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Flavonoids from *Dicliptera chinensis* (L.) Nees Grown in Vietnam and their Anti-Inflammatory Activities

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Abstract

From the leaves of Dicliptera chinensis (L.) Nees (Acanthaceae) collected in Nam Dinh province, five flavonoids (1-5) were isolated by chromatographic methods. These compounds were identified as: Kaempferol-3-O-β-D-glucopyranoside (1), Nicotiflorin (2), Kaempferol-3-O-α-L-rhamnopyranoside-7-β-D-glucopyranoside (3), Catechin (4), Quercetin (5). Their structures were elucidated by spectroscopic methods, including MS and NMR. Compound 1 and 3 were isolated from Dicliptera genus for the first time. Compound 3 and 4 also showed potential COX-2 inhibitory effects with IC50 values of 22.38 ± 1.72, 22.95 ± 1.87 µg/mL, respectively.

Keywords: Kaempferol-3-O-β-D-glucopyranoside; Nicotiflorin; Kaempferol-3-O-α-L-rhamnopyranoside-7-β-D-glucopyranoside; Catechin; Quercetin

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Introduction

The leaves of *Dicliptera chinensis* (L.) Nees (Acanthaceae) is an ethnomedicine, which has been commonly used for treatment of inflammatory in folk [1]. Phytochemical studies of this plant demonstrated the presence of glycosides, flavonoids, monoterpenoids: dicliparisides A, dicliparisides B, dicliparisides C, acid vanillic, β–sitosterol, 2,5–Dimethoxy–bezoquinone, daucosterol, lugrandoside and poliumonside [2,3]. Gao Yu-tao has isolated thirteen compounds from petroleum ester extract fraction of Dicliptera chinensis, these were hexatriacontanol, stearic acid, lupenone, lupeol, 4-sitost-4-en-3-one, stigmast-5-en-7-oxo-3β-yl palmitate, β-sitosterol, oleanolic acid, 3β,6β-stigmast-4-en-3,6-diol, 6β-hydroxy-stigmast-4-en-3-one, 3β-hydroxy-stigmast-5-en-7-one, dehydrovomifoliol, and vomifoliol [4]. Previous studies reported that *D. chinensis* had pronounced bioactivities, including antioxidant, anti-inflammatory activities. Gao Ya has showed *D. chinensis* polysaccharide was effective for liver injury induced by antituberculosis drug, and the mechanism may be associated with its anti-inflammatory action [5]. Other study demonstrated that functional components including flavonoids, polysaccharides and polyphenols from *D. chinensis* had strong free radical scavenging capacity [6]. Although *D. chinensis* is used for clinical treatment, there have been very few studies on this plant. Therefore, this paper reports on the phytochemical investigation of *D. chinensis* and on the evaluation of the anti-inflammatory activities of isolated compounds.

Spectra (ESI-MS) were recorded on an AGILENT 1260 Series LC-MS ion Trap (Agilent Technologies, USA).Melting points were measured on SMP10 BioCote in the School of Medicine and Pharmacy-VNU.Optical rotation was measured on PLR-4, MRC scientific instruments in the School of Medicine and Pharmacy -VNU.

Materials and Methods

Plant material

The leaves of *Dicliptera chinensis* (L.) Nees (Acanthaceae) is an ethnomedicine, which has been commonly used for treatment of inflammatory in folk [1]. Phytochemical studies of this plant demonstrated the presence of glycosides, flavonoids, monoterpenoids: dicliparisides A, dicliparisides B, dicliparisides C, acid vanillic, β–sitosterol, 2,5–Dimethoxy–bezoquinone, daucosterol, lugrandoside and poliumonside [2,3]. Gao Yu-tao has isolated thirteen compounds from petroleum ester extract fraction of Dicliptera chinensis, these were hexatriacontanol,

Column chromatography was performed on silica gel (0.040-0.063 mm, Nicalai Tesque Inc., Japan), YMC ODS-A (50 µm, YMC Co. Ltd., Japan).Organic solvents were of analytical grade.Analytical TLC was performed on Kieselgel 60 F254 (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F254 (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H2SO4, followed by heating with a heat gun.The NMR [^{1}H (500 MHz), ^{13}C (125 MHz), and DEPT-90 and 135 MHz)] spectra were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST).

The whole plants of *D. chinensis* (L.) Nees were collected from Nam Dinh province, Vietnam during June 2016 and taxonomically identified by the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The samples were stored at the School of Medicine and Pharmacy-VNU.

