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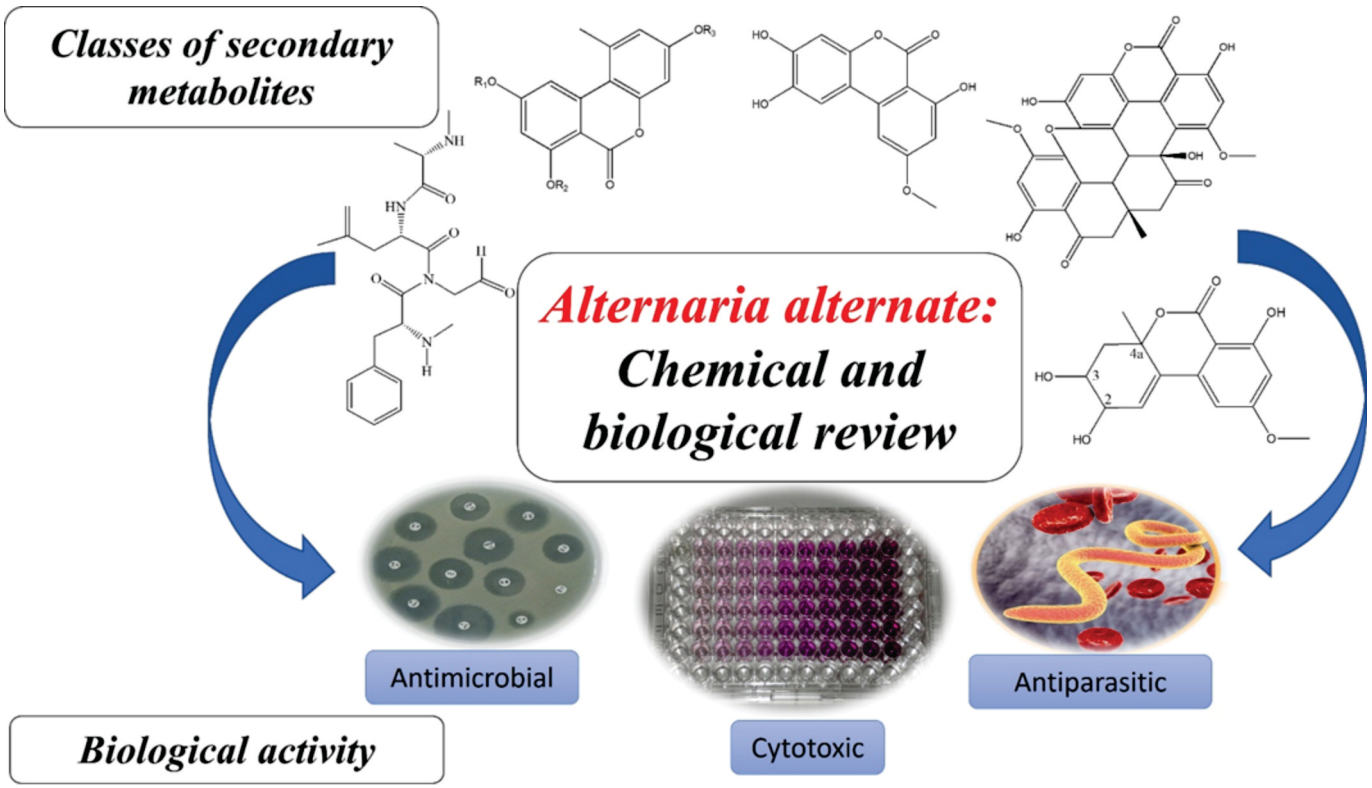

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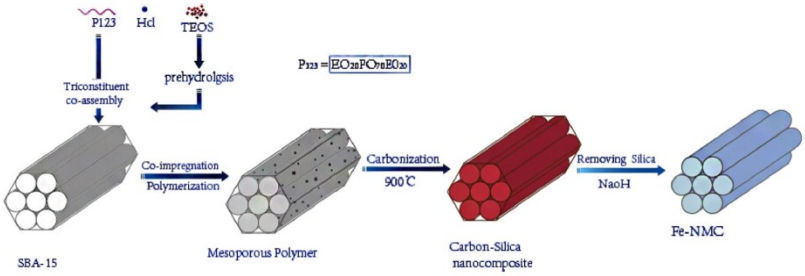

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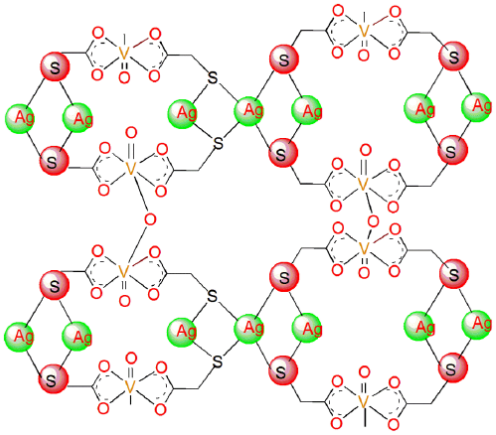

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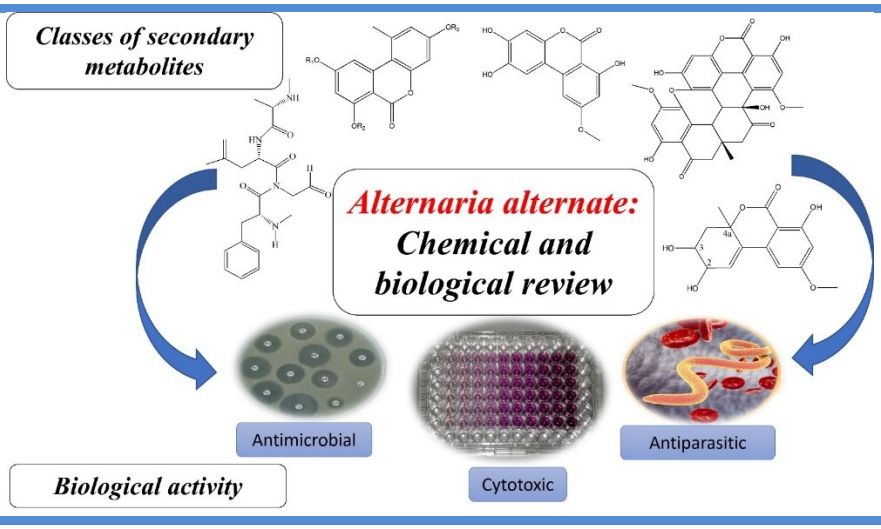

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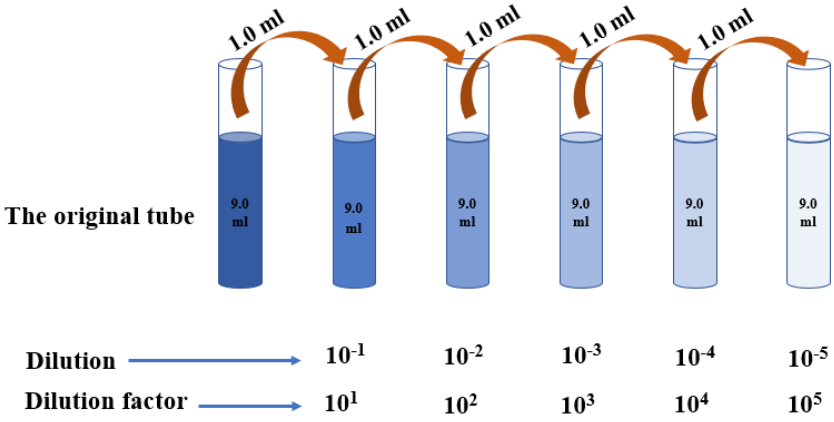

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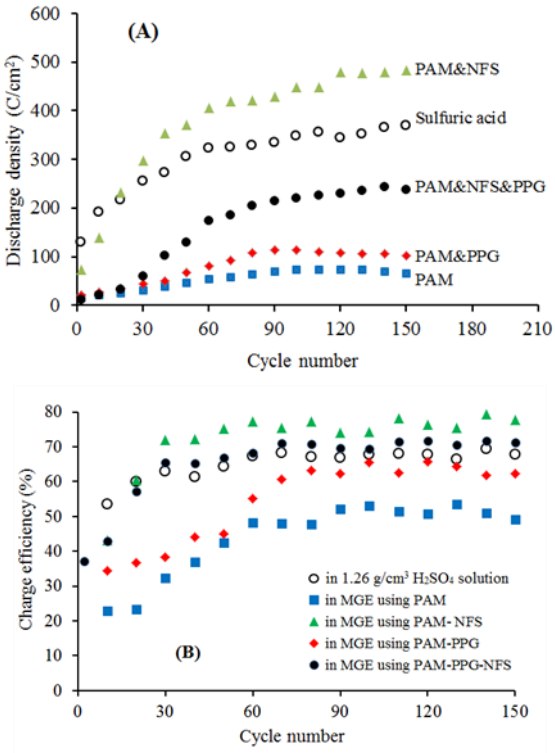

