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Synthesis and Evaluation of Mannich Base Derivatives of Hexyl Ferulate as Antibacterial and Antioxidant Agents

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Abstract
Antimicrobial resistance (AMR) has become a critical global health issue, particularly in developing countries where bacterial infections remain prevalent and treatment options are increasingly limited. The rising resistance of pathogens such as Staphylococcus aureus and Escherichia coli highlights the urgent need for new antibacterial agents with improved efficacy. In addition, oxidative stress contributes to cellular damage, making compounds with dual antioxidant and antibacterial properties highly valuable. This study aims to synthesize and evaluate Mannich base derivatives of hexyl ferulate as potential antibacterial and antioxidant agents. The research employed an experimental laboratory approach, including the synthesis of ferulic acid derivatives through esterification and Mannich reactions, followed by structural characterization using spectroscopic methods. Antioxidant activity was assessed using DPPH and FRAP assays, while antibacterial activity was evaluated through agar diffusion and dilution methods. The results showed that Mannich base derivatives exhibited significantly enhanced antioxidant activity, with lower IC_{50} and EC_{50} values compared to ferulic acid and hexyl ferulate. In antibacterial testing, the derivatives demonstrated stronger inhibition against both Gram-positive and Gram-negative bacteria, with certain compounds showing superior activity. These findings indicate that structural modification through Mannich substitution effectively improves biological activity. In conclusion, Mannich base derivatives of hexyl ferulate have strong potential as lead compounds for developing new antibacterial and antioxidant agents to address AMR challenges.

INTRODUCTION

Antimicrobial resistance (AMR) poses a major global health concern, exerting substantial effects on public health and sustainable development, especially in developing nations like Indonesia. AMR contributes substantially to increased morbidity and mortality, accounting for an estimated 34500 deaths directly attributed to resistant infections and an additional 133800 deaths linked to antimicrobial resistance in Indonesia in 2019 (IHME, 2019). The effects of AMR include prolonged illness, increased risk of death, and extended hospital stays (Walsh et al., 2023). Worldwide, most antimicrobial resistance–related infections are caused by bacterial pathogens like Staphylococcus aureus and Escherichia coli, with methicillin-resistant Staphylococcus aureus (MRSA) and E. coli resistant to cephalosporins being particularly prevalent (Murray et al., 2022). In Indonesia, MRSA resistance rates are reported at 40-50%, while E. coli resistance to cephalosporins ranges from 70-80%. Microbial resistance mechanisms, including restricted drug uptake, efflux pumps, drug inactivation, and target modification, are major challenges in controlling bacterial infections (Uddin et al., 2021).

The relationship between antioxidants and antibacterial activity lies in their ability to counteract oxidative stress in bacterial cells (Mammari et al., 2022; Mourenza et al., 2020). Antioxidants play a crucial role in neutralizing free radicals and alleviating oxidative stress

while simultaneously inhibiting bacterial metabolic processes. Several phenolic-derived antioxidant compounds have demonstrated the ability to protect cells from oxidative damage and compromise bacterial cell wall integrity, thereby impeding microbial proliferation (Zheng et al., 2024).

The development of compounds with both antioxidant and antibacterial properties offers a promising solution to combat AMR. One such compound, ferulic acid (FA), derived from Ferula foetida, has long been recognized for its antioxidant and chemopreventive effects in protecting cells from oxidative damage and DNA carcinogenesis (Rani et al., 2024). However, the low solubility and bioavailability of FA limit its therapeutic potential. Structural modifications like the synthesis of alkyl ester derivatives have been investigated to enhance both the antibacterial and antioxidant properties of FA. Hexyl ferulate exhibits notable antibacterial activity with enhanced antioxidant potential compared to ferulic acid (Song et al., 2023).

Moreover, the Mannich reaction has been applied to modify ferulic acid derivatives, enhancing their pharmacological potential such as antibacterial and antioxidant effects. The introduction of Mannich bases enhances lipophilicity, solubility, and membrane permeability, allowing the modified compounds to penetrate bacterial membranes more effectively, especially in gram-negative bacteria (Hayun et al., 2020).

