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Original Research Article

In Silico Prediction of Anti-Inflammatory Potential of Isolated Compounds from *Enhalus acoroides*

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ABSTRACT

Enhalus acoroides is a marine flowering plant with numerous biological activities. The current study aim to isolate, characterize the chemical constituents from *Enhalus acoroides* whole plant, and to evaluate their anti-inflammatory activity *in silico*. The dried powdered *E. acoroides* whole plant was subjected to extraction, portioning, and chromatographic separation for the isolation of its phytochemical constituents. The structures of the isolated compounds were elucidated by a combination of Nuclear Magnetic Resonance (NMR) spectroscopy, Mas Spectrometry (MS), and comparison of spectral data with literature. The isolated compounds were investigated for their potential anti-inflammatory activity *in silico* via molecular docking of the compounds with inducible nitric oxide synthase (iNOS). Phytochemical investigation of the whole plant of *E. acoroides* led to the isolation of ten (10) compounds. Their chemical structures were determined as methyl pheophorbide a (1), (+)-catechin-4'-*O*-*β* -D-glucoside (2), quercetin (3), rutin (4), apigenin-7-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β* -D-glucopyranoside (5), adenosine (6), uracil (7), D-mannitol (8), 6*β* -hydroxystigmast-4-en-3-one (9), and daucosterol (10) by ESI-MS, NMR spectral analysis, and comparison with published data. Molecular docking simulations of the compounds revealed their potential iNOS inhibitory activity, with compounds 1, 2, 3, 4, 5, 9, and 10 showing stronger binding affinities than the reference inhibitor, ethyl 4-[(4-methylpyridin-2-yl)amino]piperidine-1-carboxylate (AR-C95791). These findings suggest that the compounds, particularly compound 10, hold significant promise as iNOS inhibitors, potentially serving as candidates for anti-inflammatory drug development.

Keywords: *Enhalus acoroides*, Chemical constituents, Anti-inflammatory, Binding affinity.

Introduction

Enhalus acoroides (L.f.) Royle, belonging to the Hydrocharitaceae family, is a marine flowering plant that grows in the shallow coastal waters of tropical and temperate regions.[1] In Vietnam, *E. acoroides* is commonly found in the Tam Giang-Cau Hai lagoon area (Thua Thien-Hue province) and Cam Lam Beach (Khanh Hoa province). Phytochemical screening of *E. acoroides* has revealed the presence of flavonoids, steroids, monoterpenoids, diterpenoids, and aliphatic acids.[2-4] Pharmacological investigations have demonstrated that *E. acoroides* exhibits various biological activities such as antioxidant, antifeedant, antibacterial, larvicidal, antitumour, and cytotoxic effects.[1-5] However, the anti-inflammatory activity of *E. acoroides* has not been studied.

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In a targeted bioprospecting effort to discover new anti-inflammatory agents from marine flowering plants, the present study reports the isolation and characterization of ten compounds from *E. acoroides* collected in Vietnam. An *in silico* study was conducted to first evaluate the potential binding affinity of the isolated compounds for the anti-inflammatory inducible nitric oxide synthase (iNOS) target protein. The results from the *in silico* study is aimed at predicting potential active compounds in *Enhalus acoroides* that could further be investigated *in vitro* and *in vivo*.

Materials and Methods

General experimental procedures

The NMR data were recorded using Bruker AV-III 500 NMR spectrometer with TMS as the internal standard and chemical shifts were reported in δ values (ppm). The electrospray ionization mass spectrometry (ESI-MS) spectra were measured on an Agilent LC/MS-6420. The preparative HPLC was run on an Agilent 218 Purification System using the ZORBAX SB-C18 columns (100 mm × 21.2 mm × 5µm). Silica gel 230–400 mesh (0.040–0.063 mm, Merck) and YMC RP-18resins (30–50 µm, Fujisilisa Chemical Ltd.) were used for the column chromatography (CC). The thin-layer chromatography (TLC) was done on pre-coated silica gel 60-F254 plates. Spots were visualized by spraying with 10% sulfuric acid reagent, and heating. All chemicals and reagents used in this study were of analytical grade.

Plant materials

The whole plant of *E. acoroides* was collected from the Van Phong-Khanh Hoa province (12.1960803 N, 108.9950386 E), Vietnam in March 2023. The plant material was identified and authenticated by Assoc. Prof. Dr. Dam Duc Tien, a plant taxonomist in the Department

of Chemistry, Vinh University, Vietnam. A herbarium specimen with voucher No. XuanThuy-032021 was deposited at the Laboratory of the department.

Extraction and isolation

The air-dried whole plant of *E. acoroides* (14 kg) were cut into small pieces and extracted with methanol (50 L × 3 times) by maceration at room temperature. The solvent was removed by a vacuum evaporator to yield the crude methanol extract (685 g), which was further suspended in water and successively partitioned with n-hexane (8 L × 3 times), ethyl acetate (8 L × 3 times) and n-butanol (8 L × 3 times) to give n-hexane extract (EAH 157.5 g), ethyl acetate extract (EAE 238.9 g), and n-butanol extract (EAB 64.6 g), respectively. The n-hexane extract was subjected to silica gel chromatography column (CC), eluted with hexane/acetone (100:1 to 1:1, v/v) to yield seven fractions (Frs. EAH1 – EAH7). Fraction EAH3 (6.1 g) was subjected to silica gel CC eluted with hexane/ethyl acetate (50:1 to 2:1, v/v) to afford compound 9 (22.4 mg). The ethyl acetate extract was separated over a silica gel CC, eluted with chloroform/methanol (100:0 to 3:1, v/v) to produce eight fractions (Frs. EAE1-EAE8). Fraction EAE2 (16.4 g) was applied to silica gel CC, eluted with n-hexane-acetone (10:1 to 1:1, v/v) to afford compound 1 (44.7 mg). Fraction EAE3 (11.5 g) was subjected to a silica gel CC, eluted with dichloromethane/methanol (25:1 to 0:1, v/v) to yield four subfractions (Frs. EAE3.1-EAE3.4). Subfraction EAE3.3 (2.9 g) was further purified using preparative HPLC (60% methanol in water) to obtain compound 3 (20.1 mg). The n-butanol extract (EAB) was also subjected to silica gel CC eluted with gradient chloroform/methanol/water (50:1:0 to 1:1:1, v/v) to obtain nine fractions (Frs. EAB1 – EAB9). Further purification of fraction EAB2 (1.2 g) was separated on silica gel CC, eluted with chloroform/methanol (90:10 to 25:75 v/v) to give compound 4 (45.9 mg). Fraction EAB3 (45.1 mg) was purified using preparative HPLC (70% acetonitrile in water) to yield compound 5 (10.4 mg). Subfraction EAB4 (1.7 g) was separated by CC with RP-C18 eluted with methanol:water (3:1), and further purified by sephadex LH-20 CC to give compound 6. Compound 2 (42.3 mg) was isolated from subfraction EAB5 (8.1 g) by silica gel CC eluted with chloroform/methanol (7:1, 4:1, 1:1 v/v in a stepwise gradient). Fraction EAB6 (2.1 g) was purified using preparative HPLC (50% acetonitrile in water) to yield compound 7 (9.6 mg) and compound 8 (11.2 mg). The EAB8 fraction (4.2 g) was separated by CC eluted with chloroform:methanol (9:1) to obtain compound 10 (53.7 mg).