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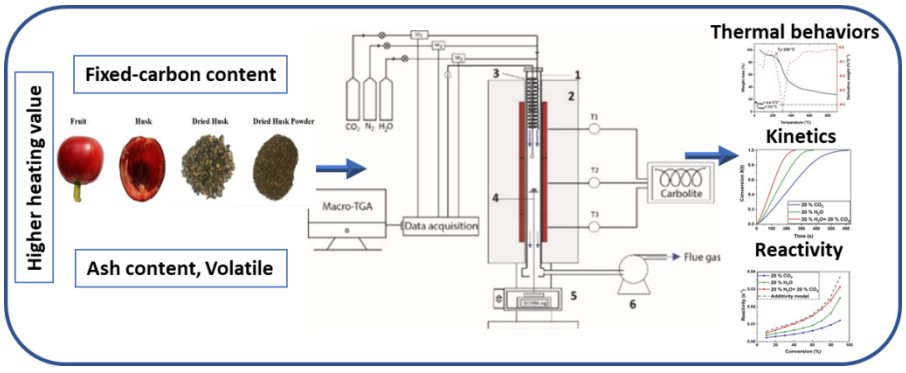

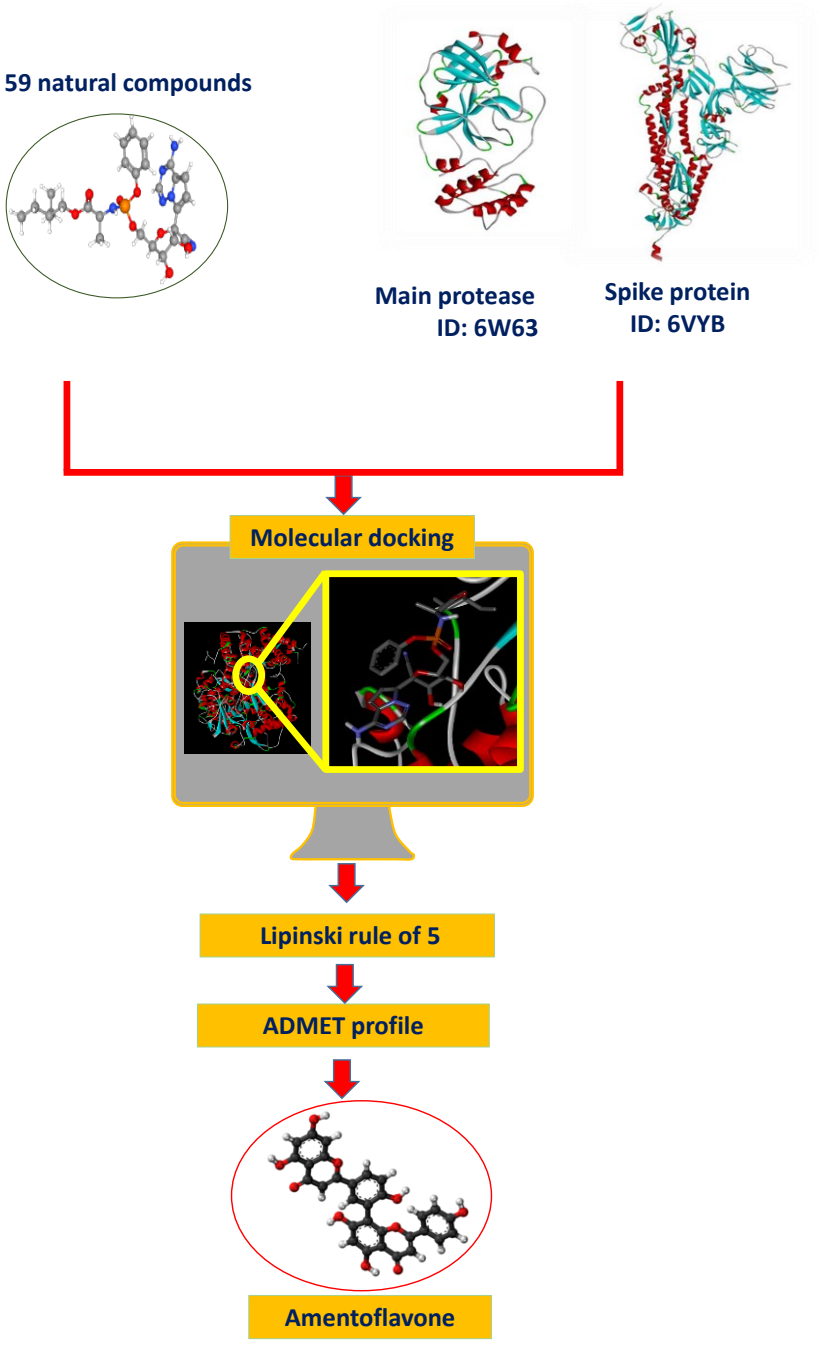

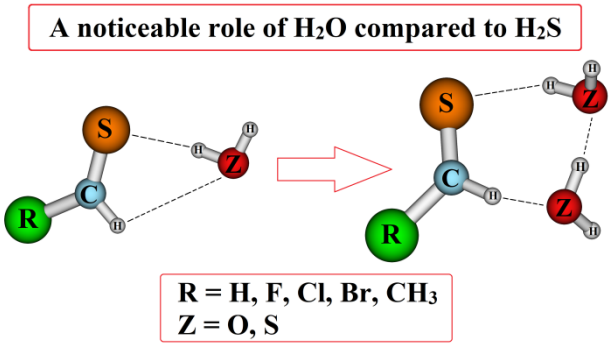

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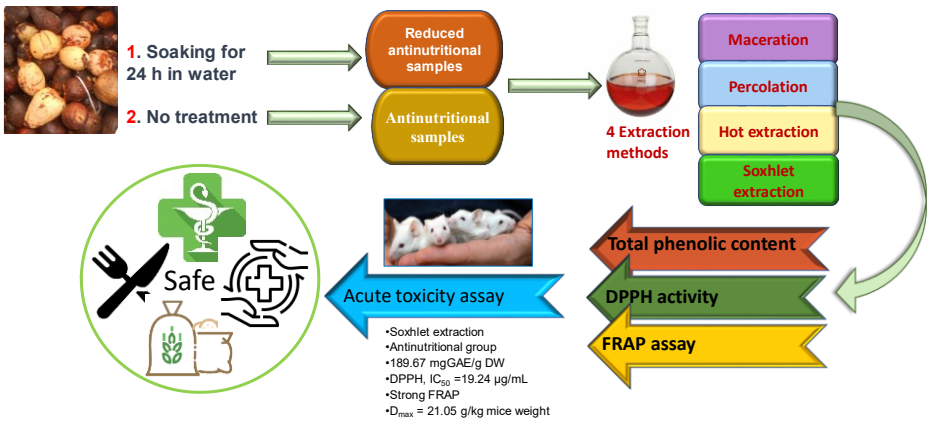

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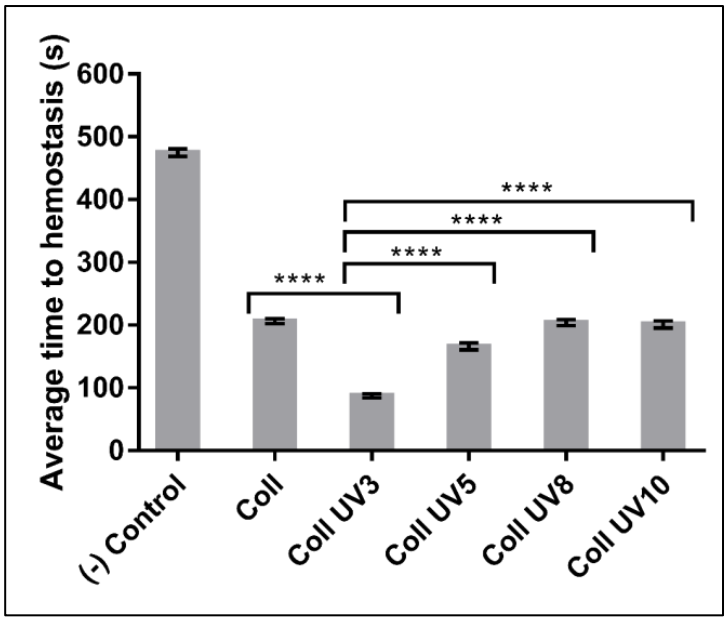

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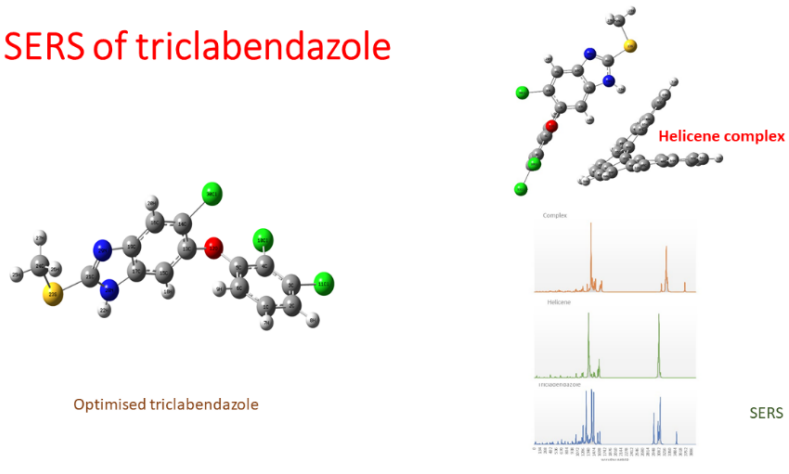