This study aimed to develop hexyl ferulate derivatives substituted with Mannich bases (such as diethylamine, dimethylamine, morpholine, and 2,6-dimethylmorpholine) as promising candidates for both antioxidant and antibacterial agents. These derivatives are expected to exhibit enhanced biological activity compared with existing antibiotics, thereby supporting the urgent pursuit of new therapeutic options against AMR. Through structural modification of ferulic acid, this study aims to develop innovative, potent, and bioavailable compounds with combined antioxidant and antibacterial properties.

Hayun et al. (2020) reported that Mannich bases derived from vanillic acid showed enhanced antioxidant activity, while Tokalı et al. (2021) demonstrated that phenolic Mannich base derivatives possessed promising bioactivity, molecular interaction potential, and ADME-related properties. These studies indicate that the introduction of amine-containing Mannich substituents can improve lipophilicity, membrane permeability, and electron-donating capacity, which are important for antibacterial and antioxidant mechanisms.

Despite these developments, research combining hexyl ferulate esterification with Mannich base substitution remains limited. Many previous studies focused either on ferulic acid esters or Mannich bases separately, rather than integrating both strategies in one scaffold. Moreover, there is still a need to compare different secondary amine substituents, such as dimethylamine, diethylamine, morpholine, and 2,6-dimethylmorpholine, in terms of their influence on antioxidant capacity, antibacterial activity, and physicochemical properties. This gap provides a strong rationale for synthesizing and evaluating Mannich base derivatives of hexyl ferulate.

The urgency of this research lies in the need to discover new lead compounds that may contribute to the development of alternative antibacterial agents. As AMR continues to reduce the effectiveness of existing antibiotics, structural modification of natural compounds offers a rational and sustainable approach to drug discovery. Natural phenolic scaffolds such as ferulic acid are attractive because they already possess biological activity, while chemical

modification may improve potency and pharmacokinetic potential. Therefore, this research is important for expanding the chemical diversity of antibacterial and antioxidant candidates.

The novelty of this study is reflected in the synthesis and biological evaluation of Mannich base derivatives of hexyl ferulate using different secondary amines. By combining esterification and Mannich substitution, this research explores how increased lipophilicity, nitrogen basicity, and substituent structure affect antioxidant and antibacterial performance. The manuscript reports that Mannich derivatives showed stronger antioxidant activity than ferulic acid and hexyl ferulate, while compound 3d produced the largest antibacterial inhibition zone and compound 3b showed strong bactericidal activity. These findings highlight the potential of Mannich-modified hexyl ferulate as a promising bioactive scaffold.

Accordingly, the purpose of this research is to synthesize, characterize, and evaluate Mannich base derivatives of hexyl ferulate as antibacterial and antioxidant agents. The main objective is to determine whether structural modification can enhance biological activity compared with the parent ferulic acid and hexyl ferulate compounds. This study contributes to medicinal chemistry by providing evidence on the structure–activity relationship of ferulic acid derivatives and offers practical benefits as a preliminary basis for developing new antimicrobial candidates. In the broader context, the research may support future drug discovery efforts aimed at addressing AMR through natural product-based structural innovation.

METHOD

Analytical-grade materials were sourced from Sigma-Aldrich (USA), Merck (Germany), and Mallinckrodt (USA). Silica gel 60 (Merck, Germany) served as the stationary phase for chromatographic purification. Melting points were measured using a Stuart apparatus (UK). UV-Vis and FTIR spectra were recorded on Shimadzu instruments (Japan), and NMR spectra (^{1}H, ^{13}C) were obtained on an Agilent 500/125 MHz spectrometer using $CDCl_3$ as the solvent.

Synthesis

The synthesis process began with the preparation of ferulic acid (1), followed by hexyl ferulate (2), and subsequently Mannich base–substituted hexyl ferulate derivatives: dimethylamine (3a), diethylamine (3b), morpholine (3c), and 2,6-dimethylmorpholine (3d) (Figure 1).