Molecular docking

The structure of inducible nitric oxide synthase in complex with AR-C95791 was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/structure/3E7G) (PDB ID: 3E7G).[6] All ions, water molecules, and co-crystallized ligands were removed, and hydrogen atoms along with Kollman charges were added using AutoDockTools v1.5.6. The 3D structures of the isolated compounds were drawn using Marvin JS software, then their energy was optimized using the UFF force field in the OpenBabel program package.[7,8] Subsequently, the ligand and protein files were converted to PDBQT format, ready for molecular docking simulations using AutoDock Vina v1.2.3.[9,10] The box coordinate parameters were set as follows: center coordinates were defined as x = 55 Å, y = 21.8 Å, z = 78.7 Å, box size X x Y x Z = 24 x 24 x 24, spacing = 1, and other parameters were set to default. The exhaustiveness value was adjusted to 400 as reported previously.[11,12] The re-docking process was performed with the co-crystallized ligand AR-C95791 (Ethyl 4-[(4-methylpyridin-2-yl)amino]piperidine-1-carboxylate) to verify the parameters for a reliable binding mode prediction. After obtaining the docking results of the isolated compounds with the iNOS protein target, Discovery Studio Visualizer was used to analyze and visualize their interactions.

Results and Discussion

Isolation and structure elucidation

The column chromatographic seperation, and preparative HPLC of the crude methanol extract of *Enhalus acoroides* whole plant led to the isolation of ten compounds. These compounds included methyl pheophorbide a (1), (+)-catechin-4'-*O*-*β* -D-glucoside (2), quercetin (3), rutin (4), apigenin-7-*O*-α-L-rhamnopyranosyl-(1→2)-*β* -D-glucopyranoside (5), adenosine (6), uracil (7), D-mannitol (8), 6*β* -hydroxystigmast-4-en-3-one (9) and daucosterol (10).

Compound 2 was obtained as a white amorphous powder. The ESI-MS spectrum of 2 exhibited a molecular ion peak at m/z 453 $[M+H]^+$, corresponding to the molecular formula of $C_{21}H_{24}O_{11}$. The ^{1}H-NMR spectra of compound 2 exhibited signals of five aromatic protons at δ_H 6.90 – 5.80 ppm and an anomeric proton signal at δ_H 4.77 (1H, d, J = 6.9 Hz, H-1'') . The ^{13}C-NMR spectrum of compound 2 exhibited 21 carbon signals, including 15 of a flavan-3-ol and 6 of a sugar, suggesting a flavonoid glycoside. The NMR data of compound 2 were compared with the literature data reported for (+)-catechin-4'-*O*-*β* -D-glucoside, [13] and were found to match.

Compound 3 was obtained as a yellow amorphous powder. The ESI-MS spectrum of 3 exhibited a molecular ion peak at m/z 301 $[M-H]^-$, corresponding to the molecular formula of $C_{15}H_{10}O_7$. The ^{1}H-NMR spectra of 3 exhibited five aromatic protons at δ_H 7.76 (1H, s, H-2'), 7.66 (1H, d, J = 8.0 Hz, H-6'), 6.91 (1H, d, J = 8.0 Hz, H-5'), 6.41 (1H, s, H-8), and 6.21 (1H, s, H-6). The ^{13}C-NMR spectra of 3 showed fifteen carbon signals including a carbonyl group at δ_C 177.4 (C-4), seven oxygenated aromatic carbons at δ_C 165.6 (C-7), 162.5 (C-5), 158.3 (C-9), 148.0 (C-2/C-4'), 146.2 (C-3'), 137.2 (C-3), two quaternary aromatic carbons at δ_C 124.2 (C-1') and 104.5 (C-10), five sp^2 methine carbons at δ_C 121.7 (C-6'), 116.2 (C-5'), 116.0 (C-2'), 99.3 (C-6), and 94.4 (C-8) were confirmed a flavonoid bearing five hydroxyl groups. Based on NMR, MS data, and published data,[14] compound 3 was characterized as quercetin.

Compound 4 was obtained as a yellow amorphous powder. Its ESI-MS spectrum of 4 showed a molecular ion peak at m/z 611 $[M+H]^+$, corresponding to the molecular formula of $C_{27}H_{30}O_{16}$. The ^{1}H-NMR spectra of 4 exhibited signals of two aromatic systems, characteristics of the quercetin compound. The ^{13}C-NMR spectra of 4 revealed 27 carbon signals, including 15 carbons of a flavonol skeleton and 12 carbon of two sugar units, suggesting a flavonoid glycoside. Based on the MS, NMR analyses, and published data,[15] compound 4 was elucidated as rutin.

The other compounds were elucidated as methyl pheophorbide a (1),[16] apigenin-7-*O*-α-L-rhamnopyranosyl-(1→2)-*β* -D-glucopyranoside (5),[17] adenosine (6),[18] uracil (7),[19] D-mannitol (8),[20] 6*β* -hydroxystigmast-4-en-3-one (9),[21] and daucosterol (10).[22] Their MS and NMR data were compared to those of the literature and found to match. To the best of our knowledge, this is the first report on the isolation and structure elucidation of chemical constituents from *Enhalus acoroides*. The structures of these components are shown in Figure 1.

