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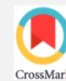

RESEARCH ARTICLE

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

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ABSTRACT
Aim and objective: In this study, the free radical scavenging of tectoquinone compound isolated from leaf of *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.
Methods: The quantitative assessment of antioxidant activity was conducted using a UV-Vis spectrophotometric approach, with measurements taken at a wavelength of 516 nm.
Results: The IC_{50} 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.
Conclusion: 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: DPPH assay, free radical, IC_{50} value, *Syzygium oblanceolatum*, Tectoquinone.

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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 systems[1]. 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 compounds[2].

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 Kalimantan[3]. 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 plants[4]. As the eighth-largest dicot plant family, it comprises approximately 5,650 species grouped into 130-150 generad[5] and is widely distributed across Africa, extending into South Asia and tropical Southeast Asian countries[6]. Due to their phytochemical content and health-promoting properties, several *Syzygium* species have garnered significant interest[7,8]. On the other hand, many other *Syzygium* species remain relatively unexplored[9], and their chemical and biological activities continue to be of interest including the class of quinones compound[10].

Quinones, a class of organic compounds, exhibit diverse medical properties[11]. They are recognized for their antioxidant capabilities[12], with compounds like Coenzyme Q10 and Vitamin K helping combat oxidative stress[13]. In cancer treatment, specific quinones, such as anthracyclines, interfere with cancer cell growth[14]. Quinones also play a role in skincare, blood clotting[15], and neurological disorders[16]. Some quinones demonstrate antibacterial[17], antifungal[18], and anti-inflammatory effects[19]. CoQ10, in particular, is studied for mitochondrial function and cardiovascular health[20]. 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.

Tectoquinone (as a member of the class of quinones compound)[21] is 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 groups[22]. 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 properties[23].

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, a UV-Vis spectrophotometric approach was utilized, 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 isolated from leaf of *Syzygium oblanceolatum* (C.B.Rob.) Merr. was collected in Maros distric, South Sulawesi-Indonesia. The collected plant was identified by a taxonomist, Dr. Wuu-Kuang Soh (Trinity College Dublin, the Republic of Ireland), 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. Tectoquinone previously identify by NMR Spectroscopy and compared with literature.

Sample extraction and isolation

The leaves of *S. oblanceolatum* (C.B.Rob.) Merr. ground into a powder (0.7 kg) were extracted three times with 20 L each of MeOH by maceration at room temperature. The filtrates were combined and evaporated under reduced pressure to yield 60 g of a dark gummy extract. The extract (10.1 g) was suspended in methanol: water (9:1) (0.2 L) and extracted with *n*-hexane (7 x 0.25 L) to obtain the *n*-hexane extract 2.7 g and methanol: water 7.3 g. The *n*-hexane extract was applied to a silica gel column and eluted with an *n*-hexane, *n*-hexane: ethyl acetate (8:1; 6:1; 4:1; 1:1) and methanol respectively to give seven major fractions (H1–H7). Fraction H2 (340.5 mg) was applied to a silica gel column and eluted with *n*-hexane: acetone (15:1) and methanol to yield nine fractions (H2A-H2I).Fraction H2H1 was separated successively by preparative reversed-phase HPLC using the eluent methanol: water (3:1) with flow rate 8 mL/min to afford three fractions (H2H1A-H2H1C). Fraction H2H1B was further purified by reversed-phase preparative thin layer chromatography PTLC eluted with methanol: water (5:1) to yield compound tectoquinone (2.0 mg).

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:

$$\%\ \text{inhibition} = \frac{A1 - A2}{A1} X100$$

Where A1 represents the absorbance of the control and A2 denotes the absorbance of the sample.

To find the IC_{50} 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 IC_{50} value was then calculated as IC_{50}=(50 - a)/b.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum showed five protons coupling with one another at δH 7.47-7.36 (m, 5H) indicating the absence of an aromatic ring. The signal at δ_H 5.40 (dd, J=12.7, 3.3 Hz, 1H) showed correlation with a geminal protons at δ_H 3.04 (dd, J = 17.0, 12.9 Hz, 1H), and δ_H 2.84 (dd, J=17.4, 2.9 Hz, 1H). In addition, broad singlet for two methyl groups δ_H 2.07 and 2.06 (d, J= 3.1 Hz, 6H), one of the protons in the downfield area at δ_H 12.26 indicated an absence of hydroxyl proton. The ^{13}C NMR (150 MHz, $CDCl_3$) showed 15 signals: three signals due to the oxygen substituted aromatic carbon at δC 160.86, 159.39, 157.72, seven signals due to carbon or hydrogen substituted aromatic at δ_C 139.02, 128.87, 128.66, 125.97, 103.05, 102.94, 102.06 and a signal each oxygen substituted carbon at δ_C 78.77, methylene at δ_C 43.56, carbonyl carbon at δ_C 196.42 and two methyl carbon at δ_C 7.7and 6.9. Spectrum data were compared with previous literature isolated compound was found as tectoquinone[24].