Synthesis of Ferulic Acid (1)

Ferulic acid (FA) was produced via a solvent-free Knoevenagel condensation between vanillin and malonic acid, adopting the procedure described by Schijndel et al. (2017) with minor modifications (Alifah et al., 2025), and was subsequently used as a precursor for esterification and Mannich base formation. The compound's purity was verified through melting point analysis and thin-layer chromatography (TLC), while structural characterization was performed using UV–Vis and FT-IR spectroscopy.

Synthesis of Hexyl Ferulate (2)

Hexyl ferulate was synthesized from ferulic acid through an esterification reaction with hexyl bromide in the presence of a base catalyst, following the general procedure reported by Vu and Prakash (2014) with slight modification. The product was purified and characterized using FT-IR and ^{1}H-NMR spectroscopy.

Synthesis of Mannich-Substituted Hexyl Ferulate Derivatives (3a–3d)

Mannich-base derivatives of hexyl ferulate were prepared based on the method described by Tokali et al. (2022) with minor modifications. The synthesis was carried out via the condensation of hexyl ferulate with paraformaldehyde and different secondary amines in ethanol under reflux conditions. The obtained products were purified and their structures verified through FT-IR, ^{1}H-NMR, and ^{13}C-NMR analyses.

Antioxidant Activity

The antioxidant activity of the synthesized compounds was assessed using two in vitro assays that complemented each other: the DPPH free radical scavenging test and the Ferric Reducing Antioxidant Power (FRAP) assay. These methods evaluate the compounds' capacity to donate electrons or hydrogen atoms to neutralise free radicals. The assay was conducted in accordance with the method outlined by Hayun et al. (2020).

Antibacterial Activity

The antibacterial assay was performed following the protocols outlined by Halpani and Satyendra (2024) and Song et al. (2023), with certain modifications.

The antibacterial activity was assessed via the agar well diffusion technique. A mixture of one milliliter of bacterial suspension and 15–20 milliliters of molten nutrient agar was then poured into Petri dishes. The wells were filled with 20 micro litres of each test compound, as well as positive controls consisting of either 30 milligrams per litre of tetracycline or 10 micro grams of gentamicin, and negative controls made up of 1% DMSO and 1% Tween 80. The plates were incubated at 37 degrees Celsius for 18–24 hours, and the inhibition zones were then measured.

The MIC and MBC were measured by broth dilution in nutrient broth at concentrations ranging from 24 to 0.375 mg/mL. The test sample was first added in a volume of 0.5 mL to each tube, which was then serially diluted by a factor of two, following the addition of the bacterial suspension and an incubation period of 18–24 hours at 37°C. The MIC was determined as the lowest concentration at which no turbidity was visible, whereas the MBC was established by subculturing clear tubes onto NA plates to identify the lowest concentration exhibiting no bacterial growth.

Physical and Spectral Data of The Synthesized Compounds

1. Ferulic Acid (1)

Yellowish white needle-like crystals, 43.48% yield, λmax (MeOH): 314, 289, and 213 nm. IR (cm^{-1}): 3736, 3649, and 3566 (broad, O–H phenol), 1697 (C=O carboxylic acid), 1653 and 1647 (C=C alkene), 1616 and 1558 (C=C aromatic), 1541 and 1508 (C–C aromatic), 1265 (C–O carboxylate), 1203 (asymmetric C–O–C ether), 1033 (C–O phenol), 941 (O–H bending), 850 (para-substituted aromatic ring), and 802 (meta-substituted aromatic ring).

2. Hexyl Ferulate (2)

Yellowish viscous liquid, 95.08% yield. IR (cm^{-1}): 3736, 3649, and 3566 (broad, O–H phenol), 2956, 2929, and 2858 (C–H aliphatic), 1695 (C=O carboxylic acid), 1631 and 1589 (C=C alkene), 1512 (C=C aromatic), 1463 and 1429 (C–C aromatic), 1261 (C–O carboxylate), 1153 (asymmetric C–O–C ether), 1033 (C–O phenol), 941 (O–H bending), 844 (para-substituted aromatic ring), and 813 (meta-substituted aromatic ring).

