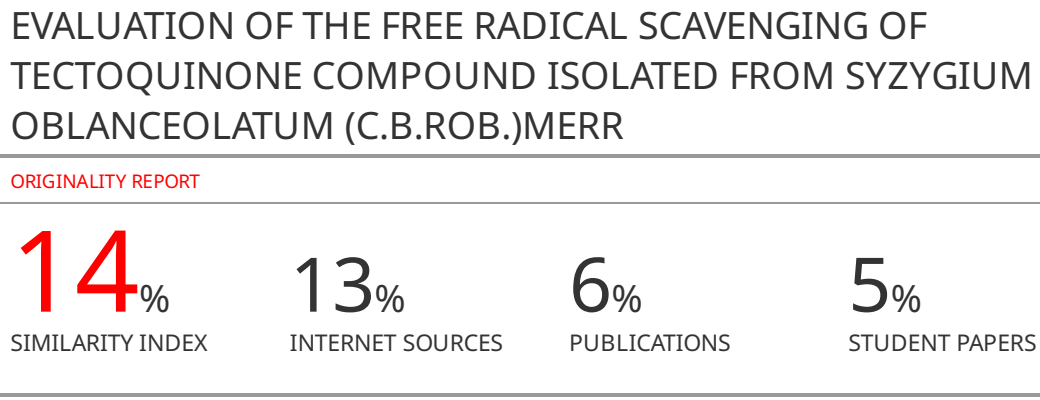
**Reviewer’s Comments**

**EVALUATION OF THE FREE RADICAL SCAVENGING OF TECTOQUINONE COMPOUND ISOLATED FROM *SYZYGIUM OBLANCEOLATUM* (C.B.ROB.)MERR**

**ABSTRACT**

In this study, the free radical scavenging of tectoquinone compound isolated from *Syzygium oblanceolatum* (C.B. Rob.) Merr was investigated by assessing their capacity to scavenge free radicals using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The quantitative assessment of antioxidant activity was conducted using a UV-Vis spectrophotometric approach, with measurements taken at a wavelength of 516 nm.The IC50 value, representing the concentration required to inhibit 50% of the DPPH radicals, was determined to be 66.362 μg/mL, indicating a moderate free radical-scavenging activity. These findings suggest that tectoquinone compounds possess a discernible ability to against free radical, though further research may be necessary to optimize their potential applications in health and medicine.

**Keywords:** Tectoquinone, *Syzygium oblanceolatum*, DPPH assay, IC50 value, free radical

**INTRODUCTION**

Oxidative stress is a fundamental contributor to various health disorders and is primarily associated with the detrimental effects of reactive oxygen species (ROS) on biological systems1. Consequently, the exploration of natural compounds with antioxidant properties has become a focal point in scientific research, as these compounds have the potential to mitigate the adverse effects of free radical compounds2.

Tectoquinone compound, isolated from the plant *Syzygium oblanceolatum* (C.B. Rob.) Merr, represents a group of compounds that have attracted attention for their potential as free radical scavenging. *S. oblanceolatum* represents the first documented report of its existence in Sulawesi, previously known to occur only in Eastern Philippines and Kalimantan3. The presence of this plant in Sulawesi, particularly in South Sulawesi, is intriguing and warrants further investigation.*S. oblanceolatum* from a family of Myrtaceae is a well-known family of vascular dicot plants4 (figure 1). As the eighth-largest dicot plant family, it comprises approximately 5,650 species grouped into 130-150 generad5and is widely distributed across Africa, extending into South Asia and tropical Southeast Asian countries6. Due to their phytochemical content and health-promoting properties, several *Syzygium* species have garnered significant interest7,8. On the other hand, many other *Syzygium* species remain relatively unexplored9, and their chemical and biological activities continue to be of interest including the class of quinones compound10.

Quinones, a class of organic compounds, exhibit diverse medical properties11. They are recognized for their antioxidant capabilities12, with compounds like Coenzyme Q10 and Vitamin K helping combat oxidative stress13. In cancer treatment, specific quinones, such as anthracyclines, interfere with cancer cell growth14. Quinones also play a role in skincare, blood clotting15, and neurological disorders16. Some quinones demonstrate antibacterial17, antifungal18, and anti-inflammatory effects19. CoQ10, in particular, is studied for mitochondrial function and cardiovascular health20. While promising, the use of quinone-based treatments should always involve consultation with healthcare professionals due to potential side effects and interactions with other medications.



**Figure 1: Plant of *Syzygium oblanceolatum* (C.B. Rob.) Merr**

Tectoquinone (as a member of the class of quinones compound)21is a naturally occurring compound that has attracted attention due to its potential antioxidant properties. It is often found in certain plant species, and researchers have been investigating its ability to scavenge free radicals, which are harmful molecules associated with oxidative stress and various health issues.

