Full paper

Synthesis of Functionalized Vanillin Analogue and Evaluation of its Antioxidant Activity

Mutie Grantone[1], Chepkwony Sarah[1], Biwott Tecla[1], Opondo Florence[1], Rotich Vincent[2]

[1] MOI University, School of Sciences and Aerospace Studies, Chemistry and Biochemistry department, P.O BOX 3900-30100, Eldoret-Kenya

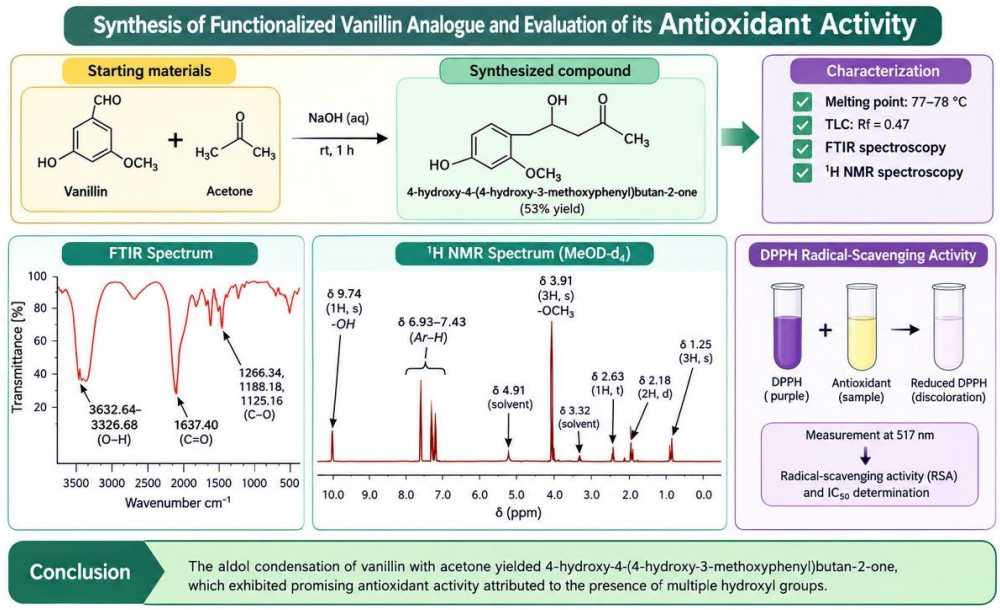
[2] Helmholtz Centre for Environmental Research- UFZ, Department of Exposure Science, Permoserstraße 15, 04318 Leipzig, Germany.

* Correspondence to: grantonemutie6@gmail.com

Abstract

Structural modification of phenolic compounds provides a means of investigating the molecular features that influence antioxidant activity. This study examined the synthesis of a functionalized vanillin analogue through the base-catalyzed aldol condensation reaction of vanillin with acetone, yielding a β-hydroxy ketone derivative. The synthesized compound was characterized using appropriate analytical techniques and evaluated for radical-scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Vanillin and vitamin C served as reference compounds for comparative evaluation. The findings provide compound specific evidence for assessing the relationship between chemical modification of the vanillin framework and antioxidant performance.

Keywords: Vanillin, aldol condensation, β-hydroxy ketone, structural modification, DPPH assay, antioxidant activity



Received 2026.09.02
Accepted 2026.10.28
Published 2026.11.17

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02

1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an aromatic phenolic compound widely used in the food, pharmaceutical, and cosmetic industries. Beyond its commercial applications, vanillin has attracted scientific interest because its molecular structure contains functional groups that can participate in different chemical reactions and influence its biological properties. The phenolic hydroxyl group, methoxy substituent, and aldehyde group provide opportunities for structural modification, making vanillin a useful starting material for the development of derivatives with potentially improved or distinct functional characteristics [1], [2] . However, the biological properties of vanillin cannot automatically be attributed to its derivatives, as changes in molecular structure may alter their chemical reactivity and biological activity [3].