^{1}H-NMR ($CDCl_3$, δ ppm): 7.62–7.59 (d, 1H, J = 15.92 Hz, Ar–CH=C), 7.02–6.99 (m, 1H, Ar–H), 6.90 (d, 1H, J = 8.21 Hz, Ar–H), 6.89 (d, 1H, J = 8.21 Hz, Ar–H), 6.31–6.27 (d,

1H, J = 15.98 Hz, C=CH–C), 3.82 (s, 3H, O–CH_3), 4.19–4.17 (t, 2H, J = 6.79 Hz, O–CH_2–C), 1.70–1.64 (m, 2H, C–CH_2–C), 1.39–1.21 (m, 6H, C–CH_2–C), and 0.90–0.87 (t, 3H, J = 6.96 Hz, C–C–CH_3).

3. Mannich-Substituted Hexyl Ferulate Derivatives (3a–3d)

a) 3a

Yellowish dry powder, 59.48% yield, mp 66–68 °C. IR (cm^{-1}): 2954, 2924, and 2856 (C–H aliphatic), 1697 (C=O carboxylate), 1672 and 1591 (C=C aromatic), 1489 and 1463 (C=C alkene), 1263 (C–O ether), 1176 (C–N amine), 1004 (C–O phenol), 864 (para-substituted aromatic ring), and 808 (meta-substituted aromatic ring).

^{1}H-NMR ($CDCl_3$, δ ppm): 7.59–7.56 (dd, 1H, J = 2.02, 15.98 Hz, Ar–CH=C), 6.99 (s, 1H, Ar–H), 6.79 (s, 1H, Ar–H), 6.28–6.25 (dd, 1H, J = 2.04, 15.86 Hz, C=CH–C), 4.20–4.17 (m, 2H, O–CH_2–C), 3.91 (s, 3H, O–CH_3), 3.67 (d, 2H, J = 2.04 Hz, Ar–CH_2–N), 2.35 (s, 6H of dimethylamine, N–CH_3), 1.71–1.67 (m, 2H, C–CH_2–C), 1.41–1.30 (m, 6H, C–CH_2–C), and 0.92–0.89 (m, 3H, C–C–CH_3).

^{13}C-NMR ($CDCl_3$, δ ppm): 167.41 (C=O), 150.05, 148.04, 144.72, 125.07, 121.71, and 109.38 (6C, aromatic carbons), 114.71 (Ar-CH=C), 64.39 (O–CH_2–C), 62.27 (Ar–CH_2–N), 55.72 (O–CH_3), 44.28 (2C of dimethylamine, N–CH_3), 31.24, 28.57, 25.52, and 22.43 (4C, C–CH_2–C), 13.91 (C–C–CH_3).

b) 3b

Brown viscous liquid, 71.31% yield. IR (cm^{-1}): 2956, 2929, and 2856 (C–H aliphatic), 1703 (C=O carboxylate), 1629 and 1595 (C=C aromatic), 1463 and 1429 (C=C alkene), 1246 (C–O ether), 1149 (C–N amine), 1087 and 1053 (C–O phenol), 840 (para-substituted aromatic ring), and 769 (meta-substituted aromatic ring).

^{1}H-NMR ($CDCl_3$, δ ppm): 7.59–7.56 (d, 1H, J = 15.92 Hz, Ar–CH=C), 6.98 (d, 1H, J = 1.97 Hz, Ar–H), 6.80–6.79 (d, 1H, J = 2.02 Hz, Ar–H), 6.28–6.25 (d, 1H, J = 15.87 Hz, C=CH–C), 4.19–4.17 (t, 2H, J = 6.75 Hz, O–CH_2–C), 3.90 (s, 3H, O–CH_3), 3.79 (s, 2H, Ar–CH_2–N), 2.66–2.62 (q, 4H of diethylamine, J = 7.16 Hz, N–CH_2–C), 1.14–1.11 (t, 6H of diethylamine, J = 7.20 Hz, C–CH_3), 1.72–1.66 (m, 2H, C–CH_2–C), 1.43–1.26 (m, 2H, C–CH_2–C), and 0.92–0.86 (m, 3H, C–C–CH_3).