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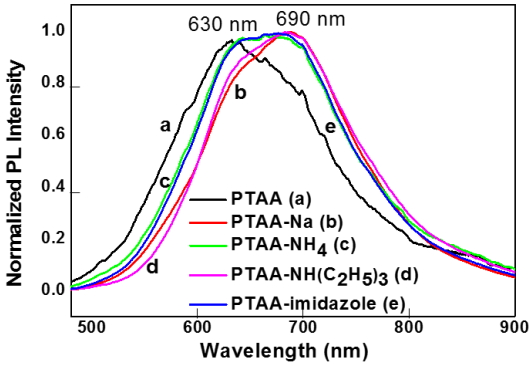

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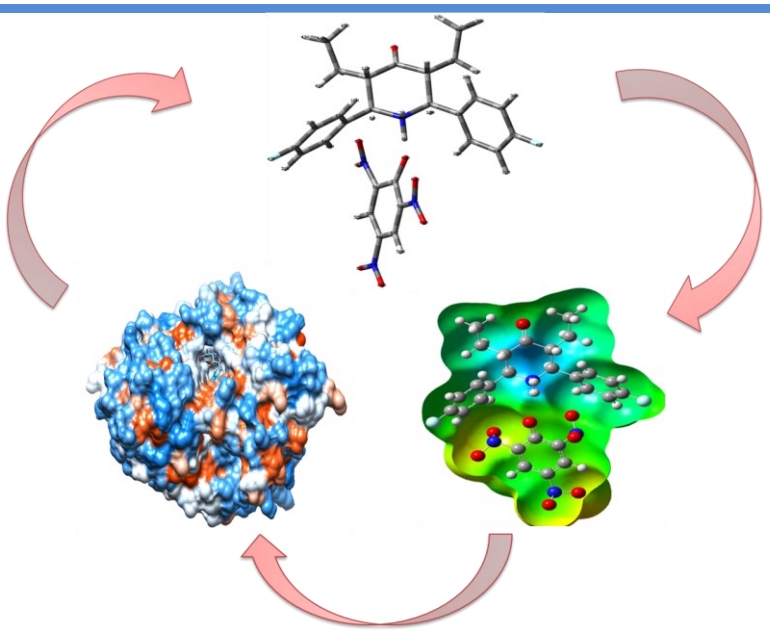
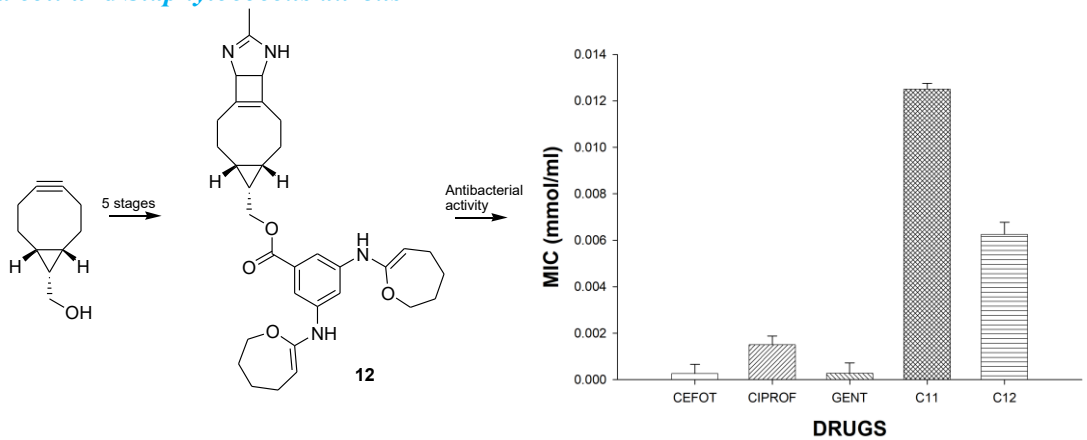
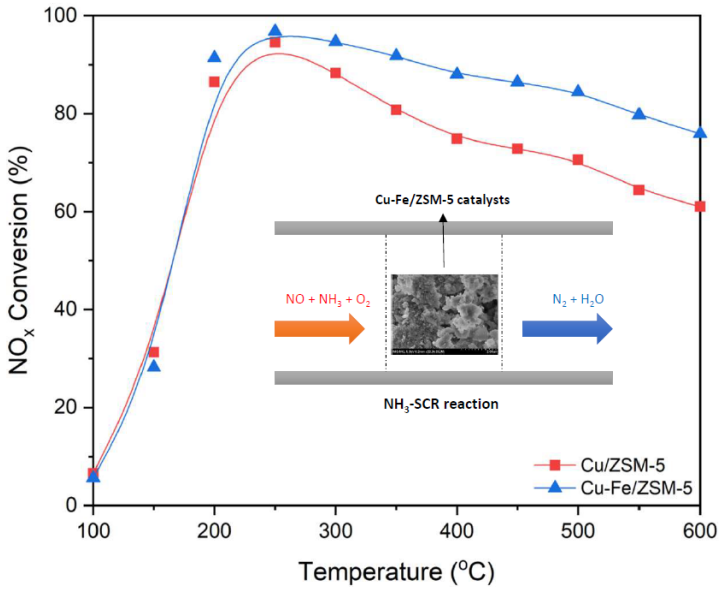
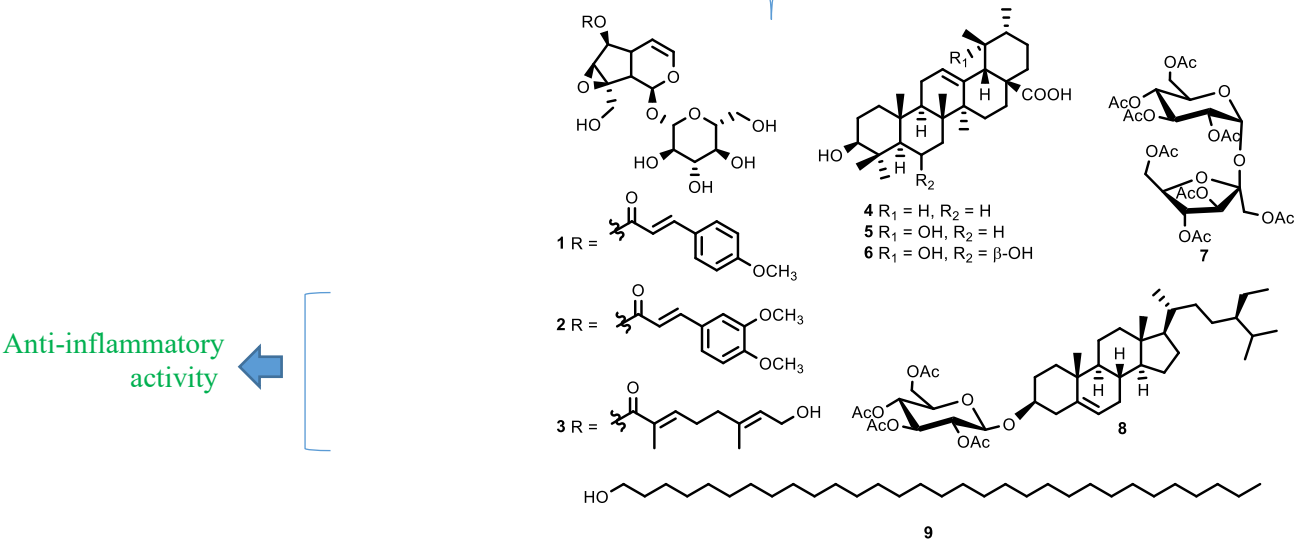






Dolichandrone spathacea → Isolation



Anti-inflammatory activity

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Phytochemistry and anti-inflammatory activity of iridoids from *Dolichandrone spathacea* collected in the mangrove forest of Phu Loc district, Thua Thien Hue province, Vietnam

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Abstract

From the leaves and barks of *Dolichandrone spathacea* collected in the mangrove forest of Hue province, three iridoids {6-*O*-[(*E*)-4-methoxy cinnamoyl]catalpol (**1**), 6-*O*-[(*E*)-3,4-dimethoxy cinnamoyl]catalpol (**2**), nemoroside (**3**)} and three ursane-type triterpenoids [ursolic acid (**4**), pomolic acid (**5**), uncaric acid (**6**)] were isolated and structurally elucidated. Besides, succrose octaacetate (**7**), daucosterol tetraacetate (**8**) and 1-hentriacontanol (**9**) were identified from the fruits of this mangrove species. It is the first time compounds **6**, **7**, **9** were isolated from *Dolichandrone spathacea*. The main constituents of this plant, iridoids **1** and **2**, were tested for the inhibition of IL-6, TNF-α factors and NO production. The anti-inflammatory activity as well as the phytochemistry of the fruits of this species are reported for the first time.