Extraction and isolation

General experimental procedures were performed as previous study with some modifications [7]. Leaves of D. chinensis was dried, powdered and then extracted with 96% ethanol (8L × 3 h × 3 times) by supersonic method. The resulting extracts were combined and then evaporated to dryness *in vacuo* to yield crude extract (630.0 g). The ethanol extract (120 g) was dissolved in water (1.2 L) and subjected to liquid-liquid partitioning (3 times) using n-hexane, ethyl acetate (EtOAc), yielding 31.0 g and 56.0 g of residue, respectively. The aqueous fraction was concentrated to yield 33.0 g of residue.

The EtOAc residue (50 g) was separated by column chromatography on silica gel 60, eluting with chloroform/ methanol (30/1 → 1/1, v/v) to obtain 4 fractions (E1 → E4). The E2 fraction (14.2 g) was submitted to chromatography on silica gel 60, eluted with chloroform/acetone/water (2/5/0.1, v/v), yielding 4 sub-fractions (E2.1 → E2.4). Fraction E2.1 (1.9 g) was further applied to an RP-18 column eluting with Acetone/H_2O (2:3, v:v), to yield 1 (56 mg). Fraction E2.3 was crystallized on solvents n-hexane/ethyl acetate (3/5, v/v) to yield 2 (54 mg).The aqueous residue (30 g) was subjected to a Diaion HP-20 column, then eluted with 25%, 50%, 75%, and 100% aqueous methanol, yielding 4 fractions (N1 → N4). Fraction N3 (10.0 g) was further separated over a silica gel column and eluted with chloroform/methanol (30/1 → 1/1, v/v) to yield four sub-fractions (N3.1 → N3.4). Fraction N3.1 (6:1:0.1, ethyl acetate: methanol: water, v/v, 1.2 g) was chromatographed on a silica gel 60 column, yielding 3 (36 mg). Fraction N3.2 (2.1 g) was applied to an RP-18 column eluting with methanol/H_2O (2/1, v:v) to yield 4 (51 mg). Finally, fraction N3.4 (2.2 g) was purified by silica gel column, and eluted with chloroform/methanol (30:1, v/v) to yield 5 (46 mg).

Compound 1: Kaempferol-3-O-β-D-glucopyranoside

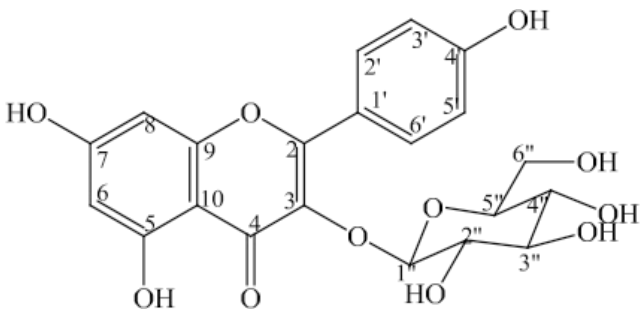

Figure 1: *Structure of compound 1.*

COX-2 assays were performed as described by Zsofia Kutil [8]. Human recombinant COX-2 (0.5 unit/reaction) was added to 180 µL of incubation mixture consisting of 100 mM tris buffer (pH 8), 5 µM porcine hematin, 18 mM L-epinephrine, and 50 µM Na2EDTA. The test substance dissolved in DMSO or pure DMSO (in case of blank) was added (10 µL) and the mixture was preincubated for 5 min at room temperature. The addition of 5 µL of 10 µM arachidonic acid started the reaction. After 20 min incubation at 37°C the reaction was stopped by 10 µL of 10% formic acid. All samples were diluted 1:15 in assay buffer and the concentration of PGE2

The percent inhibition was calculated manually using the following equation:

$$\%\ \ inhibition = \frac{Abs\ \ sample - Abs\ \ control}{Abs\ \ sample} \times 100$$

The effects of compounds were expressed by IC_{50} values. IC_{50} is defined as the concentration of inhibitor that reduces enzyme activity by 50%. All samples were tested three times to reduce the error and establish a stable baseline.