The antioxidant properties of vanillin have been investigated using different experimental approaches. Tai et al. [4] evaluated vanillin using multiple antioxidant assays and found that its measured activity differed depending on the method employed. Although vanillin showed activity in the ABTS radical-scavenging and oxygen radical absorbance capacity assays, no detectable activity was observed in the DPPH assay under their experimental conditions. These findings demonstrate that the antioxidant behavior of vanillin is influenced by the reaction system and that its activity should be interpreted in relation to the specific experimental conditions [5]. This variability also highlights the importance of investigating structurally modified vanillin compounds rather than assuming that they will exhibit the same antioxidant properties as the parent molecule.

Research on vanillin derivatives has further demonstrated the influence of chemical structure on antioxidant activity. Scipioni et al. [6] and Fayeulle et al. [7] synthesized vanillin derivatives through reductive amination and evaluated their antioxidant and protective properties. Their findings showed that certain derivatives exhibited antioxidant activity and protection against oxidative damage in experimental DNA and cellular models. Differences in activity were associated with structural features of the synthesized compounds, indicating that modification of the vanillin framework can influence its functional properties [8]-[10]. Nevertheless, these findings concern derivatives produced through reductive amination and do not establish the antioxidant behavior of vanillin analogues containing other structural modifications. Consequently, further investigation of alternative classes of vanillin derivatives is necessary to broaden understanding of the relationship between structural modification and antioxidant activity.

Despite these investigations, the available evidence from the studies discussed above does not establish the antioxidant properties of every possible vanillin derivative, particularly compounds incorporating structural changes that differ from those previously examined. This presents a research gap concerning the experimental evaluation of vanillin analogues prepared through alternative synthetic transformations. Establishing the antioxidant activity of such compounds is important for determining whether structural modification produces measurable activity and for identifying derivatives that warrant further chemical or biological investigation.

Accordingly, the present study investigates a functionalized vanillin analogue and evaluates its antioxidant activity. By examining a derivative distinct from those described in the previous studies, this work contributes to the broader investigation of vanillin-based compounds and their potential antioxidant properties.

2. Results and discussion

2.1 Synthesis and physical characterization

The aldol condensation reaction between vanillin and acetone in basic condition yielded 4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butan-2-one, which was obtained in yield of 53 % as a yellow, needle-like crystals. The reaction proceeded through enolate ion formation , nucleophilic attack on the vanillin carbonyl to form an enone and lastly protonation to form the β-hydroxy ketone. The product exhibited a melting point of 77–78 °C and a single TLC spot with an Rf value of 0.47, supporting successful formation of the product.

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02

2.2 FTIR spectral analysis

FTIR spectrum of 4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butan-2-one revealed absorption bands at 3632.64-3326.68 cm^{-1}, consistent with O-H stretching vibrations of the phenolic groups. The absorption at 1637.40 cm^{-1} was assigned to carbonyl stretching (C=O), while bands at 1266.34, 1188.18, and 1125.16 cm^{-1} were attributed to C-O stretching vibrations associated with the methoxy and secondary hydroxyl functionalities. Additional bands at 1520.14 and 1418.90 cm^{-1} were consistent with aromatic ring vibrations. Compared with vanillin (1636.26 cm^{-1}), the carbonyl band exhibited a slight shift to 1637.40 cm^{-1}, reflecting a change in the carbonyl environment following the aldol reaction.

