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Original Article

Anti-inflammatory effects, nuclear magnetic resonance identification, and high-performance liquid chromatography isolation of the total flavonoids from *Artemisia frigida*

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ABSTRACT

The aerial parts of *Artemisia frigida* Willd. are used to treat joint swelling, renal heat, abnormal menstruation, and sore carbuncle. The anti-inflammatory effects of *A. frigida* have been well-known in folk medicine, suggesting that components extracted from *A. frigida* could potentially treat inflammatory disease. With the aim of discovering bioactive compounds, in this study, we extracted total flavonoids from the aerial parts of *A. frigida* and investigated their anti-inflammatory effects against inflammation induced by carrageenan and egg albumin in rats. At the doses studied, total flavonoids (100 mg/kg, 200 mg/kg, and 400 mg/kg) and some isolated compounds (30 mg/kg) showed significant and dose-dependent anti-inflammatory effects. According to the high-performance liquid chromatography analysis of the total flavonoids from *A. frigida*, there are five major compounds, namely, 5-hydroxy-3′,4′-dimethoxy-7-O-β-D-glucuronide (F1), 5-hydroxy-3′,4′,5′-trimethoxy-7-O-β-D-glucuronide (F2), 5,7,3′-trihydroxy-6,4′-dimethoxyflavone (F3), 5,3′-dihydroxy-6,7,4′-trimethoxyflavone (F4), and 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone (F5), which may explain the anti-inflammatory activity.

1. Introduction

Inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells, which involves a complex sequence of biochemical events closely associated with the pathogenesis of various diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout, and migraine [1–3].

Nowadays, although the synthetic anti-inflammatory drugs are dominating the market, the element of toxicity from these drugs cannot be ignored. Many drugs [both nonsteroidal anti-inflammatory drugs (NSAIDs) and

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corticosteroids] have been developed but their safety profile studies have shown that none of them is clearly safe. Because of adverse reactions of synthetic medicines, that is, causing gastrointestinal irritation and reappearance of symptoms after discontinuation, herbal medicines have made a comeback to improve our basic health needs. Many plants and herbs such as ginger, turmeric, and olive oil have been shown to exhibit potent anti-inflammatory effects [4]. Currently available drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this regard, new compounds with improved pain management capacity and fewer side effects are searched every nook and corner of the world, which can serve as alternatives to NSAIDs and opiates. There is an increasing focus on evaluating the efficacy of plant-based drugs used in the traditional medicine because they are cheap and have little or no side effects [5,6].

Artemisia frigida is a member of the Compositae family and is distributed throughout Inner Mongolia, occupying 10.38% of its Steppe [7]. It is widely used in Mongolian traditional medicine for treatment of diverse diseases, especially for arthritis and rheumatoid [8]. In our previous studies, flavonoids [9–14], sesquiterpene lactone glycosides [15], and coumarins [16] were isolated from this plant.

Flavonoids are a class of secondary metabolites generally located in plant leaves, flowers, and stems [17]. These compounds are not only present in plants as constitutive agents but are also accumulated in plant tissues in response to microbial attack [18,19]. During the recent years, flavonoids have been isolated from many plants and are demonstrated to have significant pharmacological activities, such as anti-inflammatory, antioxidant, and hepatoprotective effects [20,21]. However, until recently, there have been few studies about the pharmacological effects of *A. frigida*. With the aim of discovering bioactive compounds, in this study, we extracted total flavonoids from the aerial parts of *A. frigida* and investigated their anti-inflammatory effects against inflammation induced by carrageenan and egg albumin in rats. We also describe the anti-inflammatory activity and the high-performance liquid chromatography (HPLC) analysis of the total flavonoids extracted from the aerial parts of *A. frigida*.