Keywords. *Dolichandrone spathacea*, mangrove forest, iridoids, triterpenoids, anti-inflammatory activity

1. INTRODUCTION

Dolichandrone spathacea (L.f.) L. Schum. (Bignoniaceae) is a mangrove plant distributed in India, Cambodia, Vietnam, Malaysia, Solomon Islands and New Caledonia.[1] In Vietnam, this species grows in the coastal areas, mostly from Da Nang to An Giang province.[1] Traditionally, this plant was used singular or with other ingredients to cure diseases, for instance: *D. spathacea* leaves combined with some other herbal folk medicines are good for women in menstruation and after childbirth; a folk remedy including roots and leaves of *D. spathacea* and roots of *Acanthus ilicifolius* was used for liver detoxification. In India, people use the seeds of *D. spathacea* with ginger to treat pain caused by muscle spasms. In addition, the leaves of this species were utilized to cure asthma and the root barks were used to detoxify the body.[1] Phytochemistry of this plant was investigated, indicating iridoids,[2-4] phenolics,[2,3,5] saponins,[2] triterpenoids,[2,3] phenylethanoid glycosides,[2] flavonoids[2,3] and steroids[2,5] as the main bioactive components. Anti-microbial[2,3,6,7] and antioxidant activites[6] of the barks and leaves of *D. spathacea* were studied. In this report, nine compounds including 6-*O*-[(*E*)-4-methoxy cinnamoyl]catalpol (**1**), 6-*O*-[(*E*)-3,4-dimethoxy cinnamoyl]catalpol (**2**), nemoroside (**3**), ursolic acid (**4**), pomolic acid (**5**), uncaric acid (**6**), succrose octaacetate (**7**), daucosterol tetraacetate (**8**) and 1-hentriacontanol (**9**) were isolated from the barks, leaves, and fruits of *D. spathacea* collected in Phu Loc district, Thua Thien Hue province. Compounds **1**-**6** were obtained from the barks and leaves of this plant. Compounds **7**-**9** were isolated from the fruits, of which **7** and **8** were purified from

an acetylated sub-fraction of dichloromethane extract. Compounds **6**, **7** and **9** were identified for the first time from this species. This is the first time the phytochemistry of *D. spathacea* fruits and the anti-inflammatory activity of the main iridoids **1** and **2** have been studied.

2. MATERIALS AND METHODS

2.1. Plant materials

Dolichandrone spathacea (fruits, leaves and barks) were collected in the mangrove forest in Phu Loc district, Thua Thien Hue province, Vietnam in December 2018. The sample was taxonomically identified by Dr. Do Xuan Cam, University of Agriculture and Forestry, Hue University. A voucher specimen (QNH2018) was kept in Institute of Chemistry (VAST).

2.2. Chemicals and equipment

The equipment and experimental procedures are the same as in our previous published paper.[8]

2.3. Extraction and isolation

2.3.1. Extraction and isolation of the compounds from the fruits of Dolichandrone spathacea

The dried fruits of *Dolichandrone spathacea* were ground to give 480 g powder which was extracted in EtOH 96 % (4 × 1.0 L × 4 h). The EtOH extracts were grouped and the solvent was evaporated *in vacuo* to give 38.7 g residue. This residue was extracted successively with dichloromethane and acetone (3 x 500 mL for each solvent) to yield the corresponding dichloromethane (QNQ.1 22.2 g), acetone extract (QNQ.2 3.2 g) and a remaining solid (QNQ.3 4.5 g). The dichloromethane extract QNQ.1 (22.2 g) was subjected to a silica gel column (*n*-hexane/DCM/MeOH 95:5:0-0:0:100) to obtain 18 fractions (QNQ.1.1-QNQ.1.18). Fraction QNQ.1.7 (2.1 g) was separated by silica gel column chromatography (*n*-hexane/DCM 95/5) to afford compound **9** (12.4 mg). Due to the difficulties of separation, fraction QNQ.1.13 (1.0 g) was acetylated with anhydride acetic acid (2 mL) and pyridine (2 mL) at room temperature for 24 hours. Removal of anhydride acetic acid and pyridine under *vacuo* gave the residue which was purified by silica gel column (*n*-hexane/EtOAc 9/1) to yield 9 sub-fractions (QNQ.1.13A-QNQ.1.13I). Fraction QNQ.1.13E appeared white crystals, which was precipitated in EtOH to furnish compound **8** (11.1 mg). Fraction QNQ.3 (4.5 g) was acetylated with the same condition above. After workup, the reaction mixture was subjected to a silica gel column (*n*-hexane/EtOAc/MeOH 50/50/1) to give 16 sub-fractions (QNQ.3.1-QNQ.3.16). Fraction QNQ.3.5 (200.1 mg) was purified by silica gel column chromatography (*n*-hexane/acetone/MeOH 90/10/1) to afford compound **7** (4.2 mg).

2.3.2. Extraction and isolation of the compounds from the barks and leaves of Dolichandrone spathacea

The dried barks and leaves of *Dolichandrone spathacea* were powdered to give 1.0 kg, which was extracted sequentially with *n*-hexane, DCM, acetone and MeOH/H_2O 9/1 (4 x 2 L x 4h for each solvent) to give *n*-hexane (QNV.1 80 g), DCM (QNV.2 29.0 g), acetone (QNV.3 52.0 g) and MeOH (QNV.4 152.0 g) extracts, respectively. The DCM extract (QNV.2) was separated on a silica gel column (DCM/MeOH 10:0-7:3) to yield four fractions (QNV.2.1-QNV.2.4). Fraction QNV.2.1 (2.0 g) was purified in a RP-18 column (MeOH/H_2O 1/1) to give compound **3** (5.1 mg). The acetone extract QNV.3 (52.0 g) was subjected to silica gel column (DCM/MeOH 90:10-80:2) to afford 22 sub-fractions (QNV.3.1-QNV.3.22). Fraction QNV.3.3 (230.2 mg) was separated on a silica gel column (DCM/MeOH 95:5-0:100) and further purified on a Sephadex LH-20 column (MeOH 100 %) to give compound **6** (5.0 mg). Fraction QNV.3.5 (418.2 mg) appeared white crystals, which was washed with cold DCM to yield compound **4** (7.3 mg). Fraction QNV.3.13 (6.0 g) was separated on a silica gel column (DCM/MeOH/H_2O 95:5:0-60:40:5) to afford 11 fractions. Compound **5** (12.9 mg) was obtained from fraction 1, and compound **2** (1.0 g) was afforded from fraction 2 through recrystalization in DCM/MeOH 9:1. Fraction QNV.3.17 (10.0 g) was precipitated in DCM/MeOH 9:1 to obtain compound **1** (5.0 g).

6-*O*-[(*E*)-4-methoxy cinnamoyl]catalpol (1): White amorphous powder, $C_{25}H_{30}O_{12}$, ESI-MS (*m/z*): 523.1 $[M+H]^+$, 521.0 $[M-H]^-$. ^1H NMR and ^{13}C NMR, see table 1.

6-*O*-[(*E*)-3,4-dimethoxy cinnamoyl]catalpol (2): White amorphous powder, $C_{26}H_{32}O_{13}$, (-)-ESI-MS (*m/z*): 588.2 $[M+Cl]^-$. ^1H NMR and ^{13}C NMR, see table 1.

Nemoroside (3): White amorphous powder, $C_{25}H_{36}O_{12}$, ESI-MS (*m/z*): 551.1 $[M+Na]^+$. ^1H NMR

and ^{13}C NMR, see table 1.

Ursolic acid (**4**): White amorphous powder, $C_{30}H_{48}O_3$, ESI-MS (*m/z*): 479.23 $[M+Na]^+$, 455.27 $[M-H]^-$. ^{1}H (500 MHz, DMSO-d_6), δ_H (ppm), *J* (Hz): 5.14 (1H, br s, H-12), 3.02 (1H, m, H-3), 2.15 (1H, d, *J* = 11.0, H-18), 1.06 (3H, s, H-27), 0.92 (3H, d, *J* = 6.5, H-30), 0.89 (3H, s, H-23), 0.87 (3H, s, H-25), 0.82 (3H, d, *J* = 6.5, H-29), 0.75 (3H, s, H-26), 0.68 (3H, s, H-24). ^{13}C NMR (125 MHz, DMSO-d_6), δ_C (ppm): 138.38 (C-13), 124.48 (C-12), 76.91 (C-3), 54.85 (C-5), 52.49 (C-18), 47.09 (C-17), 46.88 (C-9), 41.69 (C-14), 39.33 (C-8), 38.58 (C-20), 38.40 (C-4, C-19), 38.30 (C-1), 36.56 (C-10), 36.43 (C-22), 32.79 (C-15), 30.32 (C-7), 27.62 (C-23), 26.99 (C-2, C-21), 23.92 (C-11), 23.27 (C-27), 22.88 (C-16), 21.13 (C-30), 18.04 (C-6), 17.07 (C-29), 16.08 (C-24), 15.25 (C-25), 15.12 (C-26).