2.3 ^{1}H NMR spectral analysis

The ^{1}H NMR spectrum of the synthesized compound, recorded in MeOD-d_4, provided definitive structural confirmation of the synthesized vanillin analogue. The spectrum displayed a characteristic singlet at $\boldsymbol{\delta}$ 9.74 ppm ($\mathbf{1}$H) assigned to the hydroxyl proton bonded to the carbon group in the para position from the phenol group, while the aromatic protons appear as well resolved signals in the region of $\boldsymbol{\delta}$ 6.93–7.43 ppm corresponding to the trisubstituted benzene ring. A sharp singlet at $\boldsymbol{\delta}$ 3.91 ppm ($\mathbf{3}$H) confirms the presence of the methoxy group attached to the aromatic core. The aliphatic side chain protons are evidenced by signals at $\boldsymbol{\delta}$ 2.63 ppm ($\mathbf{1}$H, t), $\boldsymbol{\delta}$ 2.18 ppm ($\mathbf{2}$H, d), and $\boldsymbol{\delta}$ 1.25 ppm ($\mathbf{3}$H, s), consistent with the expected molecular backbone. Additionally, residual solvent and moisture peaks associated with the deuterated solvent system are observed at $\boldsymbol{\delta}$ 3.32 ppm and $\boldsymbol{\delta}$ 4.91 ppm.

2.4 DPPH radical-scavenging activity results

As illustrated by table 1 below, absorbance increased from 0.094 to 0.597 as the DPPH concentration increased from 0.0002 to 0.0020 mg/mL. The DPPH calibration curve exhibited a strong linear relationship between concentration and absorbance, with a correlation coefficient value of 0.9976. This relationship confirms the suitability of the tested range for monitoring changes in DPPH absorbance which is important for ensuring consistent spectrophotometric measurements during antioxidant analysis.

Table 1: Calibration data for DPPH reagent measured at 517 nm.

Concentration (mg/mL)	Absorbance (AU)
0.0002	0.094
0.0004	0.173
0.0008	0.297
0.0012	0.381
0.0016	0.496
0.0020	0.597

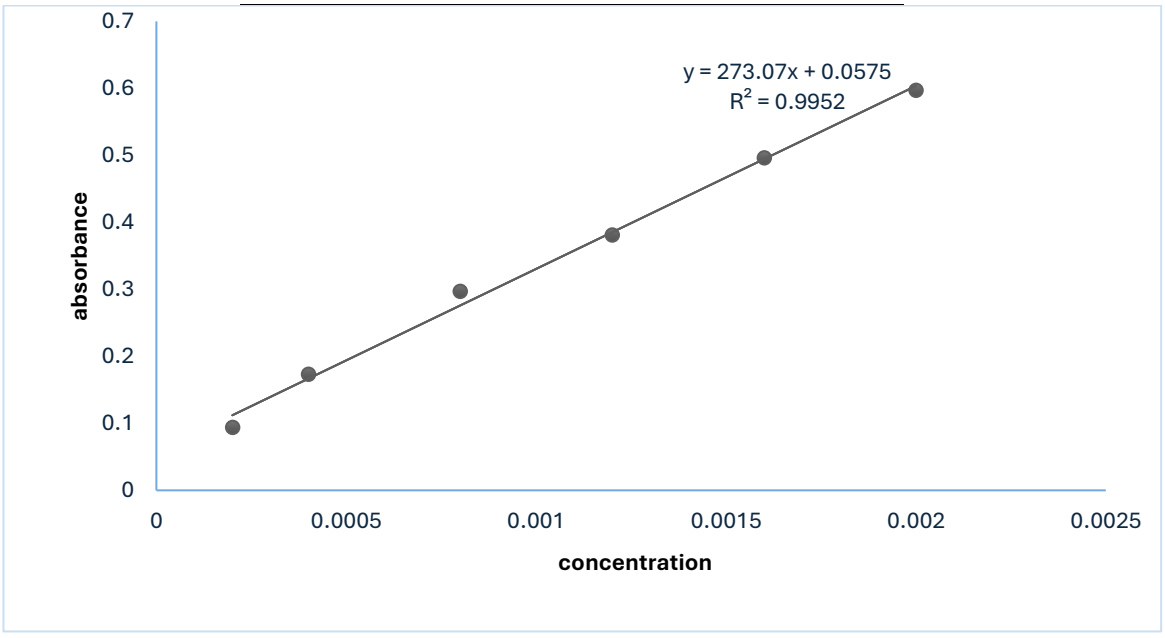


Figure 4: Calibration curve showing absorbance as a function of DPPH concentration in methanolic solution.