^{13}C-NMR ($CDCl_3$, δ ppm): 167.11 (C=O), 150.39, 147.92, 144.57, 124.70, 121.59, and 109.10 (6C, aromatic carbons), 114.36 (Ar-CH=C), 64.08 (O–CH_2–C), 56.31 (Ar–CH_2–N), 55.49 (O–CH_3), 46.01 (2C of diethylamine, N–CH_2–C), 31.13, 28.41, 25.32, and 22.20 (4C, C–CH_2–C), 13.68 and 13.66 (C–C–CH_3), 10.81 (2C of diethylamine , C-CH3)

c) 3c

Yellowish viscous liquid, 99.33% yield. IR (cm^{-1}): 2954, 2850, and 2794 (C–H aliphatic), 1708 (C=O carboxylate), 1631 and 1597 (C=C aromatic), 1496 and 1454 (C=C alkene), 1280 (C–O ether), 1110 (C–N amine), 1070 (C–O phenol), 866 (para-substituted aromatic ring), and 796 (meta-substituted aromatic ring).

^{1}H-NMR ($CDCl_3$, δ ppm): 7.59–7.55 (d, 1H, J = 15.89 Hz, Ar–CH=C), 7.00 (s, 1H, Ar–H), 6.82 (s, 1H, Ar–H), 6.29–6.26 (d, 1H, J = 15.91 Hz, C=CH–C), 4.60 (s, 1H, –OH), 4.40 (t, 2H, O–CH_2–C), 3.91 (s, 3H, O–CH_3), 3.75 (t, 4H of morpholine, CH_2–O–CH_2), 3.70 (s, 2H, Ar–CH_2–N), 2.60–2.49 (m, 4H of morpholine, CH_2–N–CH_2), 1.72–1.66 (m, 2H, C–CH_2–C), 1.43–1.13 (m, 2H, C–CH_2–C), and 0.94–0.85 (m, 3H, C–C–CH_3).

^{13}C-NMR ($CDCl_3$, δ ppm): 167.40 (C=O), 149.45, 144.59, 125.78, 122.14, 120.79, and 109.80 (6C, aromatic carbons), 148.27 (Ar-CH=C), 115.35 (C=CH-C), 66.73 (2C of morpholine, CH_2–O–CH_2), 64.56 (O–CH_2–C), 61.41 (Ar–CH_2–N), 55.93 (O–CH_3), 52.85 (2C of morpholine, CH_2–N–CH_2), 31.49, 29.70, 25.67, and 22.57 (4C, C–CH_2–C), 14.03 (C–C–CH_3).

d) 3d

Yellowish viscous liquid, 99.59% yield. IR (cm^{-1}): 2971, 2932, and 2792 (C–H aliphatic), 1708 (C=O carboxylate), 1632 and 1596 (C=C aromatic), 1493 and 1457 (C=C alkene), 1231 (C–O ether), 1142 (C–N amine), 1084 (C–O phenol), 870 (para-substituted aromatic ring), and 838 (meta-substituted aromatic ring).

^{1}H-NMR ($CDCl_3$, δ ppm): 7.12–7.09 (d, 1H, J = 15.87 Hz, Ar–CH=C), 6.58 (s, 1H, Ar–H), 6.42 (s, 1H, Ar–H), 5.85–5.82 (d, 1H, J = 15.83 Hz, C=CH–C), 4.44–4.16 (m, 2H, Ar–CH_2–N), 3.78–3.65 (m, 2H, O–CH_2–C), 3.54–3.43 (t, 3H, O–CH_3), 2.39–2.34 and 1.25–1.19 (m, 4H of 2,6-dimethylmorpholine, CH_2–N–CH_2), 2.23–1.71 (m, 2H, C–CH_2–C), 0.90–0.83 (m, 3H, C–C–CH_3), and a set of overlapping multiplets at 0.46–3.23 ppm corresponding 2,6-dimethylmorpholine (CH_3–CH–O–CH–CH_3).