Position	#δ_C	δ_C	DEPT	δ_H (J=Hz)	HMBC (H→C)
2	159.14	159.07	C	-	
3	135.46	135.47	C	-	
4	179.53	179.51	C	-	
5	163.04	163.09	C	-	
6	99.93	100.03	CH	6.23 (d, 2.0)	5,8
7	165.99	166.42	C	-	
8	94.79	94.85	CH	6.42 (d, 2.0)	7, 9, 10
9	158.51	158.57	C	-	
10	105.74	105.65	C	-	
1'	122.8	122.83	C	-	
2'	132.28	132.27	CH	8.07 (d, 9,0)	
3'	116.1	116.09	CH	6.91 (d, 9,0)	1'
4'	161.54	161.58	C	-	
5'	116.1	116.09	CH	6.91 (d, 9,0)	
6'	132.28	132.27	CH	8.07 (d, 9,0)	5', 4'
1"	104.09	104.14	CH	5.26 (d,7,5)	
2"	75.73	75.75	CH	3.45 (m)	3
3"	78.42	78.42	CH	3.24 (m)	

$_1$H and $_{13}$C-NMR spectral data of 2 were similar to those of 1, except for the presence of a rhamnose unit and the strong downfield shift of a methylene carbon at δ_C 66.93 (C-6″). The (1 → 6) glycosidic bond, of rhamnose to glucose was characterized from the cross-peak of H-1‴ (δ_H 4.38) to C-6″ (δ_C 66.93) and the downfield shift of C-6″ of the glucose unit which is shown in Table 2. By comparing physicochemical properties and spectroscopic data in the literature[10,11], 2 was identified as nicotiflorin.

The ESI-MS spectral showed molecular ion peak at m/z 291 [M+H]+ and 289 [M-H]-, consistent with the molecular formula $C_{15}H_{14}O_6$. The 13C-NMR of 4 showed 15 carbon signals including twelve aromatic carbons, two oxygenated aliphatic carbons at δ_C 82.82 and 68.79 and one aliphatic carbon at δ_C 28.42. The 1H-NMR indicated in Table 4, flavan 3-ol moiety including 1,3,4-substituted aromatic proton signals at δ_H 6.86 (1H, d, J=2.0, H-2'), 6.78 (1H, d, J=8.5, H-5'), 6.75 (1H, dd, J=8.5, 2.0, H-6'), two meta-coupled aromatic proton signals at δ_H 5.95 (1H, d, J = 2.5, H-6), 5.89 (1H, d, J=2.5, H-8) and a methene group at δ_H 2.53 (1H, dd, J=16.0, 8.0, H-4α), 2.87 (1H, dd, J=16.0, 5.5, H-4β) and two methine protons at δH 4.59 (1H, d, J=7.5, H-2), 4.01 (1H, m, H-3). The 2,3-trans configuration was confirmed from the large J value of H-2. Based on the spectroscopic evidences and comparison with literature values, 4 was determined to be (+)-catechin.

4"	71.35	71.38	CH	3.37 (m)	
5"	78.38	78.07	CH	3.45 (m)	
6"	62.61	62.65	CH2	3.71 (dd, 2.0, 11.5) 3.52 (dd, 5.5, 12.0)	

Compound 1 was isolated as light yellow powder (Figure 1). The APCI-MS data showed the protonated peak $[M+H]^+$ at m/z 449.0, which matched with the molecular formula of 1 as $C_{21}H_{20}O_{11}$. The 1H-NMR spectrum showed two doublets at δ_H 6.23 (1H, d, J=2.0 Hz, H_6) and δ_H 6.42 (1H, d, J=2.0 Hz, H8) corresponding to C6- and C8–protons of the flavonol A ring. Two doublets at δ_H 6.91 (2H, d, J=9.0 Hz, $H_{3'}$, $H_{5'}$) and δ_H 8.07 (2H, d, J=9.0 Hz, $H_{2'}$, $H_{6'}$) corresponded to their para-substituted flavonoid B-ring. The anomer proton signal at δ_H 5.26 (1H, d, J=7.5 Hz, $H_{1'}$) suggested that 1 was a flavonoid glycoside.

The $_{13}$C-NMR and DEPT confirmed the presence of 21 carbon atoms. Besides the 15 carbon signals of the flavonoid nucleus, the 13C-NMR spectrum of 1 exhibited six carbon resonances of a sugar moiety. In addition, the HMBC correlation between the anomeric proton δH 5.26 ($H_{1''}$) and the carbon signal δ_C 135.47 (C_3) revealed the linkage with the aglycone moiety which is shown in Table 1. Based on above deductions and comparing spectral data to reference [9].1 was determined as kaempferol-3- O-β-D-glucopyranoside.

Compound 2: *Nicotiflorin*

Yellow needless.

Melting point: 190-192 oC.

[α]D25=+20.2 (c = 0.5, MeOH);

Molecula fomula: $C_{27}H_{30}O_{15}$; M=594;

1H-NMR and 13C-NMR (DMSO-d_6) data: Table 2.