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02

Vitamin C exhibited the lowest absorbance values, which decreased from 0.026 to 0.000, followed by the synthesized product, which decreased from 0.039 to 0.006, and vanillin, which decreased from 0.183 to 0.023 as shown in table 2 below. This reduction in absorbance with increasing concentration indicates increased depletion of the DPPH free radical. The synthesized product demonstrated a response approaching that of vitamin C, suggesting substantial radical scavenging potential. According to Rana et al [11], the activity of this compound may be associated with the number of its hydroxyl groups, which can donate hydrogen atoms to stabilize free radicals as evidenced by increased number of hydroxyl group of the synthesized analogue.

Table 2: Absorbance of samples incubated with DPPH at different concentrations

Sample	0.005 mg/mL	0.010 mg/mL	0.020 mg/mL	0.040 mg/mL	0.080 mg/mL
Vitamin C	0.026	0.010	0.008	0.001	0.000
Vanillin	0.183	0.093	0.053	0.046	0.023
4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butan-2-one	0.039	0.022	0.015	0.009	0.006

The calculated RSA (%) values are expressed in table 3 below as well as their IC_{50} values, following the order; vitamin C (2.525 µg/mL) < aldol product (2.535 µg/mL) < vanillin (2.680 µg/mL). The findings clearly show that the synthesized product exhibited RSA and IC_{50} values close to vitamin C than the parent compound (vanillin). This demonstrates that the synthesized analogue has promising DPPH radical-scavenging activity following aldol condensation reaction.

Table 3: RSA (%) values of samples at different concentrations and their **IC_{50}**

Sample	0.005 mg/mL	0.010 mg/mL	0.020 mg/mL	0.040 mg/mL	0.080 mg/mL	IC_{50} µg/mL
Vitamin C	99.00	99.60	99.70	99.90	100	2.525
Vanillin	93.30	96.60	98.10	98.30	99.20	2.680
4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butan-2-one	98.60	99.20	99.50	99.70	99.80	2.535

3.Materials And Methods

3.1 Materials

Vanillin, acetone, sodium hydroxide, ethyl acetate, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH), vitamin C, and anhydrous sodium sulfate were used in this study. All reagents were obtained from Sigma-Aldrich, and solvents were of analytical grade. Distilled water used was obtained from engineering laboratory water distiller.

3.2 Tools

Chemistry glassware set, analytical balance, filter papers, spatula, magnetic stirrer, rotary evaporator, TLC silica gel 60 F254 plates, UV lamp, melting point apparatus, FTIR spectroscopy, 1H NMR spectroscopy and UV-Vis spectrophotometer were used in this research.

3.3 Aldol condensation reaction

The reaction was a modified procedure of Prabawati et al. [12] where, acetone (7.8 mL) was mixed with 30 % aqueous sodium hydroxide (4 mL) in a 250 mL conical flask equipped with an ice and stirred for 30 min. Vanillin (2 g) was then added, and the reaction mixture was stirred for a further 1 hour at room temperature. Distilled water (20 mL) was added, and the resulting mixture was stirred for an additional 30 min. The mixture was subsequently extracted two times with 20 mL ethyl acetate, and the organic layer was separated, dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated under reduced pressure until a solid substance was obtained. The resulting crude solid was purified by recrystallization using diethyl ether and the final yield determined.

3.4 Melting point, and thin-layer chromatography

Approximately 20 mg of the sample was placed in a capillary tube for measurement of its melting point. For thin-layer chromatography (TLC), a small amount of the sample was dissolved in chloroform and spotted onto silica gel 60 F254 plates. The plates were developed using hexane-ethyl acetate mixture (70:30, v/v) as the

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02

mobile phase. After development, the spots were visualized under UV lamp at 254 nm. The retention factor (Rf) was thus calculated as the ratio of the distance travelled by the compound to the distance travelled by the solvent front.