Characterization of the isolated compounds

Methyl pheophorbide a (1): white amorphous powder; ESI-MS: m/z 607 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.46 (1H, s, H-10), 9.30 (1H, s, H-5), 8.54 (1H, s, H-20), 7.93 (1H, dd, J = 18.0, 11.5 Hz, H-3'), 6.25 (1H, s, H-13''), 6.25 (1H, d, J = 17.5 Hz, H-3''E), 6.15 (1H, dd, J = 12.0, 1.0 Hz, H-3''Z), 4.45 (1H, *dt*, J = 7.0, 1.5 Hz, H-18), 4.20 (1H, d, J = 10.0 Hz, H-17), 3.88 (3H, s, H-13''''), 3.66 (3H, s, H-12'), 3.62 (2H, *q*, J = 7.5 Hz, H-8'), 3.57 (3H, s, H-17''''), 3.37 (3H, s, H-2'), 3.17 (3H, s, H-7'), 2.63 (1H, m, H-17'a), 2.51 (1H, m, H-17''a), 2.32 (1H, m, H-17'b), 2.25 (1H, m, H-17''b), 1.81 (3H, d, J = 7.5 Hz, H-18'), 1.66 (3H, t, J = 7.5 Hz, H-8''); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 189.6 (C-13'), 173.4 (C-17'''), 172.2 (C-19), 169.6 (C-13'''), 161.2 (C-16), 155.6 (C-6), 151.0 (C-9), 149.7 (C-14), 145.2 (C-8), 142.1 (C-1), 137.9 (C-11), 136.5 (C-3), 136.2 (C-4), 136.1 (C-7), 131.9 (C-2), 129.1 (C-12), 129.0 (C-13/C-3'), 122.8 (C-3''), 105.2 (C-15), 104.4 (C-10), 97.5 (C-5), 93.1 (C-20), 64.7 (C-13''), 52.8 (C-13''''), 51.7 (C-17''''), 51.1 (C-17), 50.1 (C-18), 31.1 (C-17''), 29.9 (C-17'), 23.1 (C-18'), 19.4 (C-8'), 17.4 (C-8''), 12.1 (C-2'/C-12'), 11.2 (C-7').

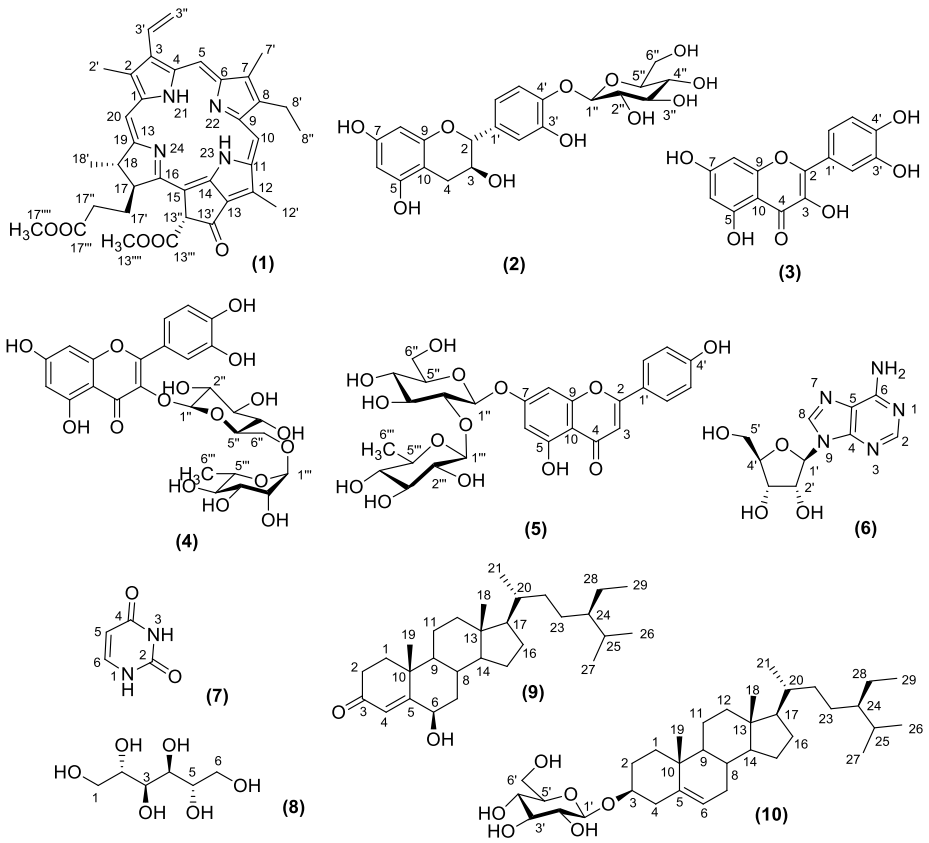


Figure 1: Structures of isolated compounds (**1** – **10**) from *Enhalus acoroides* whole plant

*(+)-Catechin-4'-O-β -D-glucoside (**2**):* white amorphous powder; ESI-MS: m/z 453 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 6.86 (1H, d, *J* = 2.0 Hz, H-2'), 6.79 (1H, m, H-5'), 6.75 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 5.95 (1H, d, *J* = 2.0 Hz, H-6), 5.88 (1H, d, *J* = 2.5 Hz, H-8), 4.77 (1H, d, *J* = 6.9 Hz, H-1''), 4.59 (1H, d, *J* = 7.5 Hz, H-2), 4.00 (1H, m, H-3), 3.80 – 3.50 (4H, m, H-2''/H-3''/H-4''/H-5''), 3.61 (1H, dd, *J* = 11.0, 5.0 Hz, H-6''a), 3.54 (1H, dd, *J* = 11.0, 6.0 Hz, H-6''b), 2.87 (1H, dd, *J* = 16.5, 5.5 Hz, H-4a), 2.53 (1H, dd, *J* = 16.0, 8.0 Hz, H-4b); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 157.8 (C-7), 157.6 (C-5), 156.9 (C-9), 146.2 (C-3'/C-4'), 132.2 (C-1'), 120.0 (C-6'), 116.1 (C-5'), 115.3 (C-2'), 100.9 (C-10/C-1''), 96.3 (C-6), 95.5 (C-8), 82.8 (C-2), 79.9 (C-3''), 75.6 (C-2''), 73.9(C-5''), 71.3 (C-4''), 68.8 (C-3), 64.4 (C-6''), 28.5 (C-4).

*Quercetin (**3**):* yellow amorphous powder; ESI-MS: m/z 301 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 7.76 (1H, s, H-2'), 7.66 (1H, d, *J* = 8.0 Hz, H-6'), 6.91 (1H, d, *J* = 8.0 Hz, H-5'), 6.41 (1H, s, H-8), 6.21 (1H, s, H-6); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 177.4 (C-4), 165.6 (C-7), 162.5 (C-5), 158.3 (C-9), 148.0 (C-2/C-4'), 146.2 (C-3'), 137.2 (C-3), 124.2 (C-1'), 121.7 (C-6'), 116.2 (C-5'), 116.0 (C-2'), 104.5 (C-10), 99.3 (C-6), 94.4 (C-8).

