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Synthesis, Spectral Characterization, and Structural Modification of a Hesperetin-Derived N-Benzylacetamide.

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ABSTRACT

Aim : This work aimed to synthesize and characterize a novel N-benzylacetamide derivative of hesperetin from Orange peel and evaluate its structural modification.

Materials Method: Intermediate 1 (75% yield) was produced by reacting hesperetin with ethyl bromoacetate in ethanol. Intermediate 2 (62% yield) was obtained after treatment with sodium carbonate in ethanol–water and acidification with hydrochloric acid. N-benzyl-2-[5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-7-yloxy]-acetamide, the last hesperetin derivative was synthesized by combining benzylamine with EDC/HOBt producing 48%. UV-visible spectroscopy, FT-IR, ^{1}H NMR, and mass spectrometry were used to confirm the structural and functional characteristics of the synthesized chemical.

Results: The synthesized compound was characterized using UV–visible spectroscopy, FT-IR, ^{1}H NMR, and mass spectrometry to confirm its structural and functional properties. The synthesis yielded the target hesperetin derivative with an overall efficiency (final yield 48%). UV–visible analysis showed a λmax at 288 nm. FT-IR spectra confirmed the presence of key functional groups, including O–H, C=O, and C–O. ^{1}H NMR spectra confirmed that the benzylacetamide molecule was successfully incorporated. Mass spectrometry exhibited a molecular ion peak at m/z 475.9 ([M+Na]$^+$), confirming the expected molecular structure.

Conclusion: A new N-benzylacetamide derivative of hesperetin was effectively synthesized and described in this study. Without changing the core flavanone, spectroscopic tests verified the structural alteration

Keywords: Synthesis, Hesperetin , Orange peel , UV-visible spectroscopy, FT-IR, ^{1}H NMR

INTRODUCTION

In the citrus industry, oranges are very known for their huge consumption, and the production of orange juice can lead to important amounts of peel wastes (Murador et al., 2019). Orange peel can represent a reliable source of bioactive compounds such as limonoids, enzymes, dietary fibers, carotenoids, and phenolic compounds (Jokić et al., 2020; Obafaye and Omoba, 2018). These valuable compounds are also rich in antioxidants with a wide range of health benefits, preventing chronic diseases, reducing the incidence of cancer and exerting anti-inflammatory effects (Anticona et al., 2020; Meléndez-Martínez et al., 2021; Sagar et al., 2018). Furthermore, the valorization of orange byproducts could be processed for bioactive compound extraction and isolation for their use in the food, cosmetic and pharmaceutical industries (Panić et al., 2019).

Naringin and Hesperidin are the main citrus flavonoids with physiological properties present orange juice. (Minakshi et al..2015) Hesperidin may be associated with potential benefits in the prevention of many diseases, such as decreasing capillary permeability, anti inflammatory, antimicrobial and anticarcinogenic effects. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductas (Uehara, 2006). Hesperidin and hesperetin are the main active ingredients of citrus fruits, and hesperetin is a natural product worthy of study in citrus (Kim & Lim, 2020). Application research of the natural product hesperetin is a hot frontier in related neighborhoods. Hesperetin has a variety of pharmacological activities, such as anti-inflammatory (Crespo et al., 1999), antioxidant (Wilmsen et al., 2005), antibacterial, anticancer (Tempesti et al., 2012), anti-hepatic fibrosis (Elshazly & Mahmoud, 2014) and immune regulation. However, since hesperidin and hesperetin are easily decomposed and absorbed in

the gastrointestinal tract after oral administration (Nectoux et al., 2019), the bioavailability of hesperidin and hesperetin is low, and the activity is general (Yamamoto et al., 2013). Therefore, the structural modification of hesperetin to better achieve the biologic activity of hesperetin is worthy of study

Therefore, in this work, we report the synthesis of hesperetin derivatives and evaluate the antimicrobial effects, anti-inflammatory effect of compounds. We look forward to finding compounds that can simultaneously enhance a variety of biological activities in these hesperetin derivatives and to promoting further application development.