3.5 FTIR spectroscopy

FTIR spectrum was recorded using a Bruker Alpha spectrophotometer with the attenuated total reflectance (ATR) method. The solid sample was placed directly on the ATR crystal and pressed to ensure adequate contact during spectral acquisition.

3.6 ^{1}H NMR spectroscopy

The ^{1}H NMR spectrum was acquired using an 800 MHz Bruker NMR spectrometer, with deuterated methanol (MeOD-d_4) as the solvent. Chemical shifts were processed and reported in parts per million (ppm).

3.7 Antioxidant activity determination using the DPPH free radical scavenging assay

The antioxidant potential of the samples was evaluated based on their ability to donate hydrogen atoms or electrons, resulting in reduction of the purple DPPH radical to its corresponding non-radical form. The resulting change in absorbance was measured using a UV-visible spectrophotometer at 517 nm.

3.7.1 Preparation of DPPH solution and calibration curve

A DPPH stock solution was prepared by modifying a procedure of Oladimeji et al. [13], by accurately weighing 0.0062 g of DPPH powder and dissolving it in 50 mL of methanol to obtain the required working solution (0.0124 % w/v). Serial dilutions of the DPPH working solution were then prepared to obtain standard concentrations of 0.0002, 0.0004, 0.0008, 0.0012, 0.0016 and 0.0020 % w/v. The absorbance values of the prepared DPPH solution was measured, with methanol used to calibrate the instrument before measurements were recorded. The resulting absorbance readings were used to establish the calibration curve for DPPH radical concentration.

3.7.2 Antioxidant activity

Stock solutions of the synthesized sample, vanillin and vitamin C were prepared by dissolving 100 mg in 10 mL methanol to obtain a concentration of 10 mg/mL. Appropriate aliquots of the stock solutions were subsequently diluted with methanol to prepare working solutions of 0.005, 0.01, 0.02, 0.04 and 0.08 mg/mL for the antioxidant assays. From each dilution, 4 mL was mixed with 4 mL of the prepared methanolic DPPH solution, and the mixtures were incubated for 30 minutes in the dark at room temperature (25 ± 2 °C). After incubation period, the absorbance of each reaction mixture was measured. A control containing only the DPPH solution without the antioxidant sample was used as the reference absorbance. The percentage radical scavenging activity (RSA%) was thus calculated using the equation below:

$$RSA\ (\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100 \qquad (1)$$

Where $\mathbf{A_0}$ is the absorbance of the DPPH control solution and $\mathbf{A_1}$ the absorbance of the DPPH solution after reaction with the test sample. The antioxidant concentration required to achieve 50 % inhibition of DPPH radicals ($\mathbf{IC_{50}}$) was determined.

4. Conclusion

The aldol condensation reaction successfully synthesized 4-hydroxy-4-(4-hydroxy-3-methoxyphenyl) butan-2-one, which was identified by its physical and chemical characteristics. The DPPH free radical assay further showed that the compound has a promising antioxidant potential which is attributed by the presence of hydroxyl groups in its molecular structure.

Acknowledgements

The authors acknowledge Jomo Kenyatta University of Agriculture and Technology (JKUAT) for access to analytical facilities used for FTIR spectroscopy and melting-point determination, and the Leibniz Institute of Surface Engineering (IOM), Leipzig, Germany, for conducting ^{1}H NMR analysis.

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02

References

[1] L. Cui et al., "Vanillin beyond flavor: Therapeutic potentials and emerging applications in hydrogel-based biomaterials," Gels, vol. 12, no. 1, Art. no. 16, 2026, doi: 10.3390/gels12010016.

[2] R. Verma, U. Agarwal, B. Chauhan, H. Singh, A. K. Mishra, N. Kumar, and A. Kumar, "Vanillin and its derivatives: Therapeutic strategies in modern medicine," Mini Reviews in Medicinal Chemistry, early access, Aug. 30, 2026, doi: 10.2174/0113895575461522260820050915.