*Rutin (**4**):* yellow amorphous powder; ESI-MS: m/z 611 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H (ppm): 12.59 (1H, s, 5-OH), 7.54 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 7.53 (1H, d, *J* = 2.0 Hz, H-2'), 6.83 (1H, d, *J* = 8.0 Hz, H-5'), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 5.15 (1H, d, *J* = 7.2 Hz, H-1''), 4.56 (1H, d, *J* = 1.6 Hz, H-1'''), 3.70 (1H, d, J = 10.5 Hz, H-6''a), 3.30 (1H, m, H-6''b), 3.29 (1H, m, H-3'''), 3.28 (1H, m, H-2'''), 3.25 (1H, m, H-5'''), 3.24 (1H, m, H-5''), 3.22 (1H, m, H-2''), 3.21 (1H, m, H-3''), 3.07 (1H, m, H-4'''), 3.05 (1H, m, H-4''), 0.99 (3H, d, J = 6.2 Hz, H-6'''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C (ppm): 177.4 (C-4), 164.1 (C-7), 161.2 (C-5), 156.6 (C-2), 156.4 (C-9), 148.4 (C-4'), 144.7 (C-3'), 135.3 (C-3), 121.6 (C-1'), 121.2 (C-6'), 116.3 (C-2'), 115.2 (C-5'), 103.9 (C-10), 101.2 (C-1''), 100.7 (C-1'''), 98.7 (C-6), 93.6 (C-8), 76.4 (C-3''), 76.0 (C-5''), 74.1 (C-2''), 71.8 (C-4'''), 70.6 (C-3'''), 70.4 (C-2'''), 70.0 (C-4''), 68.5 (C-5'''), 67.0 (C-6''), 17.7 (C-6''').

*Apigenin-7-O-α-L-rhamnopyranosyl-(1→2)-β -D-glucopyranoside (**5**):* yellow amorphous powder; ESI-MS: m/z 579 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 7.89 (2H, d, *J* = 8.5 Hz, H-2'/H-6'), 6.95 (2H, d, *J* = 8.5 Hz, H-3'/H-5'), 6.80 (1H, s, H-8), 6.66 (1H, s, H-3), 6.47 (1H, s, H-6), 5.31 (1H, s, H-1'''), 5.21 (1H, d, *J* = 8.0 Hz, H-1''), 1.33 (3H, d, *J* = 6.5 Hz, H-6'''); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 184.1 (C-4), 166.8 (C-2), 164.4 (C-5/C-7), 162.9 (C-4'), 159.0 (C-9), 129.7 (C-2'/C-6'), 123.1 (C-1'), 117.1 (C-3'/C-5'), 107.1 (C-10), 104.2 (C-3), 102.5 (C-1'''), 101.0 (C-6), 99.8 (C-1''), 95.9 (C-8), 79.0 (C-2''), 78.4 (C-3''), 78.3 (C-5''), 75.9 (C-4'''), 74.0 (C-4''), 72.2 (C-3'''), 70.0 (C-5'''), 71.4 (C-2'''), 62.4 (C-6''), 18.3 (C-6''').

*Adenosine (**6**):* white amorphous powder; ESI-MS: m/z 266 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$ & CD_3OD) δ_H (ppm): 8.23 (1H, s, H-2), 8.05 (1H, s, H-8), 5.86 (1H, d, *J* = 7.2 Hz, H-1'), 4.79 (1H, dd, *J* = 7.2, 4.8 Hz, H-2'), 4.35 (1H, dd, *J* = 4.8, 2.4 Hz, H-3'), 4.27 (1H, q, *J* = 2.4 Hz, H-4'), 3.97 (1H, dd, *J* = 12.4, 2.4 Hz, H-5'a), 3.77 (1H, dd, *J* = 12.4, 2.4 Hz, H-5'b); ^{13}C-NMR (125 MHz, $CDCl_3$ & CD_3OD) δ_C (ppm): 156.2 (C-6), 152.5 (C-2), 148.6 (C-4), 141.0 (C-8), 120.6 (C-5), 91.3 (C-1'), 87.6 (C-4'), 74.2 (C-2'), 71.9 (C-3'), 61.6 (C-5').

*Uracil (**7**):* white amorphous powder; ESI-MS: m/z 111 $[M-H]^-$; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H (ppm): 10.98 (1H, s, 3-NH), 10.81 (1H, s, 1-NH), 7.37 (1H, d, *J* = 7.5 Hz, H-6), 5.44 (1H, d, *J* = 7.5 Hz, H-5).

*D-mannitol (**8**):* white amorphous powder; ESI-MS: m/z 183 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H (ppm): 3.61 (2H, m, H-1a/H-6a), 3.53 (2H, t, *J* = 7.5 Hz, H-3/H-4), 3.45 (2H, m, H-2/H-5), 3.37 (2H, dd, *J* = 11.5, 6.0 Hz, H-1b/H-6b); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C (ppm): 71.5 (C-2/C-5), 69.9 (C-3/C-4), 64.1 (C-1/C-6).

*6β -hydroxystigmast-4-en-3-one (**9**):* orange-yellow needles; ESI-MS: m/z 428 $[M]^+$; ^{1}H-NMR (500Hz, $CDCl_3$) δ_H (ppm): 5.81 (1H, s, H-4), 4.34 (1H, m, H-6), 2.51 (1H, m, H-2a), 2.38 (1H, m, H-2b), 2.06 (1H, t, *J* = 3.5 Hz, H-12a), 2.04 (1H, m, H-1a), 1.99 (1H, m, H-7a), 1.95 (1H, m, H-8), 1.86 (1H, m, H-16a), 1.72 (1H, m, H-1b), 1.67 (1H, m, H-25), 1.63 (2H, m, H-15), 1.51 (2H, m, H-11), 1.38 (3H, s, H-19), 1.37 – 1.35 (2H, m, H-20/H-22a), 1.26 (2H, m, H-7b/H-16b), 1.24 (2H, m, H-28),

1.20 – 1.12 (4H, m, H-12b/H-14/H-23), 1.03 (1H, m, H-22b), 1.02 (1H, m, H-17), 0.93 (2H, m, H-9/H-24), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.85 (3H, t, *J* = 7.5 Hz, H-29), 0.84 (3H, d, 7.0 Hz, H-27), 0.82 (3H, d, *J* = 7.0 Hz, H-26), 0.74 (3H, s, H-18); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 200.4 (C-3), 168.5 (C-5), 126.3 (C-4), 73.3 (C-6), 56.1 (C-14), 55.9 (C-17), 53.7 (C-9), 45.9 (C-24), 42.6 (C-13), 39.7 (C-12), 38.6 (C-7), 38.0 (C-10), 37.1 (C-1), 36.1 (C-20), 34.3 (C-2), 34.0 (C-22), 29.8 (C-8), 29.2 (C-25), 28.2 (C-16), 26.2 (C-23), 24.2 (C-15), 23.1 (C-28), 21.0 (C-11), 19.8 (C-19), 19.5 (C-27), 19.1 (C-26), 18.8 (C-21), 12.0 (C-18/C-29).