Tectoquinone is a type of organic compound. Specifically, it belongs to the class of compounds known as quinones. Quinones are a class of organic compounds characterized by a six-membered aromatic ring containing two carbonyl (C=O) functional groups22. Tectoquinone, like other quinones, is known for its chemical structure that includes this characteristic aromatic ring with carbonyl groups, which contributes to its reactivity and potential biological activities, including its antioxidant properties23.

In this study, we investigate the capacity of tectoquinone compounds to scavenge free radicals, a critical aspect of their antioxidant activity. The widely recognized DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was employed for this purpose, offering a reliable means to assess the radical-scavenging abilities of compounds.

To provide a quantitative assessment of the free radical scavenging activity, we utilized a UV-Vis spectrophotometric approach, enabling precise measurements at a specific wavelength. This method allowed us to obtain valuable data on the antioxidant potential of the tectoquinone compound.

**MATERIALS AND METHODS**

**Materials**

The samples used in this research are tectoquinone compound previously isolated from *Syzygium oblanceolatum* (C.B.Rob.) Merr., DPPH (Sigma Aldrich), Quercetin (Sigma Aldrich).

**Methods**

The study was carried out through experimental procedures conducted in a laboratory setting, employing the free radical scavenging method

**Preparation of DPPH Stock Solution**

To prepare a DPPH solution with a concentration of 30 ppm, 1.5 mg of DPPH powder was dissolved in 50 mL of high-quality analytical methanol within a volumetric flask. Following this, the measurement of the DPPH's maximum wavelength was conducted within the range of 450 nm to 550 nm.

**Preparation Quercetin Standard Solution**

To prepare a reference quercetin solution, a concentration of 1,000 ppm was initially established. Subsequently, various concentrations, including 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1 ppm, were derived from this primary solution, with each variant comprising a 5 mL volume. A 2 mL aliquot of each quercetin solution series was then measured and transferred to separate vials. Next, 2 mL of DPPH solution was introduced into each vial. Following thorough mixing, the vials were left to incubate in a dark environment for 30 minutes. After this incubation period, the absorption at the maximum wavelength was determined using a UV-Vis spectrophotometer, namely 516 nm.

**Preparation of Tectoquinone**

Weighing 0.7 mg of the tectoquinone compound, we then dissolved it in 2.5 mL of high-quality analytical methanol, resulting in a stock solution with a concentration of 280 ppm. Following that, a range of concentrations was created by diluting 4 mL of this solution to produce concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm.

**Examination of Free Radical Scavenging**

To assess the radical scavenging of tectoquinone compound, 2 mL of test solutions with concentrations ranging from 10 ppm to 50 ppm were mixed with an equal volume of DPPH solution in vials and left to incubate in darkness for 30 minutes. Following this incubation period, their absorbance was measured at the maximum wavelength, namely 516 nm.

The percentage of DPPH radical inhibition was determined using the formula: % inhibition = (A1 - A2) / A1 x 100%, where A1 represents the absorbance of the control and A2 denotes the absorbance of the sample.

To find the IC50 value, a linear curve was established by plotting the test solution concentrations (x-axis) against the corresponding % inhibition (y-axis) using the equation y = a + bx. The IC50 value was then calculated as IC50 = (50 - a)/b.

**Statistical analysis**

**RESULTS AND DISCUSSION**

In the quantitative assessment of antioxidant potential, the DPPH scavenging method relies on the IC50 value. This value indicates the concentration of the test sample required to achieve a 50% inhibition of oxidative processes (effectively reducing or inhibiting oxidation by 50%). The outcomes of absorbance measurements, percentage inhibition, and IC50 values for both tectoquinone compound and the reference quercetin are provided in the table presented below as Table 1.

**Table 1. Results of absorbance measurements, percentage inhibition, and IC50 values**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Concertation (ppm) | Blank Absorbance | | Sample Absorbance | | Inhibition  (%) | IC50 (μg/mL) |
| Tectoquinone | 10 | | 0,596 | | 0,346 | 41,750 | 66,362 |
| 20 | | 0,596 | | 0,335 | 43,602 |
| 30 | | 0,596 | | 0,321 | 45,959 |
| 40 | | 0,596 | | 0,319 | 46,296 |
| 50 | | 0,596 | | 0,313 | 47,306 |
| Quercetin | 0,2 | | 0,596 | | 0,310 | 47.986 | 0,237 |
| 0,4 | | 0,596 | | 0,264 | 55,704 |
| 0,6 | | 0,596 | | 0,252 | 57,718 |
| 0,8 | | 0,596 | | 0,245 | 58,892 |
| 1,0 | | 0,596 | | 0,204 | 65,771 |

Based on the data above the relationship between % inhibition and the concentration of tectoquinone compounds is depicted in figure 1. Linear regression equations were established on the graphs, where concentration is plotted on the x-axis, and % inhibition is on the y-axis. Consequently, the IC50 value for tectoquinone compounds can be determined from the regression equation.