MATERIALS AND METHODS

1.1 Chemical and instruments used

All chemicals and reagents used were of analytical grade and used without further purification. Ethyl 2-bromoacetate, benzylamine, potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3), potassium iodide (KI), hydrochloric acid (HCl), and coupling agents EDC and HOBt were procured from standard suppliers. Solvents including ethanol, methanol, chloroform, ethyl acetate, and petroleum ether were of analytical grade. Anhydrous sodium sulfate (Na_2SO_4) was used as a drying agent. Silica gel (60–120 mesh) and pre-coated TLC plates were used for chromatographic analysis.

1.2 Preparation of Hesperetin derivative

The reaction of above obtained hesperetin with ethyl bromoacetate in ethanol provided intermediate 1 in 75% yields. Then, treatment of intermediate 1 with excess sodium carbonate in ethanol and H2O, followed by acidification with aqueous HCl, resulted in intermediate 2 in 62% yield. Finally, the reaction of intermediate 2 with benzylamine produced 48% yields Hesperetin derivative - N-Benzyl-2-[5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-7-yloxy]-acetamide **(Luo et al., 2016; Li et al., 2017).**

1.2.1 Synthesis of Intermediate 1

Hesperetin (3.02 g, 10 mmol), ethyl 2-bromoacetate (1.66 g, 15 mmol), anhydrous K_2CO_3 (2.76 g, 20 mmol) and catalytic amount KI were added in anhydrous ethanol (150 mL), the mixture was refluxed for 1 h. The solution was evaporated, and the residue was poured into water and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, and then concentrated to give white solid 2, which was used without further purification.

1.2.2 Synthesis of Intermediate 2

Compound 2 (2.82 g, 7.1 mmol), 5% Na_2CO_3 (10 mL), and ethanol (40 mL) were stirred at 50 °C for 8 h. Then the reaction mixture was poured into 10% HCl (200 mL), and filtered. The filter cake was recrystallized from EtOH to give yellow solid.

1.2.3 Procedures for the Preparation of hesperetin derivative

Compound 3 (0.37 g, 1 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC, 0.29 g, 1.5 mmol), 1-hydroxybenzotrizole (HOBT, 0.20 g, 1.5 mmol) and $CHCl_3$ (20 mL) were stirred at room temperature for 1 h, and then benzylamine (3 mmol) were added. The solution was stirred at room temperature overnight. Then the solvent was poured into water and extracted with ethyl acetate (20 mL × 3). The solution was dried over anhydrous Na_2SO_4 and concentrated. The residues were purified by flash chromatography with chloroform/petroleum ether (2:1, *v*/*v*) elution. White solid (48% yield) was obtained finally.

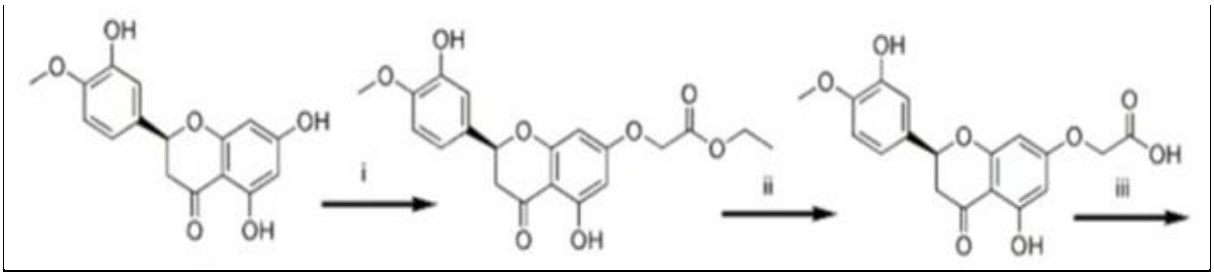


Figure 1: Synthesis of hesperetin derevative

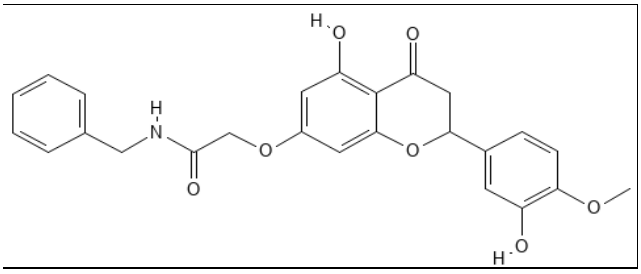


Figure 2: Structure of obtained hesperetin derivative (- N-Benzyl-2-[5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-

oxochroman-7-yloxy]-acetamide).