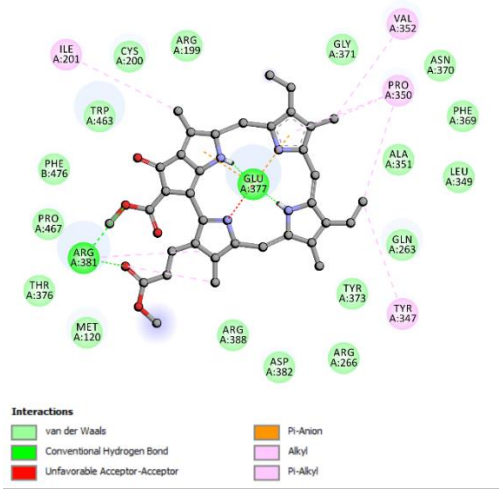
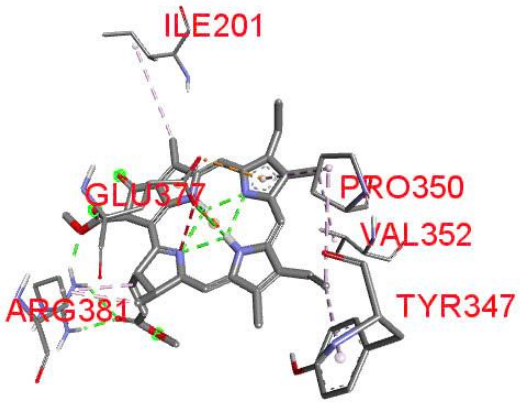
*Daucosterol (**10**):* white amorphous powder; ESI-MS: m/z 414 [M-Glc]$^+$; ^{1}H-NMR (500Hz, DMSO-d_6) δ_H (ppm): 5.33 (1H, t, J = 4.5, 1.5 Hz, H-6), 4.86 (1H, d, J = 4.5 Hz, H-6'b), 4.84 (2H, t, *J* = 10.0, 4.5, Hz, H-3'/H-4'), 4.40 (1H, t, *J* = 11.5, 5.5, H-6a), 4.22 (1H, d, *J* = 7.5 Hz, H-1'), 3.66 – 2.88 (6H, m, H-2'/H-3'/H-4'/H-5'/H-6'a/H-6'b), 2.37 (1H, dd, *J* = 10.5, 2.5 Hz, H-4a), 2.13 (1H, s, H-4b), 1.97 (1H, m, H-12), 1.90 (1H, m, H-7), 1.82 (1H, m, H-2), 1.80 (2H, m, H-1/H-16), 1.61 (1H, m, H-25), 1.52 (1H, m, H-15), 1.50 (1H, m, H-7), 1.50 (1H, m, H-11), 1.47 (1H, m, H-2), 1.41 (1H, m, H-11), 1.39 (1H, m, H-8), 1.38 (1H, m, H-20), 1.30 (1H, m, H-22), 1.24 (1H, m, H-16), 1.22 (1H, m, H-28), 1.20 (1H, m, H-28), 1.18 (2H, m, H-23), 1,17 (1H, m, H-12), 1,10 (1H, m, H-17), 1.05 (1H, m, H-15), 0.99 (1H, m, H-22), 0.98 (1H, m, H-1), 0.97 (1H, m, H-14), 0.96 (3H, s, H-19), 0.93 (1H, m, H-24), 0.91 (1H, m, H-9), 0.90 (3H, d, *J* = 6.5 Hz, H-21), 0.82 (9H, m, H-26/H-27/H-29), 0.61 (3H, s, H-18); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C (ppm): 140.4 (C-5), 121.1 (C-6), 100.8 (C-1'), 77.0 (C-3), 76.7 (C-5'), 76.6 (C-3'), 73.4 (C-2'), 70.1 (C-4'), 61.1 (C-6'), 56.2 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1 (C-24), 41.8 (C-13), 40.1 (C-4), 40.0 (C-12), 38.2 (C-1), 36.8 (C-20), 36.2 (C-10), 35.4 (C-22), 33.3 (C-7), 31.4 (C-8), 31.3 (C-2), 29.2 (C-23), 28.7 (C-16), 27.7 (C-25), 25.5 (C-15), 23.8 (C-28), 22.6 (C-11), 20.6 (C-27), 19.7 (C-19), 19.0 (C-21), 18.9 (C-26), 11.7 (C-29), 11.7 (C-18).

Molecular docking data of isolated compounds

To investigate the inhibitory potential of compounds isolated from *Enhalus acoroides* whole plant against inducible nitric oxide synthases (iNOS), molecular docking was employed. iNOS plays an important role in inflammation by producing nitric oxide (NO), an inflammatory mediator that helps protect the body against bacteria and harmful agents, but when overproduced, it can contribute to tissue damage and amplify the inflammatory response. By studying the interactions between these isolated compounds and iNOS, the drug design process can be optimized and deeper insights into the enzyme's role in inflammation pathology is gained.