2. Methods

2.1. Plant material

The aerial parts of *A. frigida* were collected from Tongliao, Inner Mongolia of China, in July 2014, and identified by Professor Buhebateer (Inner Mongolia University for Nationalities). A voucher (No. 20140714) has been deposited in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

2.2. Animals

Male Wistar rats (200–300 g) were provided by Changchun Yisheng Laboratory Animal Technology Co., Ltd. (Changchun, China). The rats were maintained under standard animal housing conditions (25 ± 5°C, 40–70% relative humidity, 12-hour light/dark cycle) and provided with standard laboratory food [rat sterile granulated feed, product executive standard: GB14924–2001, license: SCXK–(JI) 2010–0001] and water *ad libitum*. They were fasted for 24 hours before a test.

2.3. Preparation of total flavonoids from A. frigida

The air-dried aerial parts of *A. frigida* (2 kg) were crushed and extracted two times with 75% ethanol in a 50 L solvent volume for 6 hours under reflux. The ethanol solution was vacuum evaporated at 60°C. Approximately 860 g of the residual ethanol was obtained. The extract was then dissolved in 300 mL of water and added onto the D-101 macroporous adsorption resin chromatographic column (500 g). After eluting with 5 L ethanol–water (20:80), 10 L ethanol–water (85:15) eluate was concentrated in vacuum to dryness. The residue (580 g) was stored in a refrigerator (0–4°C) until further use.

2.4. HPLC isolation of total flavonoids and nuclear magnetic resonance identification of HPLC fractions

The liquid chromatography (LC) system consisted of an LC-20AT pump (Shimadzu, Japan), Shimadzu SPD-20A detector, and Shimadzu CBM-20A software for data processing. A solution of 35% methanol was passed through an HPLC column—EZ0566 (250 mm × 20 mm, 5 μm). The column temperature was maintained constant at 30°C. The flow rate was 5 mL/min and the injection volume was 200 μL. Approximately 1.5 g of the dried total flavonoids was ground into a powder form, accurately weighed, soaked with 50 mL water and acetonitrile solution (70:30, v/v) for 60 minutes at room temperature, and then sonicated for 20 minutes. The solution was filtered through a 0.45-μm membrane filter before LC isolation, and then separated by semipreparative HPLC to yield 172 mg of F1 at 99.0% purity, 164 mg of F2 at 98.5% purity, 102 mg of F3 at 96.3% purity, 91 mg of F4 at 95.2% purity, and 88 mg of F5 at 94.8% purity from 1.50 g of the total flavonoids. The purity of Compounds F1–F5 was determined by normalization of the peak areas detected by HPLC.

The structures of Compounds F1–F5 were identified by nuclear magnetic resonance (NMR). NMR spectra were measured on a Bruker Avance III-500 NMR spectrometer using tetramethylsilane as the internal reference, and chemical shifts are expressed in δ (ppm).

2.5. Anti-inflammatory activity on carrageenan-induced paw edema

The total flavonoids and compounds (F1–F5) isolated from the aerial parts of *A. frigida* were investigated for their anti-inflammatory effects against inflammation induced by carrageenan and egg albumin in rats and their inhibitory effects on granuloma induced by cotton pellet in rats according to the method described previously [22]. The animals were divided into 10 groups of eight rats. The negative control group received distilled water (0.5 mL/kg, p.o.), the positive control group received luteolin (30 mg/kg, p.o.), and the test groups received the total flavonoids at doses of 100 mg/kg p.o., 200 mg/kg p.o., and 400 mg/kg p.o. and compounds F1–F5 at

doses of 30 mg/kg p.o. The test was conducted using an electric plethysmometer 7140 (Ugo Basile, Monvalle VA, Italy). Carrageenan 2.5% (0.05 mL) was subcutaneously injected into the plantar surface of the rat's left hind paw 1 hour after the oral administration of drugs to induce a progressive swelling of the paw. The paw volume, up to the tibiotarsal articulation, was measured at 0 hours (before carrageenan injection) and 1 hour, 3 hours, 5 hours, and 10 hours later.