Pomolic acid (**5**): White amorphous powder, $C_{3O}H_{48}O_4$, HR-ESI-MS (*m/z*): 473.3630 $[M+H]^+$ (calculated for $C_{30}H_{49}O_4$ 473.3631). ^{1}H NMR (500 MHz, CD_3OD), δ_H (ppm), *J* (Hz): 5.29 (1H, t, *J* = 3.5, H-12), 3.17 (1H, dd, *J* = 11.5, 4.5, H-3), 2.57 (1H, s , H-18), 2.50 (1H, ddd, *J* = 26.5, 13.5, 4.5, H-2a), 2.03-1.93 (2H, m, H-11), 1.93-1.79 (1H, m, H-9), 1.79-1.70 (3H, m, H-15a, H-21a, H-22a), 1.70-1.62 (2H, m, H-2b, H-6), 1.62-1.50 (5H, m, H-1a, H-1b, H-16. H-21b), 1.50-1.38 (2H, m, H-2b, H-6), 1.40-1.30 (2H, m, H-1b, H-7b), 1.35 (3H, s, H-27), 0.99 (3H, s, H-23), 0.96 (3H, s, H-25), 0.94 (3H, d, *J* = 7.0, H-30), 0.93-0.0.88 (1H, m, H-5), 0.85 (3H, s, H-24), 0.80 (3H, s, H-26). ^{13}C NMR (125 MHz, CD_3OD), δ_C (ppm): 140.55 (C-13), 129.00 (C-12), 79.80 (C-3), 73.85 (C-19), 56.77 (C-5), 55.33 (C-18), 48.83 (C-17), 48.66 (C-9), 43.11 (C-20), 42.66 (C-14), 41.07 (C-8), 39.87 (C-4), 39.83 (C-10), 39.37 (C-22), 34.33 (C-7), 29.79 (C-15), 28.78 (C-23), 27.89 (C-21), 27.52 (C-16), 27.26 (C-29), 26.87 (C-2), 24.78 (C-27), 24.69 (C-11), 19.67 (C-1), 17.29 (C-24), 16.69 (C-26), 16.35 (C-30), 15.90 (C-25).

Uncaric acid (6): White amorphous powder, $C_{30}H_{48}O_5$, ESI-MS (*m/z*): 489.1 $[M+H]^+$, 471.1 $[M-H_2O+H]^+$, 523.2 $[M+Cl]^-$, 487.2 $[M-H]^-$. ^{1}H NMR (500 MHz, CD_3OD), δ_H (ppm), *J* (Hz): 5.34 (1H, t, *J* = 3.5, H-12), 4.54 (1H, br s, H-6), 3.11 (1H, dd, *J* = 12.0, 4.5, H-3), 2.58 (1H, ddd, *J* = 13.5, 13.0, 5.0, H-16a), 2.54 (1H, s, H-18), 2.12-2.00 (2H, m, H-11), 1.94-1.88 (1H, m, H-15a), 1.78-1.69 (5H, m, H-2b, H-7b, H-9, H-21b, H-22b), 1.69-1.62 (2H, m, H-16b, H-22a), 1.62-1.58 (1H, m, H-2a), 1.59-1.50 (2H, m, H-1b, H-7a), 1.42-1.35 (1H, m, H-20), 1.33 (3H, s, H-25), 1.32 (3H, s, H-27), 1.22 (3H, s, H-29), 1.18 (3H, s, H-24), 1.10 (3H, s, H-26), 1.06 (3H, s, H-23), 1.09-0.98 (1H, m, H-15b), 0.94 (3H, d, *J* = 6.5, H-30), 0.76 (1H, d, *J* = 1.5, H-5). ^{13}C NMR (125 MHz, CD_3OD), δ_C (ppm): 178.52 (C-28), 139.26 (C-13), 129.80 (C-12), 80.18 (C-3), 73.66 (C-19), 68.96 (C-6), 57.24 (C-5), 55.15 (C-18), 49.34 (C-17), 49.00 (C-9), 43.10 (C-14), 43.06 (C-20), 42.06 (C-1), 41.90 (C-7), 40.71 (C-4), 40.21 (C-8), 39.01 (C-22), 37.63 (C-10), 29.59 (C-15), 28.43 (C-23), 28.04 (C-2), 27.30 (C-21), 27.09 (C-29), 26.67 (C-16), 24.79 (C-27), 24.61 (C-11), 18.48 (C-26), 17.61 (C-24), 17.27 (C-25), 16.57 (C-30).

Succrose octaacetate (7): White amorphous powder, $C_{28}H_{38}O_{19}$, ESI-MS (*m/z*): 654.1 $[M-COCH_3+H_2O+H]^+$. ^{1}H NMR (500 MHz, CD_3OD), δ_H (ppm), *J* (Hz): 5.73 (1H, d, *J* = 4.0, H-1), 5.49 (1H, t, *J* = 5.5, H-1'), 5.47-5.45 (3H, m, H-2, H-3, H-3'), 5.40 (1H, t, *J* = 5.5, H-4'), 5.07 (1H, t, *J* = 9.5, H-4), 4.87 (1H, dd, *J* = 10.5, 3.5, H-6'a), 4.35-4.16 (6H, m, H-5, H-6, H-1', H-5', H-6'b), 2.18 (s, 3H, -OCOC<u>H</u>$_3$), 2.12 (s, 3H, -OCOC<u>H</u>$_3$), 2.11 (s, 6H, 2 x -OCOC<u>H</u>$_3$), 2.09 (s, 3H, -OCOC<u>H</u>$_3$), 2.08 (s, 3H, -OCOC<u>H</u>$_3$), 2.05 (s, 3H, -OCOC<u>H</u>$_3$), 2.02 (s, 3H, -OCOC<u>H</u>$_3$). ^{13}C NMR (125 MHz, CD_3OD), δ_C (ppm): 172.44, 172.32, 171.88, 171.72, 171.63, 171.26 (8 x -O<u>C</u>OCH$_3$), 105.31 (C-2'), 91.32 (C-1), 80.49 (C-5'), 77.30, 76.47, 71.79, 70.98, 69.96, 69.81 (C-2, C-3, C-4, C-5, C-3', C-4'), 64.86, 64.18, 63.27 (C-6, C-6', C-1'), 20.71, 20.63, 20.61, 20,55 (8 x -OCO<u>C</u>H$_3$).

Daucosterol tetraacetate (8): White needles, $C_{43}H_{68}O_{10}$, ESI-MS (*m/z*): 762.2 $[M+H_2O]$. ^{1}H NMR (500 MHz, $CDCl_3$), δ_H (ppm), *J* (Hz): 5.35 (1H, d, *J* = 5.0, H-6), 5.20 (1H, t, *J* = 9.5, H-3'), 5.07 (1H, t, *J* = 9.5, H-4'), 4.97 (1H, dd, *J* = 9.5, 8.0, H-2'), 4.58 (1H, d, *J* = 8.0, H-1'), 4.26 (1H, dd, *J* = 12.5, 5.0, H-6'a), 4.10 (1H, dd, *J* = 12.5, 2.5, H-6'b), 3.69-3.65 (1H, m, H-5'), 3.51-3.45 (1H, m, H-3), 2.27-2.15 (2H, m, H-4), 2.07 (3H, s, -OCOC<u>H</u>$_3$), 2.04 (3H, s, -OCOC<u>H</u>$_3$), 2.01 (3H, s, -OCOC<u>H</u>$_3$), 2.00 (3H, s, -OCOC<u>H</u>$_3$), 1.99-1.98 (1H, m, H-12a), 1.98-1.95 (1H, m, H-7a), 1.88-1.86 (1H, m, H-2a), 1.84-1.81 (2H, m, H-1a, H-16a), 1.68-1.64 (1H, m, H-25), 1.58-1.57 (1H, m, H-2b), 1.56-1.55 (1H, m, H-15a), 1.49-1.48 (1H, m, H-11a), 1.45-1.39 (2H, m, H-7b, H-8), 1.35-1.32 (1H, m, H-20), 1.29-1.27 (1H, m, H-22a), 1.26-1.24 (2H, H-28), 1.23-1.17 (2H, m, H-16b, H-2b), 1.17-1.11 (3H, m, H-12b, H-23), 1.09-1.08 (1H, m, H-1b), 1.07-1.04 (2H, m, H-15b, H-17), 1.03-1.02 (1H, m, H-22b), 1.01-0.99 (1H, m, H-14), 0.98 (3H, s, H-19), 0.93-0.92 (1H, m, H-24), 0.91 (3H, d, *J* = 7.5, H-21), 0.89-0.87 (1H, m, H-9), 0.85 (3H, t, *J* = 7.5, H-29), 0.84-0.83 (1H, m, H-11b, overlap with H-27), 0.83 (3H, d, *J* = 7.0, H-27), 0.81 (3H, d, *J* = 7.0, H-26), 0.67 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$), δ_C (ppm): 170.65, 170.32, 169.38, 169.27 (4 x -<u>C</u>OCH$_3$), 140.38 (C-5), 122.14 (C-6), 99.65 (C-

1'), 80.08 (C-3), 72.96 (C-3'), 71.72 (C-5'), 71.55 (C-2'), 68.62 (C-4'), 62.15 (C-6'), 56.78 (C-14), 56.09 (C-17), 50.20 (C-9), 45.88 (C-24), 42.34 (C-13), 39.76 (C-12), 38.93 (C-4), 37.21 (C-1), 36.73 (C-10), 36.13 (C-20), 33.98 (C-22), 31.95 (C-7), 31.89 (C-8), 29.46 (C-2), 29.21 (C-25), 28.22 (C-16), 26.14 (C-23), 24.29 (C-15), 23.10 (C-28), 21.06 (C-11), 20.73 (C-27), 20.68, 20.61, 20.58, 19.80 (4 x -CO<u>C</u>H$_3$), 19.34 (C-19), 19.04 (C-26), 18.78 (C-21), 11.98 (C-29), and 11.85 (C-18).