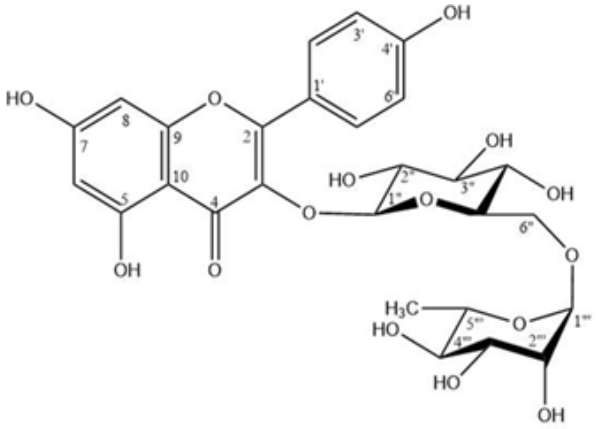

Figure 2: *Structure of compound 2.*

Table 2: *NMR spectral data for compound 2, $^{\#13}$C-NMR data of nicotiflorin [8], :*overlap.*

Position	$^{\#}\delta_C$	δ_C	DEPT	δ_H (J=Hz)	HMBC (H→C)
2	158.5	156.89	C	-	-
3	135.5	133.27	C	-	-
4	179.4	177.42	C	-	-
5	163	161.23	C	-	-
6	100	98.79	CH	6.20 (d, 2.0)	5, 7, 8, 10
7	166.1	164.26	C	-	
8	94.9	93.8	CH	6.40 (d, 2.0)	6, 7, 9, 10
9	159.4	156.55	C	-	-
10	105.6	104	C	-	-
1′	122.7	120.94	C	-	-
2′, 6′	132.4	130.9	CH	7.98 (d, 8.5)	2, 2′, 3′, 4′, 5′, 6′
3′, 5′	116.1	115.14	CH	6.88 (d, 9.0)	1′, 2′, 3′, 4′, 5′, 6′
4′	161.5	159.92	C	-	-
1″	104.6	101.4	CH	5.31 (d, 7.5)	3, 3″
2″	75.7	74.22	CH	3.16 *	1″
3″	78.1	75.78	CH	3.24*	2″
4″	71.4	69.98	CH	3.06*	3″, 6″
5″	77.2	76.42	CH	3.22 (m)	6″
6″	68.5	66.93	CH2	3.27*	
				3.69 (d 10.5)	4″
1‴	102.4	100.79	CH	4.38 (br s)	6 ″, 2‴, 3‴, 5‴
2‴	72.1	70.38	CH	3.42*	3‴, 4‴
3‴	72.3	70.65	CH	3.28*	2‴, 4‴
4‴	73.9	71.88	CH	3.09*	2‴, 3‴, 6‴
5‴	69.7	68.27	CH	3.27*	3‴
6‴	17.9	17.72	CH3	0.98 (d, 6.0)	4‴, 5‴

Compound 2 was isolated as yellow needless (Figure 2). The $_1$H and $_{13}$C-NMR spectral data of 2 were similar to those of 1, except for the presence of a rhamnose unit and the strong downfield shift of a methylene carbon at δ_C 66.93 (C-6″). The (1 → 6) glycosidic bond, of rhamnose to glucose was characterized from the cross-peak of H-1‴ (δ_H 4.38) to C-6″ (δ_C 66.93) and the downfield shift of C-6″ of the glucose unit which is shown in Table 2. By comparing physicochemical properties and spectroscopic data in the literature[10,11], 2 was identified as nicotiflorin.

Compound 3: Kaempferol-3-O-α-L-rhamnopyranoside-7-β-D-glucopyranoside

Compound 3 was isolated as yellow powder (Figure 3).

UV λmax (MeOH): 265 nm, 344 nm.

The ESI-MS data showed in Table 3, the protonated peak [M +H]+ at *m/z* 595.1, which matched with the molecular formula of 3 as $C_{27}H_{30}O_{15}$. The 1H-NMR spectrum of 3 showed meta-coupled aromatic protons (A-ring) at δ_H 6.51 (1H, d, J=2.0 Hz, H_6) and δ_H 6.76 (1H, d, J=2.0 Hz, H_8); ortho-coupled aromatic protons (B-ring) at δ_H 7.82 (2H, d, J=9.0 Hz, $H_{2'}$, $H_{6'}$) and δ_H 6.97 (2H, d, J=8.0 Hz, $H_{3'}$, $H_{5'}$), suggesting the structure of kaempferol aglycone to 3. The presence of rhamnose and glucose moieties was also suggested by two anomer proton signals at δH 5.42 (1H, d, J=1.5Hz, $H_{1''}$) and δH 5.09 (1H, d, J=7.0 Hz, $H_{1'''}$), one methyl group at δ_H 0.96 (3H, d, J=6.0 Hz, $H_{6''}$), one oxymethilene group at δ_H 3.74 (1H, m, $H_{6'''}\alpha$), δ_H 3.97 (1H, dd, J=12.0/ 2.0 Hz, $H_{6'''}\beta$).