^{13}C-NMR ($CDCl_3$, δ ppm): 166.45 (C=O), 149.13, 144.32, 125.18, 121.94, 120.80, and 109.70 (6C, aromatic carbons), 147.77 (Ar-CH=C), 114.69 (C=CH-C), 80.87 (O–CH_2–C), 71.33–71.04; 55.73–53.81 (4C of 2,6-dimethylmorpholine, CH_3–CH–O–CH–CH_3), 66.06 (Ar–CH_2–N), 63.74 (O–CH_3), 58.00 (CH_2–N–CH_2), 31.07, 28.37, 25.28, and 22.12 (4C, C–CH_2–C), and 13.65 (C–C–CH_3).

RESULT AND DISCUSSION

Chemistry

Based on the characterization results, the synthesis of ferulic acid (1) was successfully achieved, as confirmed by the agreement of its UV-Vis and FTIR spectra with reported literature. The UV-Vis spectrum of compound 1 exhibited three absorption maxima consistent with the findings of Kalinowska et al. (2014). A bathochromic shift compared with the precursor (vanillin) indicated the formation of an extended conjugated vinyl system within the FA structure (Dachriyanus, 2014; Kalinowska et al., 2014). The FTIR spectrum displayed characteristic absorption bands at 3736 cm^{-1} (O–H phenolic), 1697 cm^{-1} (C=O carboxylic), 1605–1510 cm^{-1} (C=C aromatic), and 1265 cm^{-1} (C–O carboxylic), in agreement with Sajjadi et al. (2012) and Field et al. (2008).

The synthesis of hexyl ferulate (2) was confirmed through FTIR and ^{1}H-NMR spectral analysis, both consistent with the expected structure and the literature data. The FTIR spectrum exhibited key absorption bands at 2956–2858 cm^{-1} (C–H aliphatic), 1695 cm^{-1} (C=O ester), and 1261 cm^{-1} (C–O ester), indicating successful esterification of ferulic acid with a hexyl chain. The ^{1}H-NMR spectrum further supported the formation of 2, showing characteristic and aliphatic chain protons at δ 4.19–0.87 ppm (Dachriyanus, 2014). These spectral features collectively confirm the successful synthesis of hexyl ferulate with the expected structural integrity.

Subsequent Mannich reactions of 2 with formaldehyde and various secondary amines produced four new Mannich base derivatives (3a–d) with yields ranging from 59–99%. The IR spectra displayed new absorptions at 1176–1110 cm^{-1} corresponding to C–N amin, while the

broad phenolic O–H band at 3500–3700 cm^{-1} disappeared, indicating hydrogen bonding between the nitrogen and the former phenolic hydroxyl group. Vibrations at 2700–2900 cm^{-1} confirmed the retention of the hexyl aliphatic chain after the Mannich modification.

All derivatives retained characteristic ferulate signals, including trans-alkene doublets around δ 7.6–5 ppm (J ≈16 Hz), aromatic protons at δ 6.9–7.0 ppm, and methoxy signals near δ 3.9 ppm with corresponding $^{13}C \approx 55$ ppm, confirming the ferulate core (Dachriyanus, 2014). Compound 3a showed a singlet at δ 2.35 ppm and δ 3.67 ppm corresponding to $N(CH_3)_2$ and $Ar–CH_2–N$ protons, respectively, indicating dimethylamine substitution. In 3b, the appearance of quartet (δ 2.66–2.62 ppm) and triplet (δ 1.14–1.11 ppm) signals confirmed the presence of a diethylamine group, while 3c exhibited distinct methylene signals (δ 3.70 ppm and δ 2.60–2.49 ppm) and ^{13}C at 52.85 ppm, consistent with a morpholine ring. Compound 3d displayed additional downfield methoxy and methylene resonances and splitting patterns suggesting a 2,6-dimethylmorpholine substituent, with duplicated signals attributed to cis/trans isomerism (Untung et al., 2017). Overall, the combined spectral data confirm the successful Mannich modification of hexyl ferulate with dimethylamine, diethylamine, morpholine, and 2,6-dimethylmorpholine moieties.