1-Hentriacontanol (9): White needles, $C_{31}H_{64}O$, ESI-MS (*m/z*): 453.3 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d$_6$), δ_H (ppm), *J* (Hz): 3.63 (2H, t, *J* = 7.5), 1.55 (2H, m), 1.25 (56H, m, 28xCH_2), 0.88 (3H, t, *J* = 7.0). ^{13}C NMR (125 MHz, DMSO-d$_6$), δ_C (ppm): 63.10, 32.83, 31.92, 29.69, 29.65, 29.61, 29.59, 29.43, 29.34, 25.74, 22.67, and 14.08.

2.4. Anti-inflammation bioassays: Inhibition of IL-6, TNF-*α* cytokine and NO production

The anti-inflammation bioassays was carried out according to the method described in the literature.[10]

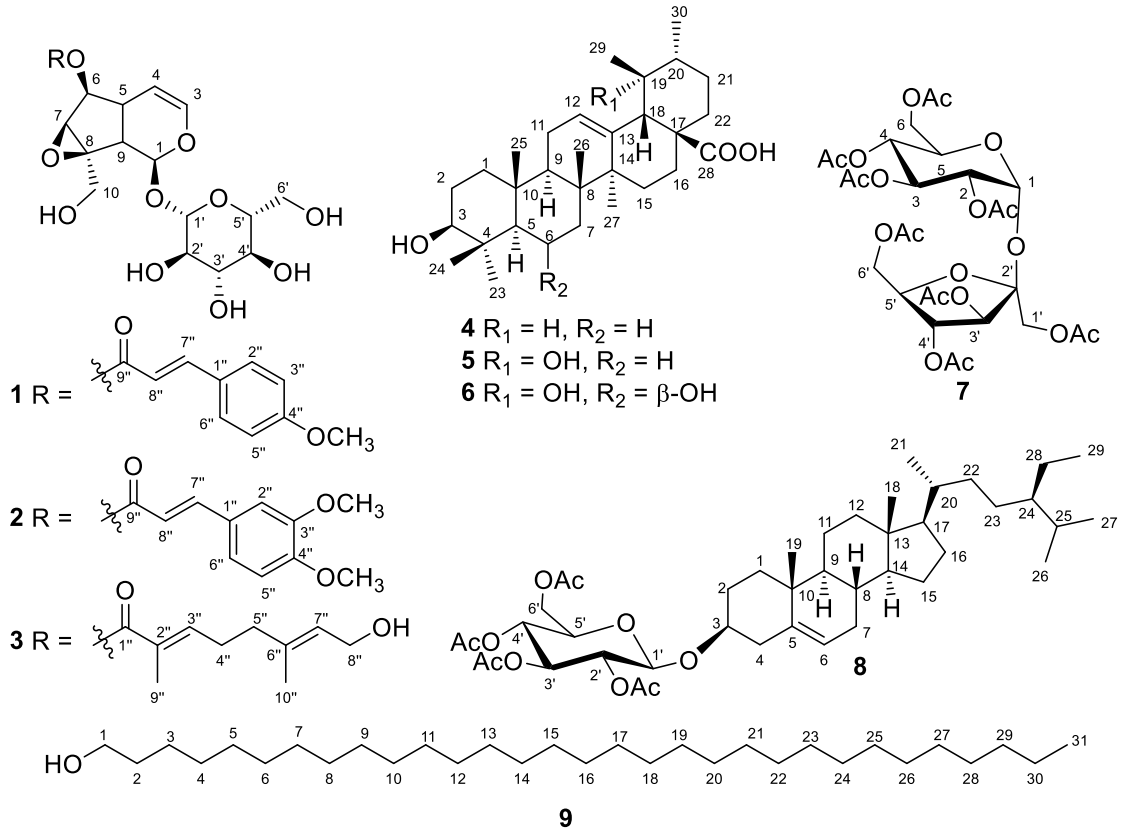

Figure 1: The isolated compounds from the barks, leaves (**1-6**) and fruits (**7-9**) of *Dolichandrone spathacea*

3. RESULTS AND DISCUSSION

3.1. Structural elucidation of the isolated compounds

Compound **1** was isolated as a white amorphous powder from the barks and leaves of *Dolichandrone spathachea*. The ^{1}H NMR spectrum of **1** appeared two olefinic proton at δ_H 6.36 (1H, dd, *J* = 6.0, 1.5) and 5.00 (1H, dd, *J* = 6.0, 4.5), together with three oxygenated methines at δ_H 5.15 (1H, d, *J* = 9.0), 5.05 (1H, dd, *J* = 7.5, 1.0), 3.73 (1H, br s), an oxygenated methylene at δ_H 4.16 (1H, d, *J* = 13.0)/3.87 (1H, d, *J* = 13.0), two methines at δ_H 2.67-2.64 (1H, m) and 2.63-2.61 (1H, m). Besides, the ^{1}H NMR spectrum of **1** indicated the signals of four aromatic protons at δ_H 7.55 (2H, d, *J* = 8.0) and 6.95 (2H, d, *J* = 8.0), demonstrating the presence of an AA'BB' ring. Two olefinic protons at δ_H 7.69 (1H, d, *J* = 16.0) and 6.41 (1H, d, *J* = 16.0) were characteristic for an alkene group with (*E*) configuration, based on the coupling constant *J* = 16.0 Hz. In addition, a methoxy signal was determined to be attached to aromatic ring at δ_H 3.84 (3H, s, OC<u>H</u>$_3$). The proton signals from 3.94-3.32, together with an anomer proton at δ_H 4.81 (1H, d, *J* = 8.0, H-1′) were assigned for a *β*-glucose moiety.

The ^{13}C-NMR and DEPT spectrum of **1** displayed 25 carbon signals, including four quaternary carbons, eighteen methines, two methylenes and one methyl. In details, two olefinic carbons at δ_C 142.17 (C-3), 102.74 (C-4), three oxygenated methines at δ_C 94.88 (C-1), 81.09 (C-6), 60.05 (C-7), one quaternary carbon at δ_C 66.67 (C-8), one oxygenated methylene at δ_C 61.12 (C-10) along with two methine groups at δ_C 36.46 (C-5), 42.89 (C-9) were observed, suggesting a catalpol iridoid skeleton in the structure

Table 1: ^{1}H NMR and ^{13}C NMR spectral data of compounds **1**-**3**

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_H (ppm)[a], *J* (Hz)	δ_C (ppm)[b]	δ_H (ppm)[a], *J* (Hz)	δ_C (ppm)[b]	δ_H (ppm)[c], *J* (Hz)	δ_C (ppm)[d]
1	5.15 (d, 9.0)	94.88	5.05-5.00 m	94.26	5.06 (d, 9.5)	95.08
3	6.36 (dd, 6.0, 1.5)	142.17	6.34 (dd, 4.5, 2.0)	141.05	6.26 (dd, 6.0, 1.5)	142.38
.4	5.00 (dd, 6.0, 1.5)	102.74	5.05-5.00 m	102.06	4.86-4.83 m	102.91
5	2.63-2.61 m	36.46	2.65-2.64 m	35.17	2.47-2.45 m	36.67
6	5.05 (dd, 7.5, 1.0)	81.09	5.05-5.00 m	79.65	4.86-4.83 m	81.62
7	3.73 br s	60.05	3.74 br s	59.12	3.57 s	60.17
8	-	66.67	-	65.42	-	66.79
9	2.67-2.64 m	42.89	2.71-2.66 m	41.76	2.52 (d, 8.0)	43.17
10	4.16 (d, 13.0) 3.87 (d, 13.0)	61.12	4.03 (d, 13.0) 3.95 m	60.53	4.05 (d, 13.0) 3.72 (d, 13.0)	62.93
1’	4.81 (d, 8.0)	99.47	4.84 (d, 8.0)	98.58	4.69 (d, 8.0)	99.72
2’	3.33-3.32 m	74.50	3.39-3.36 m	73.02	3.30 (t, 9.0)	77.72
3’	3.46-3.42 m	77.38	3.52-3.48 m	75.97	3.24-3.20 m	78.65
4’	3.30-3.28 m	71.39	3.39-3.36 m	69.29	3.19-3.14 m	71.78
5’	3.35-3.34 m	78.25	3.52-3.48 m	76.41	3.19-3.14 m	74.85
6’	3.94 (dd, 12.0, 2.0) 3.68 (dd, 12.0, 2.0)	62.63	3.88 (dd, 12.0, 5.5) 3.78 (dd, 12.0, 5.0)	60.91	3.83 (dd, 12.0, 1.5) 3.54 (dd, 12.0, 6.5)	61.27
1’’	-	127.92	-	127.01	-	169.34
2’’	7.55 (d, 8.0)	130.90	7.09 (d, 1.5)	111.00	-	128.60
3’’	6.95 (d, 8.0)	115.26	-	149.10	6.74 (dd, 7.0, 6.5)	144.15
4’’	-	162.98	-	151.30	2.29-2.24 m	28.06
5’’	6.95 (d, 8.0)	115.26	6.90 (d, 8.5)	109.72	2.09-2.06 m	39.06
6’’	7.55 (d, 8.0)	130.90	7.14 (dd, 8.5, 1.5)	122.86	-	138.37
7’’	7.69 (d, 16.0)	146.69	7.68 (d, 15.5)	145.90	5.31-5.29 m	125.74
8’’	6.41 (d, 16.0)	115.31	6.38 (d, 15.5)	114.64	3.99 (d, 6.5)	59.37
9’’	-	168.61	-	167.32	1.77 s	12.47
10’’	-	-	-	-	1.60 s	16.18
3’-OMe				55.81		
4’-OMe	3.84 s	55.81	3.92 s	55.76	-	-