Table 3: *NMR spectral data for compound 3,#: ^{13}C-NMR data of kaempferol-3-0-α-L-rhamnopyranoside-7-β-D-glucopyranoside in CD_3OD[5]*

Position	#δ_C	δ_C	DEPT	δ_H Mult., (J inHz)	HMBC (H → C)
2	159.7	159.85	C	-	
3	136.4	136.46	C	-	
4	179.7	179.8	C	-	
5	162.7	162.8	C	-	
6	100.8	100.86	CH	6.51 (d, 2.0)	5,7,8,10
7	164.6	164.66	C		
8	95.7	95.82	CH	6.76 (d, 2.0)	6,7,9,10
9	158	158	C		
10	107.6	107.69	C		
1'	122.3	122.42	C		
2'	132	132.02	CH	7.82 (d, 9.0)	2,4'
3'	116.5	116.57	CH	6.97 (d, 8.0)	1',4'
4'	161.7	161.75	C		
5'	116.5	116.57	CH	6.97 (d, 8.0)	1',4'
6'	132	132.02	CH	7.82 (d, 9.0)	2,4'
1"	103.4	103.48	CH	5.42 (d, 1.5)	3, 5"
2"	71.2	71.27	CH	3.36 (m)	
3"	72.1	72.07	CH	3.74 (m)	
4"	73.1	73.17	CH	3.36 (m)	
5"	71.8	71.89	CH	3.74 (m)	
6"	17.6	17.64	CH3	0.96 (d, 6,0)	4", 5"
1"'	101.5	101.58	CH	5.09 (d, 7,0)	7
2"'	75.1	74.71	CH	3.52 (m)	
3"'	78	77.81	CH	3.54 (m)	
4"'	72.1	72.12	CH	3.36 (m)	
5"'	78.3	78.36	CH	3.54 (m)	
6"'	62.4	62.46	CH2	3.74 (m)3.97 (dd, 12.0, 2.0)	

The 13C-NMR and DEPT spectra showed 27 carbon resonances for 3,15 of which were identical to kaempferol. The 1H and 13C NMR spectral data together with ESI-MS supported the presence of two hexose units in 3. A HMBC experiment performed with 3, showed correlations between the anomeric proton at δ_H 5.42 (1H, d, J=1.5 Hz, H1") and the quaternary carbon atom at δC 136.46 (C3); the proton at δH 5.09 (1H, d, J=7.0 Hz, $H_{1'''}$) and the carbon atom at δC 164.66 (C7), indicating the sites of glycosidation. The NMR data of 3 showed strong agreement with those of kaempferol 3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranoside [12]. By comparison with previously reported literature, the structure of 3 was deduced as kaempferol 3-O-α-L- rhamnopyranoside-7-O-β-D-glucopyranoside.

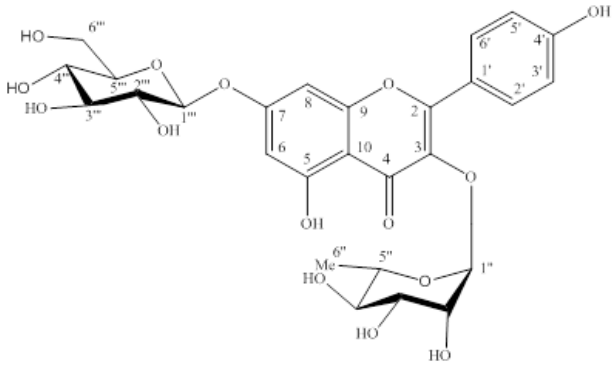

Figure 3: *Structure of compound 3*

Compound 4: *(+)-catechin*

Compound 4 was isolated as yellow powder, melting point: 175-177°C (Figure 4).