1.3 Characterization hesperetin derivative

1.3.1 UV-visible Spectroscopy:-

The synthesized hesperetin derivative were scanned over a wavelength range of 200 to 800 nm using a UV-Visible spectrophotometer (Shimadzu UV-1700), and the characteristic peaks were detected and recorded**(Kapoor et al., 2022).**

1.3.2 Characterization of synthesized hesperetin derivative by FT-IR spectroscopy:-

FT-IR spectroscopy was performed using a Perkin Spectrum 95763 spectrophotometer to identify functional groups in the synthesized hesperetin derivative **(Sant et al., 2025).**

1.3.3 Characterization of hesperetin derivative by NMR spectroscopy:-

The synthesized compound was characterized using nuclear magnetic resonance (NMR) spectroscopy. ^{1}H NMR spectra was recorded on a JNM EC-500 NMR spectrometer operating at [400/500/600] MHz for ^{1}H, respectively **(Mistry et al., 2017).**

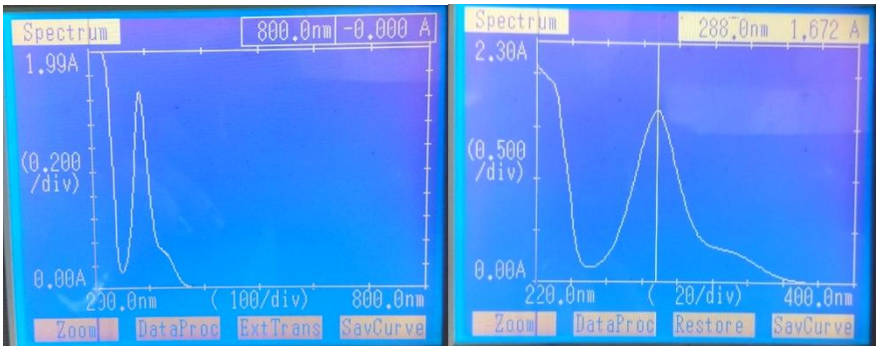
1.3.4 Characterization of synthesized hesperetin derivative by Mass spectroscopy:-

Mass spectrometry converts molecules into ions, which are then separated and detected based on their mass-to-charge (m/z) ratios. It was used to determine the molecular weights of the synthesized hesperetin derivative. The measurements were recorded using a micrOTOF-Q mass spectrometer **(Kapoor et al., 2022).**

2. Results

3.1 Spectral analysis of isolated fraction F7 (hesperetin)

3.1.1 UV spectroscopy of isolated fraction F7 (hesperetin)



Graph 1: UV Graph of isolated fraction F7 (hesperetin)

The maximum absorption wavelength (λmax) of the isolated fraction F7 (hesperetin) was observed at 288 nm.

3.1.2 IR spectra of isolated fraction F7 (hesperetin)

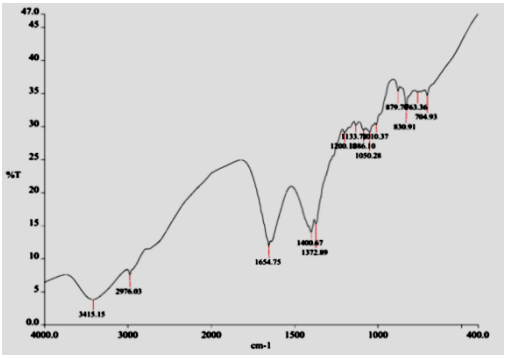


Figure 3: IR spectra of isolated fraction F7 (hesperetin)

IR Spectra of isolated fraction F7 (hesperetin) showed strong and broad absorbance at 3415.15 cm^{-1} due to the presence of O-H bond. Stretching of C-H bond at 2976 cm^{-1}denotes the presence of alkanes. Strong, sharp peak at 1654 cm^{-1} shows C=O stretch and peak at 1372 cm^{-1} confirms the presence of C-O bond of alcohols and ethers. Weak absorbance at 1086 cm^{-1} shows C-C stretching of alkanes.