[a]CD_3OD+$CDCl_3$, 500 MHz; [b]CD_3OD+$CDCl_3$, 125 MHz; [c]CD_3OD, 500 MHz; [d]CD_3OD, 125 MHz.

of **1**. The *β*-glucose unit was located at C-1 of iridoid ring, deducing from HMBC correlation of anomer proton H-1′ (δ_H 4.81)/C-1 (δ_C 94.88). The correlation from proton H-6 (δ_H 5.05) to C-9″ (δ_C 168.61; COO-) in HMBC spectrum proved the attachment of (*E*)-4-methoxycinnamoyl to C-6. The methyoxy

group was determined to attach to C-4″, confirming by the correlation between –OC$\underline{H}_3$ (δ_H 3.84) and C-4″ (δ_C 162.98). The (+)-ESI-MS of **1** gave an $[M+H]^+$ ion at *m/z* 523.1. By analysing and comparison of the spectroscopic data of **1** with the literature, compound **1** was elucidated to be 6-*O*-[(*E*)-4-methoxy cinnamoyl] catalpol.[11] This compound was first obtained from *Westringia fruticosa* and *Westrigia viminalis.*[11]

Compound **2** was obtained as a white powder from the barks and leaves of *D. spathacea*. The ^{1}H- and ^{13}C-NMR data of **2** displayed similar signals to compound **1**, sharing the same skeletone in the structure, except for an additional methoxy group in the aromatic ring. This addition was confirmed by analysis of the ESI-MS spectra (*m/z* 588.2 $[M+Cl]^-$) of **2** and the signals of aromatic protons in an ABX ring system at δ_H 7.09 (1H, d, *J* = 1.5, H-2″), 6.90 (1H', d, *J* = 8.5, H-5″), 7.14 (1H, dd, *J* = 8.5, 1.5, H-6″), together with the signals of two methoxy groups at δ_H 3.92 (6H, s)/δ_C 55.81 and 55.76 Comparing the NMR data of **2** with the literature determined **2** to be 6-*O*-[(*E*)-3,4-dimethoxycinnamoyl]catalpol.[12] This compound was firstly isolated from *Buddleja globosa.*[12]

From the barks and leaves of *D. spathacea*, compound **3** was obtained as a white amorphous powder. A comparison between 1D-NMR spectrum of **3** with those of **1** and **2** showed that compound **3** had the same structural feature. Instead of a cinnamoyl moeity, a monoterpene side chain was observed in ^{13}C NMR spectrum of **3,** including four olefinic carbons at δ_C 144.15 (C-3''), 138.37 (C-6''), 128.60 (C-2''), 125.74 (C-7''), a carbonyl ester group at δ_C 169.34 (C-1''), an oxygenated methylene at δ_C 59.37 (C-8''), two methylenes at δ_C 28.06 (C-4'') and 39.06 (C-5''), two methyl carbons belonging to the vinyl groups at δ_H/δ_C 1.77/12.47 (H-9''/C-9''), 1.60/16.18 (H-10''/C-10''). The structure of iridoid **3** was confirmed by analysis of 2D-NMR spectra (COSY, HSQC và HMBC). The monoterpene side chain was attached to iridoid ring at C-6, which was confirmed through the HMBC correlation of H-6 (δ_H 4.85) to C-1'' (δ_C 169.34). The ESI-MS spectra displayed a molecular ion peak at *m/z* 551.1 $[M+Na]^+$. By comparison of the above spectral data with those in the literature, compound **3** was determined to be nemoroside.[13] This compound was isolated previously from *Dolichandrone spathacea* and *Penstemon nemorosus*.[3,13]

Compounds **4** and **5** was obtained from the barks and leaves of this plant, which were identified as ursolic and pomolic acid, respectively. The ESI-MS and NMR spectral data of **4** and **5** were analyzied and compared with our previous report on phytochemistry of *D. spathacea* collected from Soc Trang province.[9]

Compound **6** was purified as a white amorphous powder from the barks and leaves. The ^{13}C NMR of **6** exhibited thirty carbon signals presenting for a carboxylic triterpene: a carboxylic acid group at δ_C 178.52 (C-18), two olefinic carbons at δ_C 139.26 (C-13) and 129.80 (C-12), two oxygenated methine groups at δ_C 80.18 (C-3) and 68.96 (C-6), an oxygenated quaternary carbon at δ_C 73.66 (C-19), four methines at δ_C 57.24 (C-5), 55.15 (C-18), 49.00 (C-9) and 43.06 (C-20), together with the signals of five quaternary carbons, eight methylenes and seven methyls. The ^{1}H NMR exhibited an olefinic proton at δ_H 5.34 (1H, t, *J* = 3.5, H-12), a methine proton at δ_H 3.11 (H-3) with large coupling constant value $^3J_{H\text{-}3ax/H\text{-}2ax}$ = 12.0, indicating the α-orientation of the proton H-3 and the β-orientation of the hydroxyl group at C-3 as well. Protons H-6 was orientated at the same side to the axial proton H-5, evidenced by the small coupling constant of H-6 [δ_H 4.54, 1H, brs]. Thus, the hydroxyl group at C-6 was aligned in β-direction. Proton H-18 (δ_H 2.54) presented a singlet signal, suggesting a hydroxyl substitution at C-19. The mass spectra exhibited molecular ion peaks at *m/z* 489.1 $[M+H]^+$, 523.2 $[M+Cl]^-$ and 487.2 $[M-H]^-$. The NMR and MS spectral data of **6** were in agreement with uncaric acid.[2] This compound was obtained firstly from *Uncaria elliptica.*[14] Uncaric acid was isolated from *D. spathacea* for the first time.

Compound **7** was isolated as an acetylated product from the fruits. The NMR data of compound **7** displayed signals of a fully acetylated disaccharide. Eight acetyl groups at δ_H 2.18 (s, 3H, -OCOC$\underline{H}_3$), 2.12 (s, 3H, OCOC$\underline{H}_3$), 2.11 (s, 6H, 2 x -OCOC$\underline{H}_3$), 2.09 (s, 3H, OCOC$\underline{H}_3$), 2.08 (s, 3H, -OCOC$\underline{H}_3$), 2.05 (s, 3H, OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), together with the carbonyl acetyls (-O$\underline{C}$OCH$_3$) in ^{13}C-NMR spectrum at δ_C 172.44, 172.32, 171.88, 171.72, 171.63, 171.26 were observed. In addition, the ^{13}C-NMR spectrum appeared an anomer carbon at C-1 (δ_C 91.32), a quaternary carbon at δ_C 105.31 (C-2'), seven oximethine carbons, and three oxygenated methylenes (3 x -CH_2OH). The ^{1}H-NMR spectrum indicated fourteen methine protons signals of disaccharide shifting to the downfield due to the acetylation. The anomer proton with small coupling constant was resonated at 5.73 (1H, d, *J* = 4.0, H-1). The (+)-ESI-MS spectra showed a molecular ion peak at *m/z* 654.1 $[M\text{-}COCH_3+H_2O+H]^+$. By analysis the spectroscopic data and the comparison with the literature,[15] compound **7** was deduced to be α-D-glucopyranosyl (1→2)-β-D-fructofuranose or succrose octaacetate.

Compound **8** was an acetylated product purified from the fruits extract as amorphous powder. The mass spectra exhibited a molecular peak at *m/z* 762.2 [M+H_2O]. The ^{13}C NMR of **8** contained the signal characteristic for a *β*-sitosterol unit and an acetylated glycoside moiety. Resulting from the acetylation, the chemical shift of the sugar protons was deshielded to lower field at δ_H 5.20 (1H, t, *J* = 9.5, H-3'), 5.07 (1H, t, *J* = 9.5, H-4'), 4.97 (1H, dd, *J* = 9.5, 8.0, H-2'), 4.26 (1H, dd, *J* = 12.5, 5.0, H-6'a), 4.10 (1H, dd, *J* = 12.5, 2.5, H-6'b) and 4.58 (1H, d, *J* = 8.0, H-1'). Moreover, four acetyl signals in ^{1}H NMR spectrum at δ_H 2.07, 2.04, 2.01 and 2.00 (4s x 3H, 4x -OCOC$\underline{H_3}$) confirmed the full acetylation of glycoside moiety. The ^{13}C NMR spectrum of **8** also indicated the signals of *β*-sitosterol skeleton. The proton and carbon signals of **8** were assigned by 2D NMR (COSY, HSQC, HMBC, NOESY). The HMBC cross-peak between H-3 (δ_H 3.51-3.45) and C-1' (δ_C 99.65) confirmed the attachment of the glucose moiety to C-3. The spectroscopic data above were compatible with the published one[16], so that compound **8** was determined to be daucosterol tetraacetate.