The ESI-MS spectral showed molecular ion peak at m/z 291 [M+H]+ and 289 [M-H]-, consistent with the molecular formula $C_{15}H_{14}O_6$. The 13C-NMR of 4 showed 15 carbon signals including twelve aromatic carbons, two oxygenated aliphatic carbons at δ_C 82.82 and 68.79 and one aliphatic carbon at δ_C 28.42. The 1H-NMR indicated in Table 4, flavan 3-ol moiety including 1,3,4-substituted aromatic proton signals at δ_H 6.86 (1H, d, J=2.0, H-2'), 6.78 (1H, d, J=8.5, H-5'), 6.75 (1H, dd, J=8.5, 2.0, H-6'), two meta-coupled aromatic proton signals at δ_H 5.95 (1H, d, J = 2.5, H-6), 5.89 (1H, d, J=2.5, H-8) and a methene group at δ_H 2.53 (1H, dd, J=16.0, 8.0, H-4α), 2.87 (1H, dd, J=16.0, 5.5, H-4β) and two methine protons at δH 4.59 (1H, d, J=7.5, H-2), 4.01 (1H, m, H-3). The 2,3-trans configuration was confirmed from the large J value of H-2. Based on the spectroscopic evidences and comparison with literature values, 4 was determined to be (+)-catechin.

Table 4: *NMR spectral data for compound 4, #: ^{13}C-NMR data of (+)-catechin [13]*

Position	δ_C [3]	δ_C	DEPT	δ_H Mult., (J inHz)	HMBC (H → C)

2	82	82.82	CH	4.59 (7.5) d	3, 4, 9, 1′, 2′, 6′
3	67.9	68.79	CH	4.01 m	2, 10, 1′
4	28.1	28.42	CH2	2.53 (8.0, 16.0) dd	
				2.87 (5.5, 16.0) dd	
					2, 3, 5, 10
5	156.3	156.89	C		
6	96.7	96.42	CH	5.95 (2.5) d	5, 7, 8, 10
7	156.9	157.79	C		
8	95.7	95.62	CH	5.89 (2.5) d	6, 7, 9, 10
9	156.7	157.51	C		
10	100.9	100.91	C		
1′	131.5	132.27	C		
2′	115.6	115.3	CH	6.86 (2.0) d	3′, 6′
3′	145.4	146.18	C		
4′	145.5	146.2	C		
5′	116.6	116.14	CH	6.78 (8.5) d	3′, 4′
6′	120.3	120.05	CH	6.75 (2.0, 8.5) dd	4′

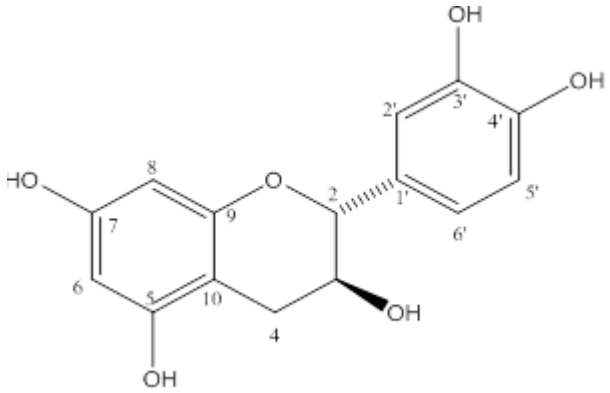

Figure 4: *Structure of compound 4*

Compound 5: *Quercetin*

Yellow needless. Melting point: 313-314 °C.

Molecula fomula: $C_{15}H_{10}O_7$; M= 302.

1H-NMR and 13C-NMR (CD_3OD) data: Table 5

Table 5: *NMR spectral data for compound 5, #: ^{13}C-NMR dat of quercetin [14]*

Position	#δ_C	δ_C	δ_H (J=Hz)
2	146.8	147.72	
3	135.6	137.19	
4	175.7	177.27	
5	160.6	162.45	
6	98.1	99.22	6.19 (d, 2.0)
7	163.8	165.51	
8	93.3	94.4	6.39 (d, 2.0)
9	156.1	158.19	
10	103	104.49	
1′	121.9	124.13	
2′	115.1	115.98	7.74 (d, 2.0)
3′	145	146.17	
4′	147.6	148.72	
5′	115.5	116.2	6.89 (d, 8.5)
6′	119.9	121.67	7.64 (dd, 2.0, 8.5)