3.1.3 ^{1}H NMR spectra of Hesperetin:–

(A)$_{1}$H NMR spectra of Hesperetin :–

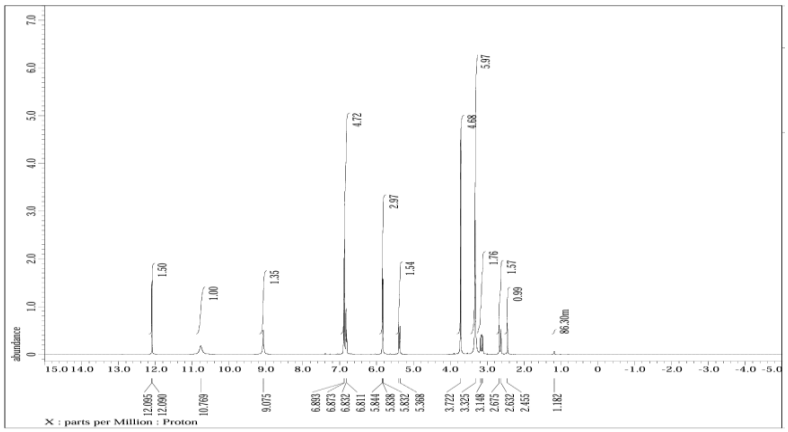


Figure 4: lH-NMR spectra of the isolated fraction F7 (hesperetin)

3.1.4 Mass – Spectroscopy:-

A mass spectrum of isolated fraction F7 (hesperetin) was recorded on Bruker micrOTOF-Q mass spectrometer. According to fragmentation pattern number of atom C (16), O (6), and H (14) with molecular formula concluded is $C_{16}H_{14}O_6$.

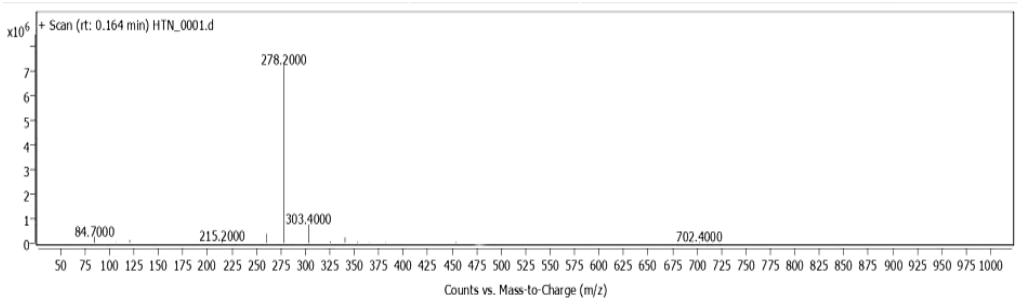
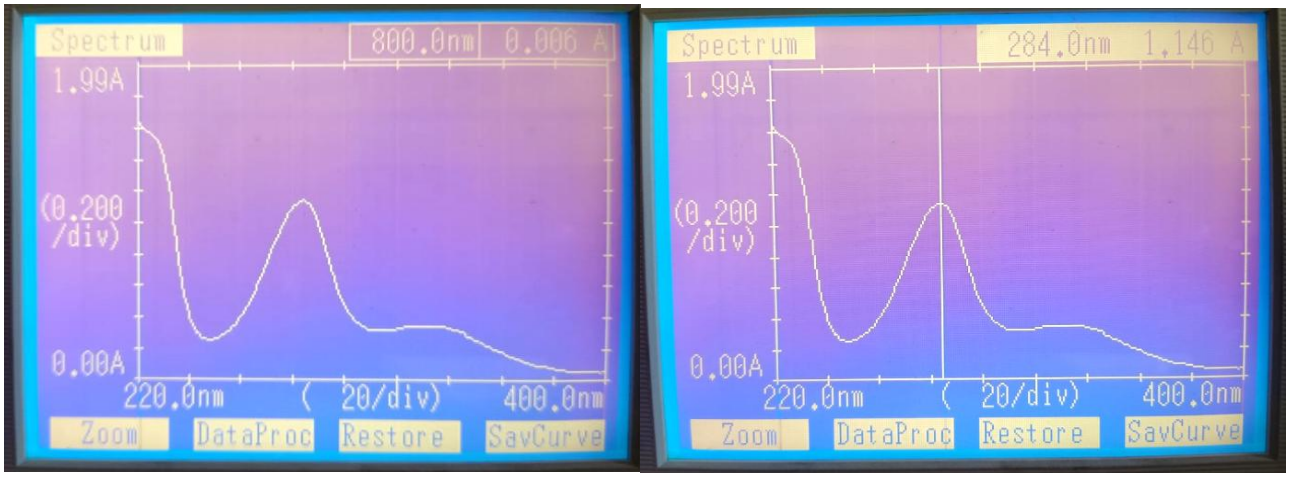


Figure 5: Mass spectrum of isolated fraction F7 (hesperetin)

3.2 Spectral analysis of hesperetin derivative

3.5.1 UV spectra of hesperetin derivative-



Graph 2: UV Graph of hesperetin derivative

The λ max of the hesperetin derivative was found to be 288 nm.