2.6. Egg albumin-induced inflammation in rats

To test the effects of total flavonoids and compounds (F1–F5) isolated from the aerial parts of *A. frigida* against inflammation, the method described by Ghisalberti [23] was adopted. In brief, rats were grouped into ten. The negative control group received distilled water (10 mL/kg, p.o.), the positive control group received luteolin (30 mg/kg, p.o.), and the test groups received the total flavonoids at the doses of 100 mg/kg p.o., 200 mg/kg p.o., and 400 mg/kg p.o. and compounds F1–F5 at doses of 30 mg/kg p.o. All the animals were injected with 0.1 mL of fresh egg albumin subcutaneously into the left hind paw 30 minutes after the administration of aforementioned compounds/drug. The volume of paw edema in each rat was measured using a digital plethysmometer (LE 7500) prior to and 60 minutes after the albumin injection and at every 60 minutes up to 240 minutes.

2.7. Acute toxicity

For the assessment of acute toxicity, Wistar rats, male and female, were divided into groups of 10 animals. The total flavonoids were administered orally to rats in Groups 1–6 at doses of 100 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg, and 3000 mg/kg, respectively. The control group received distilled water (10 mL/kg, p.o.). The mortality rate within the 72-hour period was determined and the lethal dose 50% (LD_{50}) was estimated according to the method described previously [24].

2.8. Statistical analysis

Data are presented as means ± standard error. Statistical analyses were carried out using Student *t*-test. A p value less than 0.05 is considered significant.

3. Results and discussion

3.1. HPLC isolation of total flavonoids and NMR identification of compounds F1–F5

The HPLC assay method developed in this study was applied to the isolation of total flavonoids from *A. frigida*. The HPLC chromatogram is shown in Fig. 1, which mainly contains five compounds (F1–F5). The structures of compounds F1–F5 were identified by spectroscopic methods including ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), and by comparing the data obtained with those reported in the literature [9–14].

Compound F1 was obtained as a yellow powder. In the ^{1}H NMR spectrum, the signals of five aromatic protons at δ 6.42 (1H, d, J = 2.0 Hz, H-6), 6.95 (1H, d, J = 2.0 Hz, H-6), 7.63 (1H, d, J = 2.0 Hz, H-2′), 6.98 (1H, d, J = 8.0 Hz, H-5′), and 7.61 (1H, dd, J = 8.0, 2.0 Hz, H-6′) indicated the presence of an AB system and an ABC system, and a characteristic signal of H-3 of a flavone at δ 6.93 (1H, s), which was confirmed based on the heteronuclear multiple-bond correlations (HMBC) from δ 6.93 (1H, s) to δ_C 182.5 (C-4), 164.2 (C-2), 105.3 (C-10), and 121.3 (C-1′). Moreover, the HMBC from CH_3O– (δ 3.93) to C-4′ and from CH_3O– (δ 3.91) to C-3′ indicated that the methoxy groups were attached to C-4′ and C-3′, respectively.

The ^{13}C NMR spectrum of F1 showed the presence of six carbon signals except for the glycone carbons at δ 102.2, 73.5, 74.0, 71.8, 76.5, and 171.3, which belong to a β-D-glucuronyl group based on the coupling constant of the anomeric protons at δ 5.09 (1H, d, J = 7.0 Hz). In addition, the HMBC from δ 5.09 (1H, d, J = 7.0 Hz) to C-7 (δ 163.0) indicated that the β-D-glucuronyl group was attached to C-7. Compound F1 was thus established as 5-hydroxy-3′,4′-dimethoxy-7-O-β-D-glucuronide.

Compound F2 was obtained as a yellow powder. ^{1}H and ^{13}C NMR spectral data of F2 were similar to those of F1, except for the B ring. In particular, the ABX system in F1 was substituted by the A_2 system in F2, and this was confirmed by the proton signals at δ 7.35 (2H, s, H-2′, H-6′) and at δ 3.89 (6H, s) and 3.91 (3H, s). The HMBC from the anomeric protons at δ 5.11 to C-7 revealed that the sugar moiety was linked to the C-7 of the aglycone. The anomeric configuration in the sugar moiety was determined as β according to the coupling constant (7.0 Hz). Therefore, the structure of F2 was elucidated as 5-hydroxy-3′,4′,5′-trimethoxy-7-O-β-D-glucuronide.