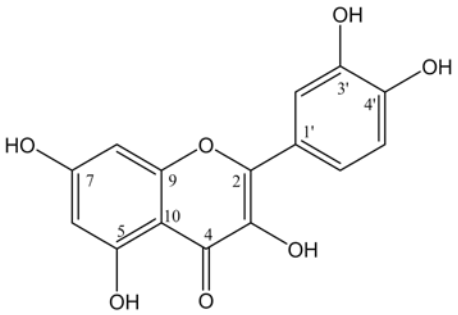

Figure 5: *Structure of compound 5*

Compound 5 was obtained as yellow needless (Figure 5). The 1H-NMR spectrum of 5 showed 1,3,4-substituted aromatic proton signals at δH 7.74 (1H, d, J=2.0, H-2'), 6.89 (1H, d, J=8.5, H-5'), 7.64 (1H, dd, J=8.5, 2.0, H-6'), two meta-coupled aromatic proton signals at δ_H 6.19 (1H, d, J=2.0, H-6), 6.39 (1H, d, J=2.0, H-8). The 13C-NMR spectrum indicated that 5 has a flavonol skeleton with 15 carbons, including five aromatic CH; ten quaternary carbons (one carbonyl, five O-bearing, and four aliphatic), suggesting that it is 3,5,7,3′,4′-pentahydroxyflavone, commonly known as quercetin [14].

Anti-inflammatory activities of isolated compounds

Table 6: *Inhibitory effect of isolated compunds on COX-2 enzyme*

Compound	Concentration (ug/ml)	% Inhibition	IC_{50}
Kaempferol-3-O-β-D-glucopyranosid	3.125	9.24 ± 1.8	38.01 ± 1.89
	6.25	15.74 ± 1.2	
	12.5	32.59± 1.2	
	25	40.25 ± 1.5	
	50	56.81 ± 2.7	

	100	86.57 ± 1.2	
Kaempferol-3-O-α-L-rhamnopyranosid-7-β-D-glucopyranosid	3.125	12.35 ± 2.3	22.38 ± 1.72
	6.25	19.47 ± 2.1	
	12.5	32.57 ± 2.6	
	25	48.71 ± 2.3	
	50	69.34 ± 1.8	
	100	87.11 ± 1.7	
Catechin	3.125	14.17 ± 2.7	22.95 ± 1.87
	6.25	24.29 ± 2.1	
	12.5	36.57 ± 2.7	
	25	52.39 ± 1.2	
	50	69.53 ± 2.4	
	100	87.08 ± 1.8	
Quercetin	3.125	10.78 ± 1.8	37.15 ± 1.35
	6.25	19.67 ± 1.2	
	12.5	30.14 ± 1.5	
	25	44.29 ± 2.1	
	50	53.78 ± 1.7	
	100	78.24 ± 1.9	
Nicotiflorin	3.125	12.36 ± 1.9	32.35 ± 1.94
	6.25	19.87 ± 2.3	
	12.5	28.34 ± 2.1	
	25	45.89 ± 2.2	
	50	61.27 ± 1.4	
	100	87.24 ± 2.4	
Celecoxib (positive drug control)	0.1563	12.36	1.43 ± 0.12
	0.3125	19.87	
	0.625	28.34	
	1.25	45.89	
	2.5	61.27	
	5	87.24	
	10	98.25	

Table 7: *IC_{50} of isolated compunds on COX-2 enzyme*

Sample	$LogIC_{50}$	IC_{50}
Kaempferol-3-O-α-L-rhamnopyranosid-7-β-D-glucopyranosid	1.35	22.38
Catechin	1.36	22.95
Quercetin	1.57	37.15
Kaempferol-3-O-β-D-glucopyranosid	1.58	38.01
Nicotiflorin	1.51	32.35

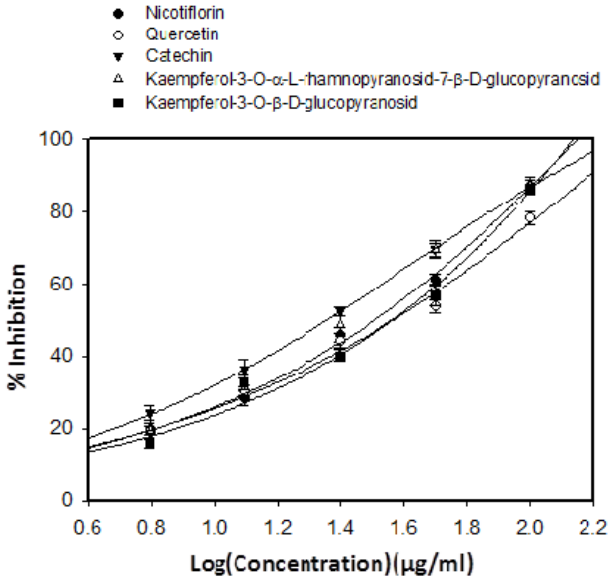

Figure 6: *Graph showing COX-2 inhibition (%) plotted against concentrations of isolated compounds. The Ic_{50} values of isolated compunds were calculated on a graph and converted from log [µg/mL] to µg/mL.*