3.5.2 IR spectra of hesperetin derivative

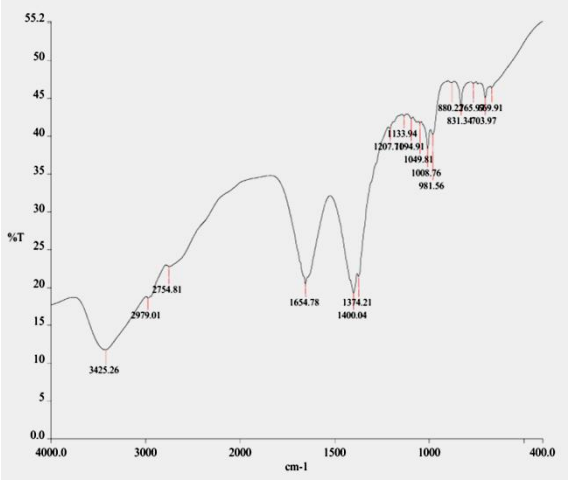


Figure 6: IR spectra of hesperetin derivative

IR Spectra of hesperetin derivative showed O-H bond stretching via strong and broad absorbance at 3425cm^{-1}. Stretching at 2979 cm^{-1} of C-H bond shows the presence of alkanes. Strong, sharp peak at 1654cm^{-1} shows C=O stretch could be attributed to ketone and ether functional group. Confirmation of alcohols and ethers was achieved by the strong and sharp absorbance at 1374cm^{-1} of C-O bond. Weak absorbance at 1008cm^{-1} shows C-C stretching of alkanes.

3.5.3 NMR – Spectroscopy hesperetin derivative–

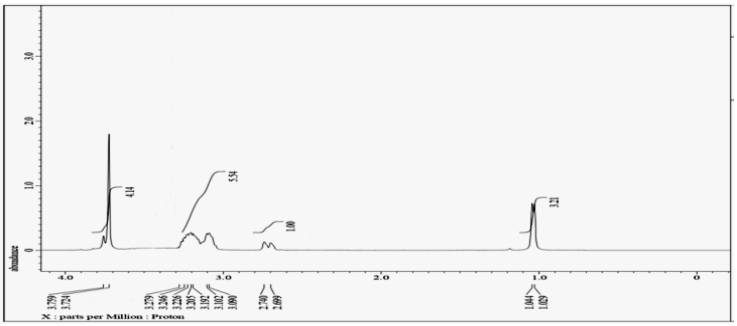


Figure 7: ^{1}H-NMR spectra of the hesperetin derivative.

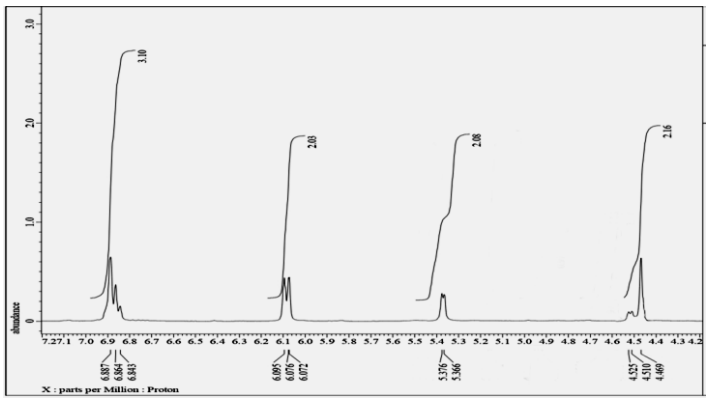


Figure 8: ^{1}H-NMR spectra-2 of the hesperetin derivative.

H-1 proton on cyclic alkane adjacent to ether functional group appeared at 3.1 ppm as triplet. H-2 proton on cyclic alkane adjacent to functional group ketone appeared at 2.74 and 2.69 ppm as doublet.