Antioxidant Activity

Figures 2 and 3 illustrate the relationship between the concentrations of the synthesized compounds and their antioxidant activities, as determined by the DPPH and FRAP assays, demonstrating a concentration-dependent increase. The corresponding IC_{50} and EC_{50} values are presented in Table 1.

According to DPPH results, ascorbic acid showed very strong antioxidant properties, with an IC_{50} value of 8.44 µM or 1.49 µg/mL (Melinda et al., 2024), whereas ferulic acid (1) (35.32 µM) and hexyl ferulate (2) (26.66 µM) displayed lower but still substantial activity. It's worth noting that all Mannich base derivatives (3a–d) exhibited very strong antioxidant capacity, with IC_{50} values ranging from 1.89 to 22.67 µM, indicating that Mannich modification had a significant impact on free-radical scavenging potential.

The antioxidant activity of the synthesized compounds is governed by multiple structural factors, particularly the presence of basic amine groups that increase the number of hydrogen-bond acceptors and facilitate electron and proton transfer. Mannich modification with amines of higher basicity enhances radical-scavenging activity by stabilizing the phenoxyl radical through resonance and inductive effects, thereby accelerating radical quenching, as previously reported by Hayun et al. (2020). Among the tested compounds, 3d showed the highest antioxidant activity, where the 2,6-dimethylmorpholine substituent provides a favorable balance of hydrogen-donor and -acceptor properties. The presence of an oxygen atom in the morpholine ring enables additional hydrogen-bonding interactions and possible intramolecular hydrogen bonding, which lowers the phenolic O–H bond dissociation energy and facilitates hydrogen atom donation (Plech et al., 2013).

The results of the FRAP assay were consistent with this trend. Outperforming ascorbic acid compounds 3b (EC_{50} = 0.34 µM) and 3d (EC_{50} = 2.20 µM) exhibited strong ferric reducing activity. Both compounds outperformed ferulic acid at a concentration of 5.26 µM and hexyl ferulate at 3.03 µM, suggesting that the Mannich modification enhanced electron transfer ability. Compound 3b exhibited the lowest EC_{50} value in the FRAP assay, which is plausibly attributed to the high basicity of the diethylamine moiety ($pK_a \approx 9.77$) that enhances its

electron-donating character. The strong electron-donating effect of the diethylamine group increases electron density on the phenolic aromatic ring, thereby facilitating more efficient electron transfer to Fe^{3+} ions in the FRAP system. Consistently, Hayun et al. (2020) reported that Mannich derivatives bearing diethylamine substituents showed the lowest FRAP EC_{50} values among related analogues, indicating superior reducing capacity associated with increased amine basicity.

The structure-activity relationship (SAR) suggests that this improvement comes from the combined impacts of lipophilicity, nitrogen basicity, and steric properties. The esterification of ferulic acid enhanced its lipophilicity, rising from a logP value of 1.36 to 3.53, thereby facilitating interaction with hydrophobic radical sites in lipid environments. The introduction of tertiary amines with variable basicity (5.8-8.7) via Mannich substitution stabilizes phenoxyl radicals via resonance and inductive effects. Compounds 3b (dietylamine) and 3d (2,6-dimethylmorpholine) achieved an optimal balance due to 3b being highly lipophilic (logP 4.25) as it efficiently donates hydrogen, and 3d benefiting from an additional hydrogen-bonding site from the morpholine oxygen, which enhances radical stabilization (Plech et al., 2013). In comparison, compounds 3a (dimethylamine) and 3c (morpholine) showed reduced activity due to unsuitable steric size or excessive polarity, which restricted radical interaction. Overall, the antioxidant potency of Mannich-modified ferulate derivatives arises from synergistic electronic and lipophilic interactions. Substituents containing nitrogen promote the donation of hydrogen and electrons, whereas increased lipophilicity facilitates interaction with lipid-phase radicals. Previous studies (Plech et al., 2013) have found that Mannich bases of phenolic compounds possess better abilities to scavenge radicals and act as reducing agents compared to their parent compounds.