The docking results are detailed in Tables 1 and 2, with the binding energies of compounds 1 – 10 ranging from -10.15 to -4.675 kcal/mol. Notably, seven of these compounds demonstrated stronger binding affinity than the reference inhibitor, AR-C95791 (-7.534 kcal/mol). Among the compounds, compound 10 emerged as the topmost candidate, showing the strongest binding to iNOS with an ΔG of -10.15 kcal/mol. This compound formed hydrogen bonds with Arg381 and Gln263, as well as pi-alkyl/alkyl interactions with Trp463, Val352, Arg199, Phe369, Cys200, Tyr489, Met355, Ala197, Leu209 and pi-sigma interactions with Trp194. Compound 5, with a binding energy of -10.15 kcal/mol, exhibited pi-sigma and pi-sulfur interactions with Met355, alongside pi-alkyl and alkyl interactions with Ala197, Cys200, and Val352. It also formed a hydrogen bond with Trp372 and pi-pi stacked interactions with Trp194. Compound 1 formed hydrogen bonds with Arg381 and Glu377, while also forming pi-anion interactions with Glu377, and pi-alkyl/alkyl interactions with Ile201, Val352, Pro350, Tyr347, and Glu377. Compound 3, which demonstrated a binding energy of -9.479 kcal/mol, formed hydrogen bonds with Tyr489 and engaged in pi-pi stacked and amide-pi stacked interactions with residues Trp194, Phe369, and Asn370. Meanwhile, compound 4 exhibited hydrogen bonds with Gln263, Arg109, Trp463, and Asp382, along with a pi-anion interaction with Glu377, and pi-alkyl/alkyl with Arg381, Pro350, contributing to a binding affinity of -8.696 kcal/mol. These results suggest that the compounds, particularly compound 10, hold significant promise as iNOS inhibitors, potentially offering a new avenue for the development of anti-inflammatory agents.

Table 1: Binding affinity, 2D- and 3D- interactions of the top compounds with the iNOS enzyme

No.	Binding affinity (kcal/mol)	2D interaction	3D interaction
1	-9.554		

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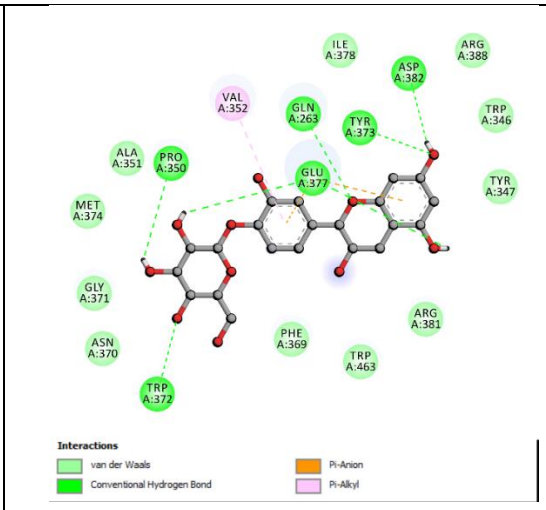
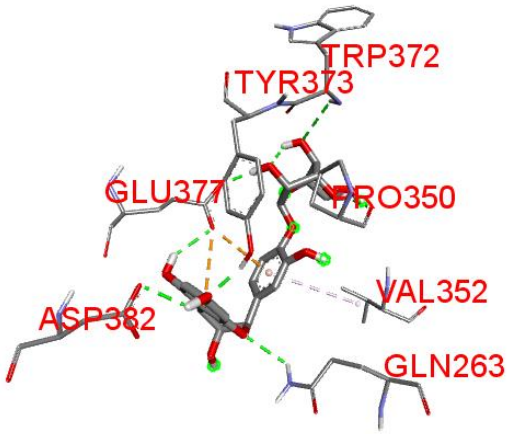
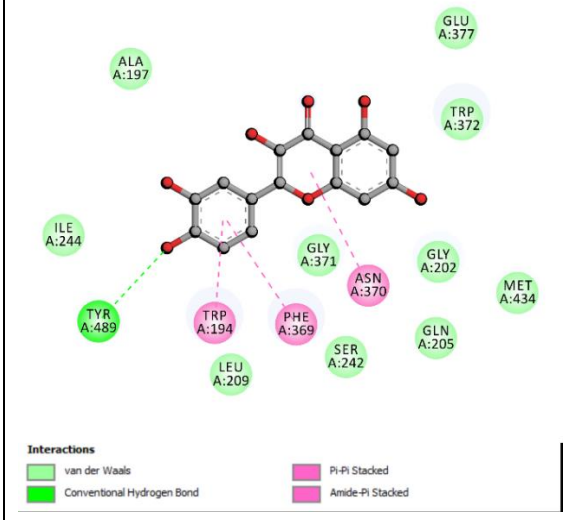
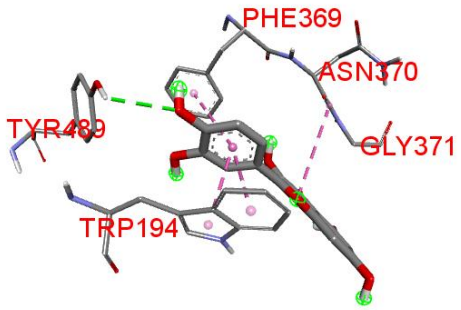
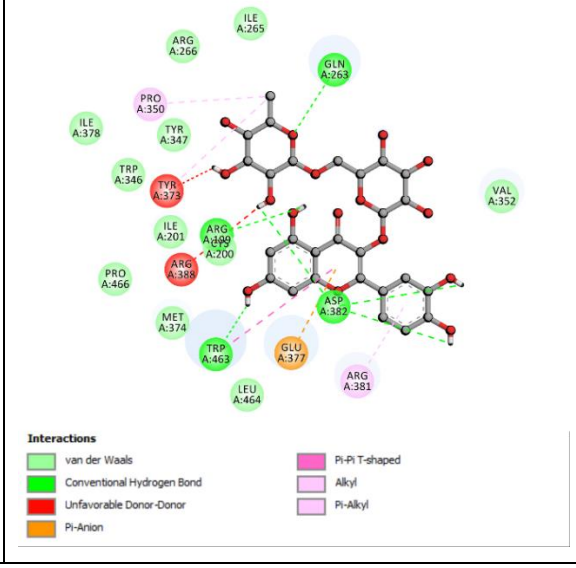
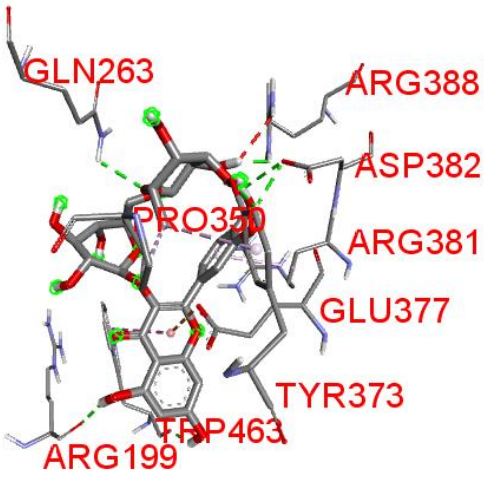
2	-8.195		
3	-9.479		
4	-8.696		