3.5.4 Mass – Spectroscopy hesperetin derivative-

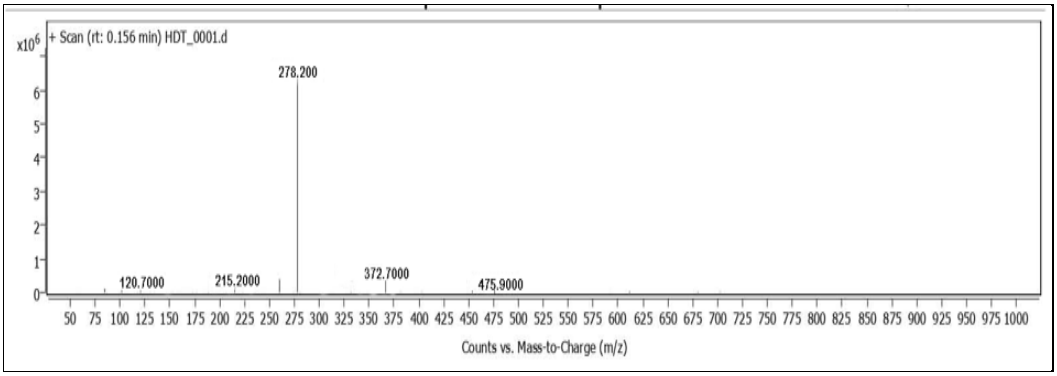


Figure 9: Mass spectrum of hesperetin derivative

Mass spectra of the obtained hesperetin derivative showed molecular ion [M^+] peak at 475.900. Peak at 327 m/z results due to loss of N-phenmethylacetamide. Base peak at 278 m/z is due to alpha cleavage at ether, resulting in loss of Rutinose sugar moiety followed by decarboxylation and rearrangement of molecular ion. Generation of 2-methoxy phenol cation results in peak at m/z 120. The data corresponds to $[M + Na]^+$ ($C_{25}H_{23}NNaO_7$) molecular formula $C_{25}H_{23}NNaO_7$ of F7D according to their fragments.

DISCUSSION

The N-benzylacetamide hesperetin derivative was successfully synthesized using a three-step procedure, but the yield gradually decreased (75% → 62% → 48%), which is common for multi-step syntheses that need purification at each stage. Although satisfactory for research-scale synthesis, the final yield of 48% for the target product indicates that overall efficiency may be increased by optimizing the final amidation reaction conditions. A spectroscopic investigation verified that the hesperetin core structure has been successfully modified. The parent hesperetin and its derivative share the same λmax at 288 nm, according to the UV-Vis spectra, suggesting that the essential flavanone chromophore was unaffected by the synthetic alterations. With very slight changes from the original compound due to the changing structural environment, the derivative's IR spectra showed all anticipated functional group absorptions, including the distinctive O-H, C=O, and C-O stretches. The ^1H NMR data provided definitive evidence for the successful introduction of the benzylacetamide moiety, with new signals corresponding to the protons on the modified cyclic alkane structure. Finally, mass spectrometry confirmed the molecular weight and formula, with the molecular ion peak at m/z 475.900 corresponding to [M + Na]+ ($C_2{}^2H_2{}^3NNaO_7$), clearly distinguishing it from the parent hesperetin (m/z 302). This structural alteration, which adds a more lipophilic benzylacetamide group at the 7-hydroxy position, was purposefully created to potentially improve membrane permeability and decrease premature gastrointestinal metabolism in order to address native hesperetin's limited bioavailability. The final success of this molecular engineering technique can only be confirmed by further biological activity testing to ascertain whether the anticipated improvements in antibacterial and anti-inflammatory activities have been achieved, even though the physicochemical characterization is finished

CONCLUSION

This study successfully synthesized a novel N-benzylacetamide hesperetin derivative via a three-step pathway, with its structure confirmed by comprehensive spectroscopic analysis. The strategic modification at the 7-hydroxy position was designed to address the low bioavailability of native hesperetin, potentially enhancing its pharmacological profile. While the chemical synthesis and characterization are complete, the true value of this derivative lies in its yet-to-be-determined biological activities. This work establishes a crucial foundation for future investigations into its antimicrobial and anti-inflammatory properties, representing a significant step toward developing more effective bioactive compounds from citrus byproducts for pharmaceutical applications

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