[3] M. Suleiman, M. Sankaranarayanan, K. T. Das, S. I. Amran, V. Berezin, B. Andrey, and J. Jamalis, "Vanillin derivatives in drug design: Structure–activity relationship (SAR) hotspots and synthetic pathways for enhanced pharmacological activity," ACS Omega, vol. 10, no. 48, pp. 57846–57875, Nov. 2025, doi: 10.1021/acsomega.5c02176.

[4] A. Tai, T. Sawano, F. Yazama, and H. Ito, "Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays," Biochimica et Biophysica Acta (BBA) - General Subjects, vol. 1810, no. 2, pp. 170–177, Feb. 2011, doi: 10.1016/j.bbagen.2010.11.004.

[5] B. M. Upton and A. M. Kasko, "Strategies for the conversion of lignin to high-value polymeric materials: Review and perspective," Chemical Reviews, vol. 116, no. 4, pp. 2275–2306, Feb. 2016, doi: 10.1021/acs.chemrev.5b00345.

[6] M. Scipioni, G. Kay, I. Megson, and P. Kong Thoo Lin, "Novel vanillin derivatives: Synthesis, anti-oxidant, DNA and cellular protection properties," European Journal of Medicinal Chemistry, vol. 143, pp. 745–754, Jan. 2018, doi: 10.1016/j.ejmech.2017.11.072.

[7] F. Fayeulle, E. Trudel, A. Damiens, A. Josse, N. Ben Hadj Youssef, et al., "Antimicrobial and antioxidant activities of amines derived from vanillin as potential preservatives: Impact of the substituent chain length and polarity," Sustainable Chemistry and Pharmacy, vol. 22, Art. no. 100471, 2021, doi: 10.1016/j.scp.2021.100471.

[8] M. Wang, L. Yuan, S. Chen, and R. Fu, "Catalyst-free synthesis of next-generation biobased high-toughness polyesters enabled by Knoevenagel chemistry," Macromolecules, vol. 58, no. 16, pp. 8845–8853, Aug. 2025, doi: 10.1021/acs.macromol.5c01106.

[9] R. Ma, Y. Hou, W. Zhang, and Y.-T. Pan, "MOF-based nanocomposites in polymer matrix: Progress and prospects," Advanced Composites and Hybrid Materials, vol. 8, no. 5, Sep. 2025, doi: 10.1007/s42114-025-01435-6.

[10] G. Dai, S. Wang, S. Huang, and Q. Zou, "Enhancement of aromatics production from catalytic pyrolysis of biomass over HZSM-5 modified by chemical liquid deposition," Journal of Analytical and Applied Pyrolysis, vol. 134, pp. 439–445, Sep. 2018, doi: 10.1016/j.jaap.2018.07.010.

[11] M. S. Rana, N. M. A. Rayhan, M. S. H. Emon, M. T. Islam, K. Rathry, M. M. Hasan, M. M. I. Mansur, B. C. Srijon, M. S. Islam, A. Ray, M. A. Rakib, A. Islam, M. K.-E.-Zahan, M. F. Hossen, and M. A. Asraf, "Antioxidant activity of Schiff base ligands using the DPPH scavenging assay: An updated review," RSC Advances, vol. 14, no. 45, pp. 33094–33123, Oct. 2024, doi: 10.1039/D4RA04375H.

[12] S. Y. Prabawati, D. Iskandar, and L. P. Suci, "The synthesis and evaluation of vanillin derivatives as the fruit fly attractant," Oriental Journal of Chemistry, vol. 34, no. 4, 2018, doi: 10.13005/ojc/3404063.

[13] H. O. Oladimeji, E. S. Idiong, U. Akpan, and V. U. Anah, "Cycloalkanol from derivatization studies on vanillin: Evaluation of antioxidant activity of obtained derivatives," American Journal of Chemistry and Materials Science, vol. 6, no. 1, Jan. 2022, doi: 10.11648/j.ajqcms.20220601.11.

Copyright: © 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license, (https://creativecommons.org/licenses/by/4.0/) (SR-license)

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i4a02