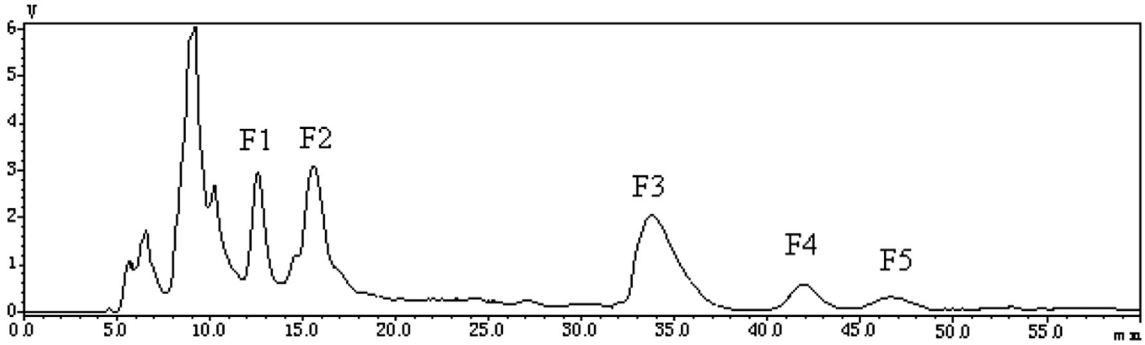

Fig. 1 – High-performance liquid chromatography chromatogram of the total flavonoids from *Artemisia frigida*.

Table 1 – ^{1}H-NMR (500 MHz, *J* in Hz) spectral data for compounds F1–F5 in DMSO-d_6.

No.	F1	F2	F3	F4	F5
2	—	—	—	—	—
3	6.93 (s)	7.04 (s)	6.56 (s)	6.61 (s)	—
4	—	—	—	—	—
5	—	—	—	—	—
6	6.42 (d, 2.0)	6.44 (d, 2.0)	—	—	—
7	—	—	—	—	—
8	6.95 (d, 2.0)	6.92 (d, 2.0)	6.91 (s)	6.93 (s)	6.93 (s)
9	—	—	—	—	—
10	—	—	—	—	—
1′	—	—	—	—	—
2′	7.63 (d, 2.0)	7.35 (s)	7.52 (d, 2.0)	7.60 (d, 2.0)	7.65 (d, 2.0)
3′	—	—	—	—	—
4′	—	—	—	—	—
5′	6.98 (d, 8.0)	—	6.95 (d, 8.0)	6.94 (d, 8.0)	6.97 (d, 8.0)
6′	7.61 (d, 8.0, 2.0)	7.35 (s)	7.56 (dd, 8.0, 2.0)	7.62 (dd, 8.0, 2.0)	7.61 (dd, 8.0, 2.0)
1″	5.09 (d, 7.0)	5.11 (d, 7.0)			
2″	3.24 (m)	3.28 (m)			
3″	3.25 (m)	3.32 (m)			
4″	3.12 (m)	3.18 (m)			
5″	3.63 (m)	3.65 (m)			
6″	—	—			
OCH_3	3.93 (s)	3.89 (s)	3.88 (s)	3.94 (s)	3.93 (s)
	3.91 (s)	3.91 (s)	3.76 (s)	3.90 (s)	3.91 (s)
		3.89 (s)		3.74 (s)	3.89 (s)
					3.78 (s)

DMSO-d_6 = dimethyl sulfoxide-d_6; NMR = nuclear magnetic resonance.