Antibacterial Activity

The antibacterial properties of the synthesized Mannich base derivatives (3a–d) were evaluated through the agar diffusion assay (Table 2). The inhibition zones increased proportionally with concentration, supporting a concentration-dependent antibacterial effect. The ferulic acid Mannich derivatives exhibited higher activity than both ferulic acid (1) and its ester derivative (2), in agreement with previous studies reporting that ferulic acid shows only weak to moderate inhibition against S. aureus, B. subtilis, and E. coli, and is inactive against P. aeruginosa (Dua et al., 2022; Song et al., 2021). Of the tested compounds, 3d exhibited the strongest inhibition, showing broad-spectrum activity with inhibition zones up to 26.90 mm at a concentration of 3.00 mg/mL, whereas 3a and 3b displayed moderate activity primarily against Gram-positive strains.

The enhanced antibacterial activity of compounds 3a–d relative to compounds 1 and 2 is due to the Mannich modification introducing aminoalkyl substituents, which enhance lipophilicity and nitrogen basicity, thus improving membrane penetration and electrostatic interaction with negatively charged bacterial surfaces (Plech et al., 2013; Bobunja-Sonje et al., 2020). The phenolic hydroxyl group is likely responsible for hydrogen bonding and for inhibiting bacteria through redox-based mechanisms (Song et al., 2021).

Visual determination of MIC values was not possible in macrodilution tubes due to turbidity resulting from compound insolubility or suspension formation, a recognized limitation of the macrodilution method for lipophilic compounds (Balouiri et al., 2016). The MBC values ranged from 0.75 to 3.00 mg/mL (Table 3). Compound 3b exhibited the lowest

MBCs against both gram-positive and gram-negative bacteria, corresponding to its moderate lipophilicity (logP = 4.25) that promotes membrane permeability and intracellular interaction (Cloutier et al., 2018).

Overall, the antibacterial potency followed the order 3d > 3c > 3b > 3a in the diffusion assay, whereas 3b > 3d > 3c ≈ 3a was observed in the dilution assay. The superior activity of morpholine-containing derivatives (3c and 3d) could stem from their optimal balance of polarity and lipophilicity, which facilitates diffusion and target binding. The results confirm that the Mannich modification significantly enhances the antibacterial efficacy of phenolic compounds by improving their physicochemical and membrane-interacting characteristics.

All six compounds (1–3d) complied with Lipinski's Rule of Five (Table 4), indicating good potential for oral bioavailability. The molecular weights (194.18–405.53 g/mol), logP values (1.36–4.25), hydrogen bond donors (1–2), and acceptors (4–6) were within acceptable parameters, with TPSA values (55.76–68.23 Å²) indicating good membrane permeability. The results imply that the compounds have favorable pharmacokinetic characteristics and have a significant potential for further development as orally active medication candidates (Wulandari et al., 2023).

CONCLUSION

The structural modification of ferulic acid into hexyl ester and Mannich base derivatives successfully enhanced its antioxidant and antibacterial activities. Compounds 3b (diethylamine) and 3d (2,6-dimethylmorpholine) exhibited the strongest antioxidant effects, with IC_{50} and EC_{50} values surpassing those of ascorbic acid and ferulic acid. Experiments testing for antibacterial properties found that samples 3c and 3d exhibited broad-spectrum effectiveness against both Gram-positive bacteria (S. aureus, B. subtilis) and Gram-negative bacteria (E. coli, P. aeruginosa), displaying the largest inhibition areas and the lowest MIC values. The improved biological activities were correlated with higher logP values, indicating increased lipophilicity and better membrane permeability. Overall, these findings suggest that Mannich base substitution on the aromatic ring of hexyl ferulate esters is an effective strategy to enhance antioxidant and antibacterial potentials, making these compounds promising candidates for the development of new antimicrobial agents to combat antibiotic resistance.

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