
J. Phys. Chem. B, vol. 122, no. 23, pp. 6198–6214, 2018.
- [19] B. I. Ngurah, "Synthetic C-methoxyphenyl calix [4] resorcinarene and its
antioxidant activity," J. Appl. Chem. Sci., vol. 5, no. 1, pp. 403–408, 2018.20.
Yuanita E, Ulfa M, Ramadhini RA, Dharmayani NK, Riyaldi A. Synthesis and
Antioxidant Activity of Calix [4] resorcinarene Derivatives Compounds. In1st Nusa
Tenggara International Conference on Chemistry (NITRIC 2022) 2023 Apr 14 (pp.
75-82). Atlantis Press.
- [20] E. Yuanita, M. Ulfa, R. A. Ramadhini, N. K. Dharmayani, and A. Riyaldi, "Synthesis
and antioxidant activity of calix [4] resorcinarene derivatives compounds," in Proc.
1st Nusa Tenggara Int. Conf. Chem. (NITRIC 2022), Atlantis Press, Apr. 14, 2023,
pp. 75–82.