Compound F3 was obtained as a yellow powder. In the ^{1}H NMR spectrum, the signals of four aromatic protons at δ 7.52 (1H, d, J = 2.0 Hz), 6.95 (1H, d, J = 8.0 Hz), and 7.56 (1H, dd, J = 8.0, 2.0 Hz) indicated the presence of an ABC system, and a characteristic signal of H-3 of a flavone at δ 6.56 (1H, s), which was confirmed according to the HMBC from δ 6.56 (H-3) to δ 181.5 (C-4), 164.3 (C-2), 106.1 (C-10), and 121.1 (C-1). The remaining aromatic signal at δ 6.91 (1H, s) was assigned to H-8 based on its correlations with δ_C 130.8 (C-6) and 101.6 (C-10). The ^{13}C NMR spectrum of F3 also showed the presence of 15 carbon signals of a flavone except for the two methoxy groups at δ 164.3 (C-2), 103.1 (C-3), 181.5 (C-4), 152.4 (C-5), 130.8 (C-6), 156.1 (C-7), 90.8 (C-8), 153.5 (C-9), 101.6 (C-10), 121.1 (C-1′), 112.7 (C-2′), 145.9 (C-3′), 149.7 (C-4′), 115.7 (C-5′), and 120.4 (C-6′). In addition, the HMBC from δ 3.76 (3H, s) to C-6 (δ 130.8) and from 3.88 (3H, s) to C-4′ (δ 149.5) indicated that the two methoxy groups were attached to C-6 and C-4′, respectively. Compound F3 was thus established as 5,7,3′-trihydroxy-6,4′-dimethoxyflavone.

Compound F4 was obtained as a yellow powder. ^{1}H and ^{13}C NMR spectral data of F4 were similar to those of F3, except for the appearance of one additional CH_3O–. The HMBC from CH_3O– (δ 3.74) to C-6, from CH_3O– (δ 3.90) to C-4′, and from CH_3O– (δ 3.94) to C-7 indicated that the methoxy groups were attached to C-6, C-4′, and C-7, respectively. Therefore, the structure of F4 was elucidated as 5,3′-dihydroxy-6,7,4′-trimethoxyflavone.

Compound F4 was obtained as a yellow powder. ^{1}H and ^{13}C NMR spectral data of F5 were also similar to those of F4, except for the appearance of one additional CH_3O–. The HMBC from CH_3O– (δ 3.78) to C-6, from CH_3O– (δ 3.89) to C-4′, from CH_3O– (δ 3.93) to C-7, and from CH_3O– (δ 3.91) to C-3 indicated that the methoxy groups were attached to C-6, C-4′, C-7, and C-3, respectively. Therefore, the structure of F5 was elucidated as 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone.

Table 2 – ^{13}C-NMR (125 MHz) spectral data for compounds F1–F5 in DMSO-d_6.

No.	F1	F2	F3	F4	F5
2	164.2	164.2	164.3	164.0	151.2
3	103.5	103.9	103.1	103.2	137.1
4	182.5	182.0	181.5	182.2	178.3
5	161.0	161.2	152.4	152.7	151.9
6	99.0	99.5	130.8	131.8	131.2
7	163.0	163.1	156.1	152.5	158.6
8	96.1	95.2	90.8	91.7	91.8
9	157.18	156.8	153.5	158.7	155.8
10	105.3	105.1	101.6	105.2	105.6
1′	121.3	120.2	121.1	121.5	122.4
2′	115.8	104.5	112.7	115.8	115.6
3′	148.6	148.2	145.9	148.1	147.8
4′	149.5	140.3	149.7	150.9	150.2
5′	111.0	148.3	115.7	110.3	112.3
6′	119.8	104.5	120.4	120.5	120.6
1″	102.2	103.1			
2″	73.5	73.1			
3″	74.0	73.8			
4″	71.8	72.0			
5″	76.5	76.6			
6″	171.3	172.1			
OCH_3	56.3	56.5 (2)	56.1	56.4	56.3
	56.1	59.6	55.0	56.1	56.2
				55.1	55.8
					55.2

DMSO-d_6 = dimethyl sulfoxide-d_6; NMR = nuclear magnetic resonance.

Table 3 – Anti-inflammatory effects of the total flavonoids and compounds isolated from *Artemisia frigida* on carrageenan-induced hind paw edema.