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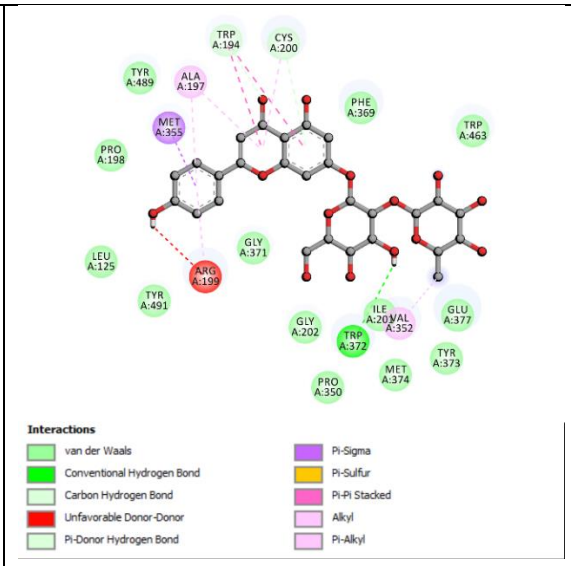
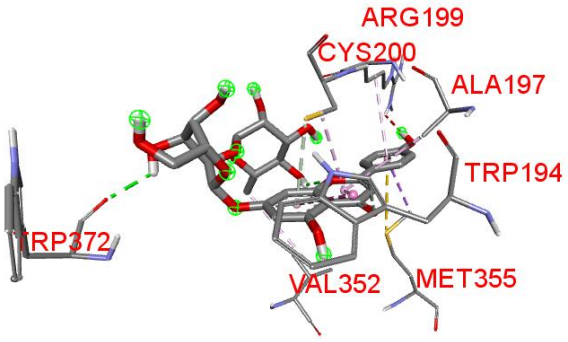
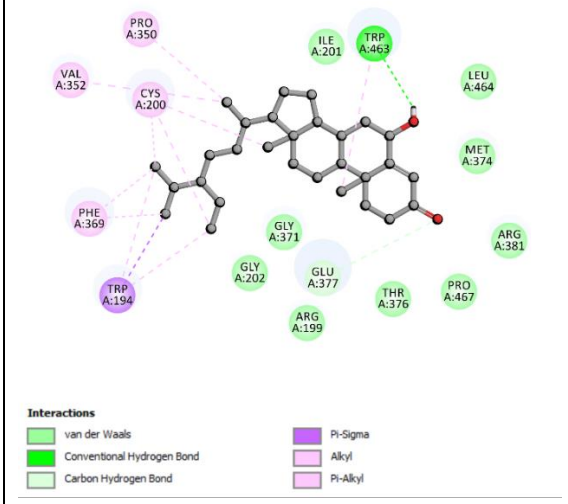
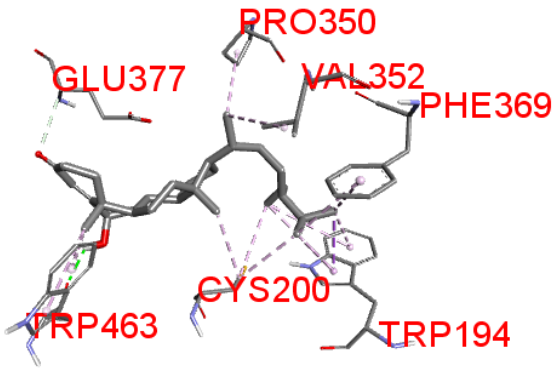
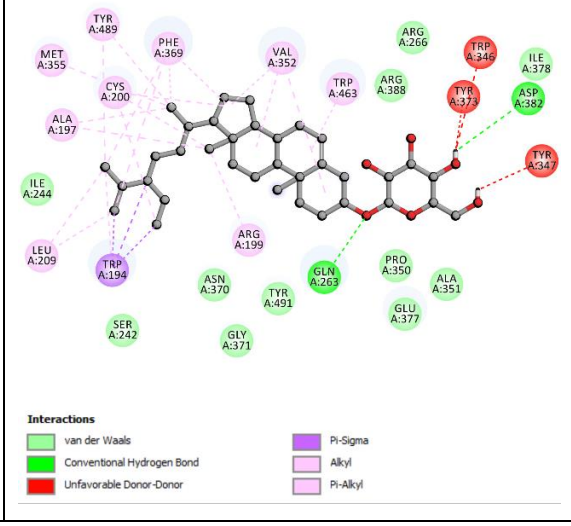
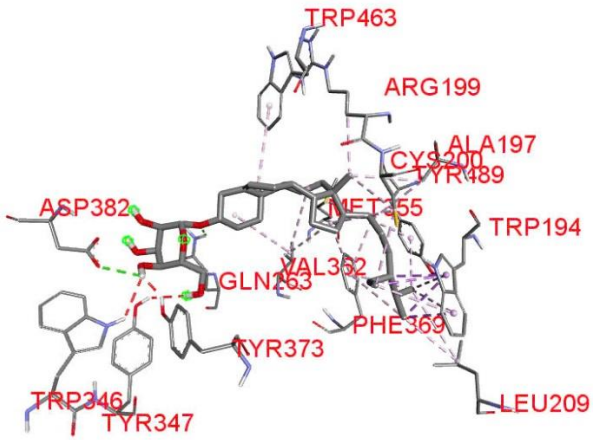
5	-10.15		
9	-8.511		
10	-10.12		

Table 2: The binding affinity of the isolated compounds **1**-**10** with the iNOS enzyme.