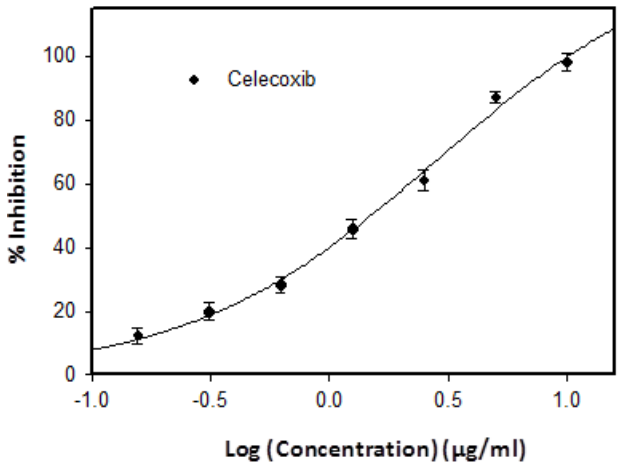

Figure 7: *Graph showing COX-2 inhibition (%) plotted against concentrations of Celecoxib*

Cyclooxygenase enzyme (COX, prostaglandin endoperoxide synthase) catalyzes two reactions, the first reaction adds an atom of oxygen to arachidonic acid to form prostaglandin G2 (PGG2) and the second reaction catalyzes the conversion of PGG2 to PGH2. Thus, the COX enzyme performs an important step in converting arachidonic acid into proinflammatory elements, including prostaglandins, thromboxanes and prostacyclins. Prostaglandins regulate smooth muscle contraction, blood pressure and platelet aggregation, regulate pain and fever. The nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit activity of cyclooxygenase enzyme and therefore it is used for the treatment of analgesic, antipyretic,

anti-inflammatory and anti-thrombotic [15]. COX-1 isoform is responsible for regulating activity of the physiological prostanoids in the body. In contrast, COX-2 is an isoform that is involved in inflammatory processes[16]. Long-term administration and uncontrolled NSAIDs cause some serious side effects, such as intestinal bleeding due to inhibiting COX-1 enzyme [15].

New-generation COX-2 inhibitors do not induce intestinal bleeding but can cause cardiovascular-related adverse events [17]. Steroid drugs play an important role in the treatment of inflammatory diseases, but it is only used in the short term due to their toxicity. Therefore, finding natural compounds with anti-inflammatory effects but with few side effects is very urgent. Medicinal plants play an important role in the search for potential compounds for drug development. The screening process of natural anti-inflammatory compounds has been increasing in recent years [18]. In this study we evaluated the COX-2 inhibitory effect of the isolated compounds shown in Figure 6. Celecoxib was used as positive control to inhibit COX2 enzyme shown in Figure 7 and its IC_{50} value was of 1.43 ± 0.12 µg / mL. Previous published showed that the COX-2 inhibitory effect of Celecoxib had an IC_{50} of 0.34 µg/mL [19]. which is shown in Table 7. This shows that our results are consistent with previous publications. Among five isolated flavonoids, two compounds showed potential COX-2 inhibitory effects, Kaempferol-3-O-α-L-rhamnopyranosid-7-β-D-glucopyranosid (IC_{50}=22.38 ± 1.72 µg/mL), and catechin (IC_{50}=22.95 ± 1.87µg/mL). Previous study showed that kaempferol is a potent strong inhibitor COX-2 which could be chemopreventive agent against cancer [20,21]. In-Ja Par has demonstrated that catechin could decrease the cyclooxygenase-2 (COX-2) expression and prostaglandin E_2 (PGE_2) levels and reduce the excessive ROS [22]. From the results of this study, we selected catechin and kaempferol-3-O-α-L-rhamnopyranoside-7-β-D-glucopyranoside for further studies of their anti-inflammatory activities.

Conclusion

From the leaves of *D. chinensis* (L.) Nees (Acanthaceae) collected in Nam Dinh province five flavonoids (1-5) were isolated by chromatographic methods. On the basis of spectroscopic analyses and by spectral comparison with published literature, the isolated compounds were identified as Kaempferol-3-O-β-D-glucopyranoside (1), Nicotiflorin (2), Kaempferol-3-O-α-L-rhamnopyranoside-7-β-D-glucopyranoside (3), Catechin (4), Quercetin (5). This is the first report on the isolation of 1 and 3 from Dicliptera genus. Among five isolated compounds, 3 and 4 also showed significant COX-2 inhibitory effects, with IC_{50} values of 22.38 ± 1.72, 22.95 ± 1.87 µg/mL, respectively which is shown in Table 6.

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