Tectoquinone: pale yellow solid; $[\alpha]_D^{23}$=-72.81 (*c*=0.070, $CHCl_3$); DART$^+$*m/z* 285.042 $[M+1]^+$ (Cal $C_{17}H_{16}O_4$ 285.105 $[M+1]^+$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.26 (s, 1H), 7.47-7.36 (m, 5H), 5.40 (dd, J=12.7, 3.3 Hz, 1H), 3.04 (dd, J = 17.0, 12.9 Hz, 1H), 2.84 (dd, J = 17.4, 2.9 Hz, 1H), 2.07 (d, J = 3.1 Hz, 6H) and ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.42 (C-4), 160.86 (C-7), 159.39 (C-5), 157.72 (C-9), 139.02 (C-1’), 128.87 (C-3’; C-5’), 128.66 (C-4’), 125.97 (C-2’; C-6’), 103.05 (C-10), 102.94 (C-8), 102.06 (C-6), 78.77 (C-2), 43.56 (C-3), 7.7, 6.9

In the quantitative assessment of antioxidant potential, the DPPH scavenging method relies on the IC_{50} 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 IC_{50} values for both tectoquinone compound and the reference quercetin are provided in the Table 1.

Table 1: Results of absorbance measurements, percentage inhibition, and IC_{50} values.

Sample	Concertation (ppm)	Blank Absorbance	Sample Absorbance	Inhibition (%)	IC_{50} (µg/mL)
	10	0.596	0.346	41.750	
Tectoquinone	20	0.596	0.335	43.602	
	30	0.596	0.321	45.959	66.362
	40	0.596	0.319	46.296	
	50	0.596	0.313	47.306	
	0.2	0.596	0.310	47.986	
	0.4	0.596	0.264	55.704	
Quercetin	0.6	0.596	0.252	57.718	0.237
	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 IC_{50} value for tectoquinone compounds can be determined from the regression equation.

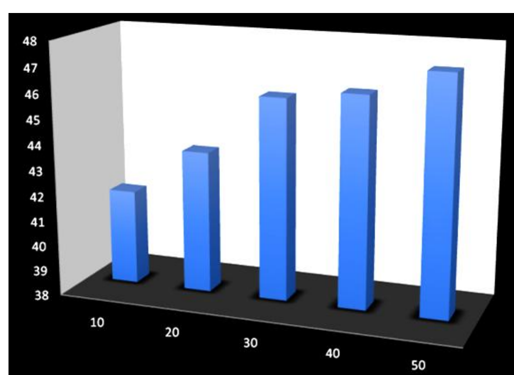

Figure 1: Relationship between tectoquinone concentrations and % inhibition.

The linear regression obtained from the quercetin reference is y=3.8758x+45.587 with an R^2 value of 0.9175, and for tectoquinone compounds, it is y = 0.138x + 40.842 with an R^2 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 IC_{50} value.

A compound is considered a very strong antioxidant if the IC_{50} 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 IC_{50} value is above 250 µg/mL[25]. From the results obtained in Table 1, the IC_{50} value for the quercetin reference is 0.237 µg/mL, classifying it as a very strong antioxidant because it falls within the IC_{50} range of <10 µg/mL. Meanwhile, the IC_{50} value for tectoquinone compounds is 66.362 µg/mL, categorizing it as a moderate antioxidant within the 50-100 µg/mL range. 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 anti-oxidants, protecting cells and biomolecules from the damaging effects of oxidative stress caused by free radicals.

Limitations of the study

The limitation of this study is that it primarily discusses the general properties and mechanisms of quinone compounds in inhibiting free radicals, with a specific focus on tectoquinone. However, it lacks specific experimental data or results related to tectoquinone's antioxidant activity or its comparison with other known antioxidants. While the theoretical background on quinones is valuable, the study does not provide empirical evidence of tectoquinone's antioxidant efficacy. To draw meaningful conclusions about tectoquinone's potential as an antioxidant, further experimental research, including *in vitro* or *in vivo* assays, is required to assess its actual free radical-scavenging capacity and compare it with established antioxidants. Additionally, the study does not address potential side effects, bioavailability, or practical applications of tectoquinone, which are essential considerations for its use in health and medicine.

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.

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