The linear regression obtained from the quercetin reference is y = 3.8758x + 45.587 with an R2 value of 0.9175, and for tectoquinone compounds, it is y = 0.138x + 40.842 with an R2 value of 0.9329. These equations can then be rearranged into the form y = bx + a, where y represents 50% inhibition, and x is the IC50 value. A compound is considered a very strong antioxidant if the IC50 value is <10 µg/mL, strong if it falls between 10-50 µg/mL, moderate if it ranges between 50-100 µg/mL, weak if it falls between 100-250 µg/mL, and inactive if the IC50 value is above 250 µg/mL24.

From the results obtained in Table 1, the IC50 value for the quercetin reference is 0.237 µg/mL, classifying it as a very strong antioxidant because it falls within the IC50 range of <10 µg/mL. Meanwhile, the IC50 value for tectoquinone compounds is 66.362 µg/mL, categorizing it as a moderate antioxidant within the 50-100 µg/mL range.

y = 0.138x + 40.842  
R² = 0.9329

**Figure 2:Relationship between tectoquinone concentrations and % inhibition**

As explained previously mention that tectoquinone belongs to the quinone class compound. Quinone compounds inhibit free radicals through their unique redox properties, enabling them to participate in electron transfer reactions. When quinones encounter free radicals, they donate electrons to neutralize these highly reactive species, rendering them less harmful. This process transforms quinones into semiquinone radicals, which are relatively stable and less reactive. In some cases, semiquinone radicals can further react to regenerate the original quinone molecule, allowing quinones to continue their antioxidant action through multiple redox cycles. This mechanism makes quinones effective antioxidants, protecting cells and biomolecules from the damaging effects of oxidative stress caused by free radicals.

**LIMITATIONS OF THE STUDY**

**CONCLUSION**

The results indicate that tectoquinone compounds exhibit a noticeable capability against free radicals with moderate potential activity, however, additional research might be needed to enhance their potential uses in the field of health and medicine.

**AUTHOR'S CONTRIBUTION**

All authors have worked equally in this study.

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**CONFLICT OF INTEREST**

No conflict of interest is associated with this work.

**REFERENCES**

1. Anik MI., Mahmud N., Masud A Al., Khan MI., Islam MN., Uddin S., et al. Role of reactive oxygen species in aging and age-related diseases: a review. ACS Appl Bio Mater. 2022;5(9):4028–54, https://doi.org/10.1021/acsabm.2c00411.

2. Jia W., Wu X., Kang X. Integrated the embedding delivery system and targeted oxygen scavenger enhances free radical scavenging capacity. Food Chem X. 2023:100558, https://doi.org/https://doi.org/10.1016/j.fochx.2022.100558.

3. Ashton P. Notes on northern Borneo Myrtaceae. Sandakania. 2016;22:55–75.

4. Armijos C., Ram\’\irez J., Vidari G. Poorly Investigated Ecuadorian Medicinal Plants. Plants. 2022;11(12):1590, https://doi.org/https://doi.org/10.3390/plants11121590.

5. Rocchetti G., Lucini L., Ahmed SR., Saber FR. In vitro cytotoxic activity of six Syzygium leaf extracts as related to their phenolic profiles: An untargeted UHPLC-QTOF-MS approach. Food Res Int. 2019;126(August):108715, https://doi.org/10.1016/j.foodres.2019.108715.

6. Pham NKT., Nguyen TD., Doan TDC., Ha TD., Tran NMA., Tran TD., et al. Stercufoetin A, new oleanane-type triterpenoid from the leaves of Sterculia foetida L. Nat Prod Res. 2021;35(7):1226–31, https://doi.org/10.1080/14786419.2019.1644508.

7. Kumar M., Zhang B., Nishad J., Verma A., Sheri V., Dhumal S., et al. Jamun (Syzygium cumini (L.) Skeels) Seed: A Review on Nutritional Profile, Functional food properties, health-promoting applications, and safety aspects. Processes. 2022;10(11):2169, https://doi.org/https://doi.org/10.3390/pr10112169.

8. Amir Rawa MS., Mazlan MKN., Ahmad R., Nogawa T., Wahab HA. Roles of Syzygium in Anti-Cholinesterase, Anti-Diabetic, Anti-Inflammatory, and Antioxidant: From Alzheimer’s Perspective. Plants. 2022;11(11):1476, https://doi.org/https://doi.org/10.3390/plants11111476.