Compound **9** was obtained from the fruits of this plant as white needles. The ESI-MS spectra displayed a molecular ion peak at *m/z* 453.3 $[M+H]^+$. The ^{1}H-NMR of compound **9** showed the signals of a saturated fatty alcohol, comprising a methyl group at δ_H 0.88 (3H, t, *J* = 7.0), 29 methylenes at δ_H 1.25 - 1.55 (m, 58H), and an oxygenated methylene at δ_H 3.63 (2H, t, *J* = 7.0). Corresponding to the ^{1}H NMR spectrum, the ^{13}C-NMR and DEPT spectra displayed the signal at δ_C 14.08 (CH_3), 22.67-32.83 (CH_2), and an oxygenated methylene at δ_C 63.10 (-CH_2OH). The above spectral data was compatible with the literature.[17] Therefore compound **9** was identified as 1-hentriacontanol. This is the first report on the isolation of this compound from *Dolichandrone spathacea*.

The above investigation on phytochemistry of *Dolichandrone spathacea* collected in Thua Thien Hue province led to the identification of iridoids, triterpenoids, sterol,.. corresponding to the previous study on chemical constituents of this plant.[2-4,9] Compounds **1**, **2** (iridoid glycosides), **4**, **5** (triterpene carboxylic acids) and **8** (sterol) were also isolated from *Dolichandrone spathacea* collected in Soc Trang province, Vietnam.[4,9] It is the first time compound **6** (uncaric acid) was identified from this mangrove plant. *Dolichandrone spathacea* collected in Vinh Long province, Vietnam was reported to possess compound **3** (nemoroside) and triterpenoid saponin (28-*O*-*β*-D-glucopyranosyl-23-hydroxy-uncaric acid) with the aglycon moeity similar to **6**.[3] The presence of compound **7** (succrose octaacetate) and **9** (1-hentriacontanol) in the fruits of *Dolichandrone spathacea* collected in Thua Thien Hue province was never described before. The above results suggested that iridoid glycosides and triterpene carboxylic acids can be considered as the chemical markers of the mangrove plant *Dolichandrone spathacea*. Further research on this species in different ecological areas needed to be conducted to explore the bioactive and marker compounds.

3.2. Anti-inflammatory activity

Iridoids with various types of skeletal structure possess multiple biological activities, along with the anti-inflammation.[18-19] The two main iridoids **1** and **2** were examined for the inhibition of proinflammatory cytokines IL-6, TNF-α and NO production on LPS-stimulated macrophage RAW 264.7 cells. Compound was considered to be active if the OD values of the cells treated with samples were lower than that of the control cells. The result revealed that both compounds **1** and **2** showed the inhibitory effect on LPS-induced IL-6 and TNF-*α* on RAW 264.7 cells at concentration of 25 μg/ml. Nevertheless, iridoids **1** and **2** exhibited weak inhibitory effect on NO producéion (with IC_{50} values of 324.28 and 335.48 μM, respectively). IC_{50} of the reference compound L-NMMA (N^G-Methyl-L-arginine, acetate salt) was 30.73 μM. The above result demonstrated the anti-inflamatory capability of iridoids **1** and **2** based on the inhibition of IL-6 and TNF-α signaling pathway.

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REFERENCES

1. Vo Van Chi. Dictionary of Vietnamese medicinal plants, Medical Publishing House, Hanoi, **1999**, 911-912.
2. P. D. Nguyen, A. Abedini, S. C. Ganglof, J. Madoux, C. Lavaud. Phytochemical analysis and antimicrobial evaluation of *Dolichandrone spathacea* (Bignoniaceae), *Planta Med.*, **2016**, *81(S01)*, S1-S381.
3. P. D. Nguyen, A. Abedini, S. C. Ganglof, C. Lavaud. Antimicrobial constituents from leaves of *Dolichandrone spathacea* and their relevance to traditional use, *Planta Med.*, **2018**, *5*, e14-e23.
4. Nguyen Van Tuan, Do Le Quyen, Nguyen The Anh,

Nguyen Tuan Thanh, Tran Van Loc, Ho Ngoc Anh, Tran Van Chien, Tran Van Sung, Tran Thi Phuong Thao. New cycloartanes and new iridoids from *Dolichandrone spathacea* collected in the mangrove forest of Soc Trang province, *Vietnam*, *J. Asian Nat. Prod. Res.*, **2018**, *20(9)*, 889-896.

5. Le Minh Tri. Study on chemical constituents from the barks of *Dolichandrone spathacea*, *Master thesis, Vietnam National University,* Ho Chi Minh City, **2011**.
6. Pham Ngoc Khoi, Nguyen Thi My Duyen. Study on the extraction conditions and antioxidant, anti-microbial activities of polyphenols from the barks of *Dolichandrone spathacea*, *Journal of Science, Ho Chi Minh City University of Education*, **2017**, *14(12)*, 181-193.
7. S. J. Azmi, M. Mohtar, M. I. Mohamed, N. Hashim. Inhibitory Potential Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) of *Dolichandrone spathacea*, a mangrove tree species of Malaysia, *Lat. Am. J. Pharm*, **2011**, *30(2)*, 359-62.
8. Ly Thi Thu Hoai, Nguyen The Anh, Nguyen Thi Luu, Ngo Van Hieu, Pham Thi Ninh, Tran Van Loc, Nguyen Linh Chi, Nguyen Xuan Nam, Tran Thi Phuong Thao. Sesquiterpenoids from the barks and roots of *Michelia alba* collected in Xuan Mai town, Hanoi City, Viet Nam, *Vietnam J. Chem.*, **2021**, *59(1)*, 120-126.
9. Nguyen Van Tuan, Nguyen Tuan Thanh, Nguyen The Anh, Do Le Quyen, Tran Van Loc, Tran Van Sung, Tran Thi Phuong Thao. Phytochemistry of *Dolichandrone spathacea* collected in Soc Trang province, Vietnam, *Vietnam J. Chem.*, **2016**, *54(6e2)*, 34-39.
10. Vo Khanh Ha, Truong Thi Minh Hanh, Giang Thi Kim Lien, Mai Thi Phuong Chi, Tran Thi Phuong Thao. Biological activities evaluation of *Eurycoma longifolia* Jack root extracts collected from Ia Grai district, Gia Lai province, *Vietnam Journal of agriculture and rural development*, **2020**, *5*, 56-61.
11. D. E. Joanne, C. J. Barry, C. D. Michael, W. G. Peter. Cinnamate esters of catalpol from *Westringia fruticosa* and *Westrigia viminalis*, *Biochem. Syst. Ecol.*, **1996**, *24(1)*, 65-69.
12. H. J. Peter, H. Hiroshi. Anti-hepatotoxic activity of extracts and constituents of *Buddleja* species, *Planta Med.*, **1989**, ***55***, 123-126.
13. Junior P. Nemorosid und Nemorososid, zwei neue Iridoidglucoside aus *Penstemon nemorosus* [Nemoroside and nemorososide, new iridoid glucosides from *Penstemon nemorosus*], *Planta Med.*, **1983**, *47(2)*, 67-70.
14. D. K. K. Thushara, G. P. Wannigama, W. Aruna, J. Lalith, S. Peta. Ursene carboxylic acids of *Uncaria elliptica*, *Phytochemistry*, **1995**, *40(4)*, 1311-1312.
15. N. Petkova, D. Vassilev, F. Grudeva, Y. Tumbarski, I. Vasileva, M. Koleva, P. Deneva. "Green" Synthesis of sucrose octaacetate and characterization of its physicochemical properties and antimicrobial activity, *Chem. Biochem. Eng. Q.,* **2017***, 31****(4)***, 395-402.
16. P. Tania, H. K. Kar. Isolation and characterization of *β*-sitosterol-3-*O*-*β*-*D*-glucoside from the extract of the flowers of *Viola odorata*, *Br. J. Pharm. Res.*, **2017**, *16(4)*, 1-8.
17. Dilip Gorai. Bioactive constituents from *Peltophorum pterocarpum* (DC.) flowers, *Der. Pharma. Chemica.*, **2013**, *5(6)*, 364-368.
18. L.-Q. Zhang, K.-X. Chen, Y. M. Li. Bioactivities of natural catalpol derivatives, *Curr. Med. Chem.*, **2019**, *26(33)*, 6149-6173.
19. Z. Tiantian, Z. Liuqiang, L. Shuang, Q. Fei, L. Yiming, X. Jin-Wen. Anti-inflammatory activity comparison among scropoliosides-catalpol derivatives with 6-*O*-substituted cinnamyl moieties, *Molecules*, **2015**, *20(11)*, 19823-19836.

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