Treatment	Dose (p.o., mg/kg)	Volume of edema (mL) by hour				
		0	1	3	5	10
Distilled water		1.13 ± 0.13	1.17 ± 0.15	1.86 ± 0.18	2.58 ± 0.27	2.71 ± 0.30
Luteolin	30	1.10 ± 0.10	1.18 ± 0.23	1.61 ± 0.24*	1.89 ± 0.30**	1.89 ± 0.27***
Total flavonoids	100	1.08 ± 0.15	1.16 ± 0.44	1.89 ± 0.26	2.25 ± 0.20*	2.09 ± 0.16**
Total flavonoids	200	1.15 ± 0.16	1.23 ± 0.14	1.59 ± 0.25*	2.06 ± 0.21**	1.49 ± 0.11***
Total flavonoids	400	1.18 ± 0.20	0.96 ± 0.18*	1.48 ± 0.15**	1.55 ± 0.39***	1.84 ± 0.29***
F1	30	1.07 ± 0.18	0.98 ± 0.14*	1.55 ± 0.15**	1.91 ± 0.09***	2.16 ± 0.19**
F2	30	1.01 ± 0.17	0.82 ± 0.38*	1.39 ± 0.26**	1.82 ± 0.11***	2.25 ± 0.13**
F3	30	1.10 ± 0.09	0.91 ± 0.15*	1.62 ± 0.20*	1.54 ± 0.39***	2.30 ± 0.12**
F4	30	1.12 ± 0.17	1.13 ± 0.29	1.56 ± 0.20*	2.01 ± 0.17**	1.94 ± 0.31**
F5	30	1.14 ± 0.11	1.15 ± 0.30	1.55 ± 0.31*	2.04 ± 0.21**	1.75 ± 0.48**

Results are expressed as means ± SD; $n = 8$.
*$p < 0.05$ compared with negative control.
**$p < 0.01$ compared with negative control.
***$p < 0.001$ compared with negative control.

Compounds F1–F5 were investigated for their effect against inflammation induced by carrageenan and egg albumin in rats in the following experiments.

3.2. *Acute toxicity*

No mortality was observed in the groups of rats treated with the total flavonoids. The LD_{50} values for the total flavonoids is more than 3000 mg/kg.

3.3. *Anti-inflammatory activity*

The anti-inflammatory activities of the total flavonoids and compounds (F1–F5) isolated from the aerial parts of *A. frigida* have been evaluated using carrageenan and egg albumin in rats and the results are presented in Tables 3 and 4. The total flavonoids showed an anti-inflammatory effect at 100 mg/kg, 200 mg/kg, and 400 mg/kg (doses), which were observable at 10 hours ($p < 0.01$), 5 hours ($p < 0.01$), and 3 hours ($p < 0.01$), respectively (Table 3). The highest dose of total flavonoids showed significant anti-inflammatory activity, which were more than that of luteolin; however, the activity of the medium dose is similar to that of luteolin. The total flavonoids caused a significant and dose-dependent inhibition of increase in paw edema (Table 4). The highest dose of total flavonoids had higher anti-inflammatory effects than that of luteolin, whereas the activity of the lower dose is less than that of luteolin.

Compounds F1–F5 exhibited a significant suppression of inflammation in rats compared with the control. Compound F5 showed significant anti-inflammatory activity, which was also higher than that exhibited by luteolin on egg albumin-induced paw edema. The high activity exhibited by Compound F5 could be explained by the number of methoxy groups on its structure (Fig. 2), which are known to enhance the anti-inflammatory activity in flavones [25–27]. Compound F1 showed better anti-inflammatory activity on carrageenan-induced hind paw edema because there is a glucose–acid in its structure. Although the exact nature of the anti-inflammatory activity of the phytoconstituents has not been elucidated, the results of this study were validated from a preclinical point of view; besides, the anti-inflammatory effect

Table 4 – Effects of the total flavonoids and some isolated compounds on albumin-induced paw edema in rats.