9. Pesiu E., Lee GE., Salam MR., Salim JM., Lau KH., Yong JWH., et al. Species composition, diversity, and biomass estimation in coastal and marine protected areas of Terengganu, Peninsular Malaysia. Agronomy. 2022;12(10):2380, https://doi.org/https://doi.org/10.3390/agronomy12102380.

10. de Sousa DP., Damasceno ROS., Amorati R., Elshabrawy HA., de Castro RD., Bezerra DP., et al. Essential Oils: Chemistry and Pharmacological Activities. Biomolecules. 2023;13(7):1144, https://doi.org/https://doi.org/10.3390/biom13071144.

11. Dahlem Junior MA., Nguema Edzang RW., Catto AL., Raimundo J-M. Quinones as an efficient molecular scaffold in the antibacterial, antifungal or antitumoral arsenal. Int J Mol Sci. 2022;23(22):14108, https://doi.org/https://doi.org/10.3390/ijms232214108.

12. Prokopiev I., Sleptsov I., Serebryakov E., Sharoyko V. Antioxidant and cytotoxic activities of quinones from Cetraria laevigata. Nat Prod Res. 2023:1–5, https://doi.org/https://doi.org/10.1080/14786419.2023.2187795.

13. Cores Á., Carmona-Zafra N., Clerigué J., Villacampa M., Menéndez JC. Quinones as Neuroprotective Agents. Antioxidants. 2023;12(7):1464.

14. Kiran P., Ghosh A., Pawar V., Maske P., Khan A., Srivastava R. Hypoxia-Targeting Drugs as New Cancer Chemotherapy Agents: Molecular Insights. Hypoxia in Cancer: Significance and Impact on Cancer Therapy. Springer; 2023. p. 351–68.

15. Mishima E., Wahida A., Seibt T., Conrad M. Diverse biological functions of vitamin K: from coagulation to ferroptosis. Nat Metab. 2023:1–9.

16. Peng Y., Xu D., Mao S., Zhou X. Neurotoxicity and apoptosis induced by pyrroloquinoline quinone and its ester derivative on primary cortical neurons. Neurotoxicology. 2020;78:47–56, https://doi.org/https://doi.org/10.1016/j.neuro.2020.02.005.

17. Sahoo PMS., Behera S., Behura R., Acharya A., Biswal D., Suna SK., et al. A brief review: Antibacterial activity of Quinone derivatives. Biointerface Res Appl Chem. 2022;12:3247–58, https://doi.org/https://doi.org/10.33263/BRIAC123.32473258.

18. Pacios-Michelena S., González CNA., Herrera RR., Alvarez-Perez OB., González MLC., Valdés RA., et al. Biomass from phytopathogens and culture conditions improve Penicillium chrysogenum antimicrobial activity and antifungal compounds production. Environ Qual Manag. 2023;33(1):349–58, https://doi.org/https://doi.org/10.1002/tqem.22065.

19. Mohamad Ishak NS., Ikemoto K. Pyrroloquinoline-quinone to reduce fat accumulation and ameliorate obesity progression. Front Mol Biosci. 2023;10:1200025, https://doi.org/https://doi.org/10.3389/fmolb.2023.1200025.

20. Pu T-T., Wu W., Liang P-D., Du J-C., Han S-L., Deng X-L., et al. Evaluation of coenzyme Q10 (CoQ10) deficiency and therapy in mouse models of cardiomyopathy. J Cardiovasc Pharmacol. 2022:10–1097, https://doi.org/https://doi.org/10.3390/antiox11091698.

21. Mart\’\in JA., López R. Biological Deterioration and Natural Durability of Wood in Europe. Forests. 2023;14(2):283, https://doi.org/https://doi.org/10.3390/f14020283.

22. Zhang S., Zhu Y-L., Ren S., Li C., Chen X-B., Li Z., et al. Covalent Organic Framework with Multiple Redox Active Sites for High-Performance Aqueous Calcium Ion Batteries. J Am Chem Soc. 2023;145(31):17309–20, https://doi.org/https://doi.org/10.1021/jacs.3c04657.

23. Cárcamo-Ibarra E., Manuel Martinez-Pacheco M., Munro-Rojas A., Enrique Ambriz-Parra J., Velázquez-Becerra C. Antifungal Activity of Crude Extracts of Tectona grandis Lf against Wood Decay Fungi. Phyt. 2022;91(8), https://doi.org/https://doi.org/10.32604/phyton.2022.020578.

24. Dahlia AA., Daim M., Najib A. Comparative study of antioxidant activity of turmeric ethanol extract (Curcuma longa L.) and ethanol extract of curcuma (Curcuma xanthorrhiza Roxb.) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging method. AIP Conference Proceedings, vol. 2595. 2023.