No.	Compound	Binding affinity (kcal/mol)
1	Methyl pheophorbide a	-9.554
2	(+)-catechin-4'-*O*-*β* -D-glucoside	-8.195
3	quercetin	-9.479
4	rutin	-8.696
5	apigenin-7-*O*-α-L-rhamnopyranosyl-(1→2)-*β* -D-glucopyranoside	-10.15
6	adenosine	-6.168
7	uracil	-4.675
8	D-mannitol	-4.846
9	6*β* -hydroxystigmast-4-en-3-one	-8.511
10	daucosterol	-10.12
11	Ethyl 4-[(4-methylpyridin-2-yl)amino]piperidine-1-carboxylate (AR-C95791)	-7.534

Conclusion

Phytochemical investigation of *E. acoroides* whole plant from Vietnam led to the isolation of ten (10) compounds including methyl pheophorbide a (1), (+)-catechin-4'-*O*-*β* -D-glucoside (2), quercetin (3), rutin (4), apigenin-7-*O*-α-L-rhamnopyranosyl-(1→2)-*β* -D-glucopyranoside (5), adenosine (6), uracil (7), D-mannitol (8), 6*β* -hydroxystigmast-4-en-3-one (9), and daucosterol (10). The structures of these compounds were determined by a combination of ESI-MS and NMR spectra and comparison with literature. Molecular docking was performed to evaluate the binding affinity of the isolated compounds with the iNOS enzyme. The findings suggest that compounds 1, 2, 3, 4, 5, 9, and 10 are promising iNOS enzyme inhibitors.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Kannan RRR, Arumugam R, Anantharaman P. *In vitro* antioxidant activities of ethanol extract from *Enhalus acoroides* (LF) Royle. Asian Pac J Trop Med. 2010; 3(11):898-901. DOI: 10.1016/S1995-7645(10)60216-7.
2. Qi SH, Zhang S, Qian PY, Wang BG. Antifeedant, antibacterial, and antilarval compounds from the South China Sea seagrass *Enhalus acoroides*. Bot Mar. 2008; 51(5):441-447. DOI: 10.1515/BOT.2008.054.
3. Wang XB, Sun ZH, Fan LX, Liu YY, Feng J, Ma GX, Chen DL. Two novel diterpenes from the stems and leaves of tropical seagrass *Enhalus acoroides* in the South China sea. Nat Prod Res. 2021; 35(9):1465-1473. DOI: 10.1080/14786419.2019.1655025.
4. Kannan RRR, Arumugam R, Anantharaman P. Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. Food Chem. 2012; 135(4):2470-2473. DOI: 10.1016/j.foodchem.2012.07.070.
5. Wang X. Study on the chemical constituents of the tropical seagrass *Enhalus acoroides*. J Hainan Normal Univ (Nat Sci). 2018; 31:383-386.
6. Garcin ED, Arvai AS, Rosenfeld RJ, Kroeger MD, Crane BR, Andersson G, Getzoff ED. Anchored plasticity opens doors for selective inhibitor design in nitric oxide synthase. Nat Chem Biol. 2008; 4(11):700-707. DOI: 10.1038/nchembio.115.
7. Rappé AK, Casewit CJ, Colwell KS, Goddard IWA, Skiff WM. UFF. A full periodic table force field for molecular mechanics and molecular dynamics simulations. J Am Chem Soc. 1992; 114(25): 10024-10035. DOI: 10.1021/ja00051a040.
8. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. J Cheminform. 2011; 3:1-14. DOI: 10.1186/1758-2946-3-33.
9. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461. DOI: 10.1002/jcc.21334.
10. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2. 0: New docking methods, expanded force field, and python bindings. J Chem Inf Model. 2021; 61(8):3891-3898. DOI: 10.1021/acs.jcim.1c00203.

11. Trang HTT, Xuan HN, Hong LC, Nhung TT, Thi TGD, Thi DH, Thuy TP. *In silico* molecular docking, DFT, and toxicity studies of potential inhibitors derived from *Millettia dielsiana* against human inducible nitric oxide synthase. J Chem Res. 2024; 48(4):17475198241263837. DOI: 10.1177/17475198241263837.
12. Hieu TT, Thuy PT, Thuan VT, Ha NX, Van HN, Nguyen NH, Giang LD. Chemical composition and antimicrobial activity of essential oil obtained from the rhizomes of *Kaempferia champasakensis*: *in vitro* and molecular docking studies. J Essent Oil-Bear Plants. 2023; 26(4):958-969. DOI: 10.1080/0972060X.2023.2251512.
13. Zerbib M, Mazauric JP, Meudec E, Le Guernevé C, Lepak A, Nidetzky B, Saucier C. New flavanol O-glycosides in grape and wine. Food Chem. 2018; 266:441-448. DOI: 10.1016/j.foodchem.2018.06.019.
14. Mouffok S, Haba H, Lavaud C, Long C, Benkhaled M. Chemical constituents of *Centaurea omphalotricha* Coss. & Durieu ex Batt. & Trab. Rec Nat Prod. 2012; 6(3):292-295.
15. Dos Santos MG, Moreno FS, de Oliveira AL, Oliveira IC, Tribuiani N, Cogo JC, Oshima-Franco Y. New pharmacological insights of *Galactia glaucescens*. Nat Prod Res. 2019; 33(16):2389-2393. DOI: 10.1080/14786419.2018.1443086.
16. Ma L and Dolphin D. Nucleophilic reaction of 1, 8-diazabicyclo [5.4. 0] undec-7-ene and 1, 5-diazabicyclo [4.3. 0] non-5-ene with methyl pheophorbide a. Unexpected products. Tetrahedron. 1996; 52(3):849-860. DOI: 10.1016/0040-4020(95)00944-2.
17. Phan VK. Study of components flavonoids and megastigmane glucosides of *Ficus callosa*. Vietnam J Chem. 2011; 49(1):55-59.
18. Huang ZB, Wu Z, Chen FK, Zou LB. The protective effects of phenolic constituents from *Gastrodia elata* on the cytotoxicity induced by KCl and glutamate. Arch Pharm Res. 2006; 29:963-968. DOI: 10.1007/BF02969279.
19. Chun QIN, Xiu P, Wen AI, Zhi-Long ZH, Jia-Yun XIA, Shi-HaiXU, Yong-Hong LIU. Secondary Metabolites from Mangrove *Sonneratia apetala* Endophytic Fungus *Neofusicoccum* sp. SaBA3. Nat Prod Res Dev. 2014; 26(8):1212.
20. Lee EJ, Lee JY, Kim JS, Kang SS. Phytochemical studies on Lonicerae flos (1)-isolation of iridoid glycosides and other constituents. 2010; 16(1):32-38.
21. Ibrakaw AS, Boatwright JS, Lesch T, Cupido CN, Hussein AA. Triterpenes and other minor chemical constituents of *Boophone haemanthoides* FM Leight (Amaryllidaceae). S Afr J Bot. 2021; 136:35-39. DOI: 10.1016/j.sajb.2020.06.025.
22. Lee YG, Cho JY, Lee HJ, Lee YH, Lee SH, Han TH, Moon JH. Isolation and identification of a sterol and three glucosides from the peel of pear (*Pyrus pyrifolia* Nakai cv. Chuhwangbae). Korean J Food Sci Technol. 2013; 45(5):557-564. DOI: 10.9721/KJFST.2013.45.5.557.