Treatment	Dose (p.o., mg/kg)	Volume of edema (mL) by hour				
		0	1	2	3	4
Distilled water		1.10 ± 0.10	1.35 ± 0.18	1.60 ± 0.38	1.95 ± 0.27	1.73 ± 0.34
Luteolin	30	1.09 ± 0.12	1.21 ± 0.12	1.22 ± 0.20*	1.46 ± 0.30**	1.04 ± 0.28**
Total flavonoids	100	1.12 ± 0.09	1.20 ± 0.38	1.36 ± 0.19	1.62 ± 0.39	1.36 ± 0.23*
Total flavonoids	200	1.08 ± 0.11	1.19 ± 0.13	1.20 ± 0.31	1.60 ± 0.12*	1.15 ± 0.32**
Total flavonoids	400	1.07 ± 0.08	1.07 ± 0.18*	1.01 ± 0.22**	1.30 ± 0.14***	1.03 ± 0.15**
F1	30	1.11 ± 0.13	1.14 ± 0.16*	1.08 ± 0.30*	1.21 ± 0.20***	1.10 ± 0.12**
F2	30	1.08 ± 0.08	1.23 ± 0.17	1.17 ± 0.16*	1.55 ± 0.26**	1.22 ± 0.20**
F3	30	1.05 ± 0.17	1.07 ± 0.24*	1.03 ± 0.15**	1.08 ± 0.36***	1.20 ± 0.25**
F4	30	1.18 ± 0.20	1.21 ± 0.22	1.41 ± 0.18	1.51 ± 0.27*	1.19 ± 0.20**
F5	30	1.19 ± 0.14	1.34 ± 0.22	1.42 ± 0.23	1.48 ± 0.33*	1.17 ± 0.21**

Results are expressed as means ± SD; $n = 8$.
*$p < 0.05$ compared with negative control.
**$p < 0.01$ compared with negative control.
***$p < 0.001$ compared with negative control.

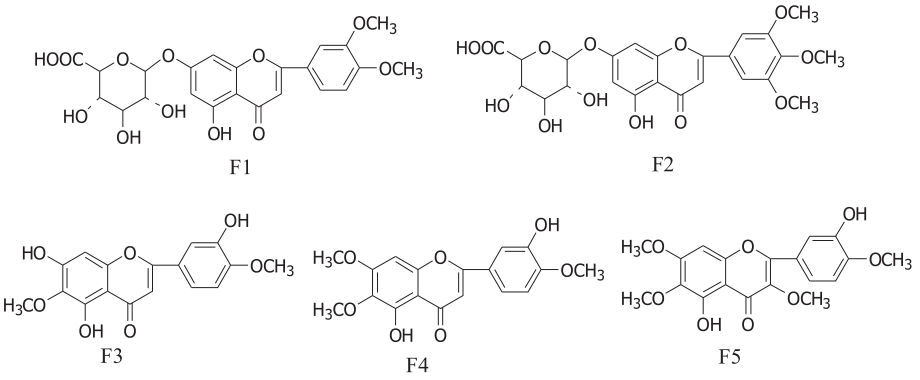

Fig. 2 – Chemical structures of Compounds F1–F5.

is also supported by its use in folk medicine for treatment of inflammatory diseases. These studies are valuable for identifying primary compounds that can be used for preparation of anti-inflammatory drugs, considering the side effects of NSAIDs and corticosteroids. Furthermore, human studies are needed to prove the safety and efficacy of long-term administration of total flavonoids from *A. frigida* as potential anti-inflammatory agent in routine clinical practice.

3.4. *Conclusions*

The total flavonoids from *A. frigida* caused a significant and dose-dependent inhibition of increase in paw edema. Results of HPLC analysis and NMR identification of total flavonoids indicated that there are five flavonoids, namely, 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone (F1), 5,7,3′-trihydroxy-6,4′-dimethoxyflavone (F2), 5,3′-dihydroxy-6,7,4′-trimethoxyflavone (F3), 5-hydroxy-3′,4′-dimethoxy-7-O-β-D-glucuronide (F4), and 5-hydroxy-3′,4′,5′-trimethoxy-7-O-β-D-glucuronide (F5). Compounds F1–F5 also exhibited a significant suppression of inflammation in rats when compared with the control. Hence, the presence of flavones may play a contributory role in the anti-inflammatory activity of total flavonoids isolated from *A. frigida*.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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