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Full Length Research Paper

Chemical composition and anti-inflammatory effects of the EtOAc extract from *Capsella bursa-pastoris* (L.) Medic.

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The aerial parts of *Capsella bursa-pastoris* (L.) Medic are used to treat nephritis, edema and enteritis. Every barber knows that *C. bursa-pastoris* had a good anti-inflammatory effect. This suggests that the extracted components from *C. bursa-pastoris* could potentially treat inflammatory disease. For discovering of the anti-inflammatory effects and chemical composition of *C. bursa-pastoris*, EtOAc extract was extracted from *C. bursa-pastoris* (EECB) and researched on EECB's anti-inflammatory effects. On the carrageenan-induced paw oedema experiment, the EECB used at the doses (100, 200 and 300 mg/kg) after 10 h ($p < 0.01$), 5 h ($p < 0.01$) and 3 h ($p < 0.01$), respectively, showed significant anti-inflammatory effects. Moreover, on the egg-albumin-induced inflammation experiment, the EECB used at the doses 200 and 300 mg/kg after 4 h ($p < 0.01$) and 2 h ($p < 0.01$), respectively, showed significant anti-inflammatory effects. In accordance with the HPLC isolation of the EECB, there are four major compounds, namely, apigenin-7-*O*-*β*-*D*-glucopyranoside (S1), luteolin-7-O-*β*-D-glucopyranoside (S2), α-adenosine (S3), and uridine (S4), which may explain the activity.

Key words: *Capsella bursa-pastoris* (L.) Medic, anti-inflammatory, high performance liquid chromatography (HPLC) isolation, flavonoids.

INTRODUCTION

It is universally acknowledged that inflammation has very important effect on the initiation and progress of many diseases such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and ancers. Inflammation can be carcinogenic with various mechanisms including furtherance of angiogenesis, inducing genomic instability alteration of the epigenetic status and enhancing cell proliferation (Woo et al., 2014; Vázquez et al., 2011; Huang et al., 2011). In today's pharmaceutical market, the synthetic anti-inflammatory drugs are in the leading position, but the toxic element of these drugs cannot be eliminated. Just because of adverse reactions of these

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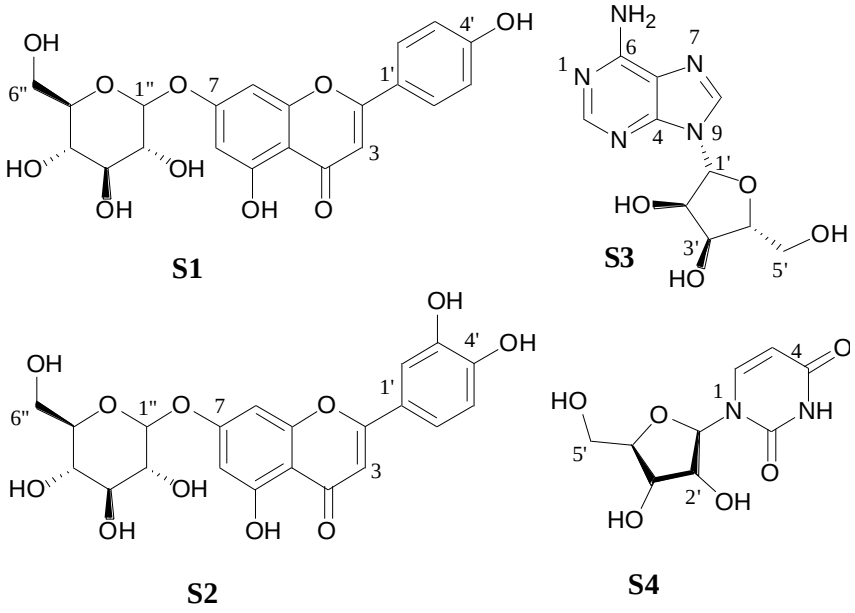

Figure 1. Structures of compounds S1-S4.

drugs, herbal medicines have returned and improved our basic health needs.

A lot of natural products like flavonoids or alkaloids isolated from plants, has been proved to have obvious anti-inflammatory effects (Hasan et al., 2012; Julião et al., 2010; Han et al., 2006) and they are cheap and have little side-effects (Dash et al., 2011; Gomase et al., 2011).

Capsella bursa-pastoris (Cruciferae family) is widely distributed throughout in China (Wang et al., 2014). The aerial parts of *C. bursa-pastoris* are one of the traditional herbal medicines commonly used for treating edema, enteritis and nephritis (Zhang and Jing, 2012; Xu et al., 2007). Flavonoids (Wang et al., 2014; Song et al., 2007) and alkaloids (Kang et al., 2012) from this plant have been reported. However, few scientific studies have been reported to support these claimed medicinal effects and therapeutic.

This current study involves the anti-inflammatory of the EtOAc extract was extracted from *C. bursa-pastoris* (EECB) and a systematic chemical study on EECB. This study will provide a reliable basis for the anti-inflammatory mechanism of the EECB.

MATERIALS AND METHODS

Plant collection and authentication

C. bursa-pastoris was collected from Tongliao, Inner Mongolia, China, in June 2015. This material was identified by Dr. Burie Bao (College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities) and a voucher specimen (NO. 20150628) has been deposited at the herbarium of the College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia, China.

Preparation of EECB

Dried and powdered plant material (aerial parts) of *C. bursa-pastoris* (2.5 kg) was extracted by EtOAc (20 L) after extracting with $CHCl_3$ (10 L). The EECB was concentrated to a residue (328 g) under reduced pressure. The dried EECB was stored in refrigerator at (4°C) before use.

Isolation and identification of EECB

HPLC isolation was performed on C_{18} semi-preparative column (250 mm × 20 mm, 5 µm). The mobile phase consisted of a mixture of MeOH (45%) in water. The flow rate was 3.0 mL/min and the injection volume was 200 µl. The quantification wavelength of these chromatograms was set at 254 nm and the column compartment was kept at the temperature of 30°C.

The dried EECB (10.0 g) was soaked with 200 ml water and acetonitrile solution (70:30, v/v) for 60 min at room temperature, then sonicated for 20 min and filtered through a 0.45 µm membrane filter. The solution separated by semipreparative HPLC to give S1 (208 mg), S2 (176 mg), S3 (124 mg) and S4 (91 mg) from 2.0 g of EECB. The purity of compounds S1 to S4 is 99.0, 98.5, 96.3 and 95.2%, respectively. The structures of compounds S1 to S4 (Figure 1) were all identified by different spectroscopic techniques.

Animals

All the experiments were carried out using Male Wistar rats (200 to 300 g), which were purchased from Changchun Yisheng Laboratory Animal Technology Co., Ltd. (Changchun, China). The rats were housed in polypropylene cages and maintained under standard laboratory conditions (25 ± 5°C, 40 to 70% relative humidity, 12 h light/dark cycle). They were fed with a standard diet (Rat sterile granulated feed, product executive standard: GB14924–2001, license: the confirmation number of SCXK–(Ji) 2010–0001) and water was given *ad libitum*. All experiments were conducted after overnight fasting but there was free access to water.

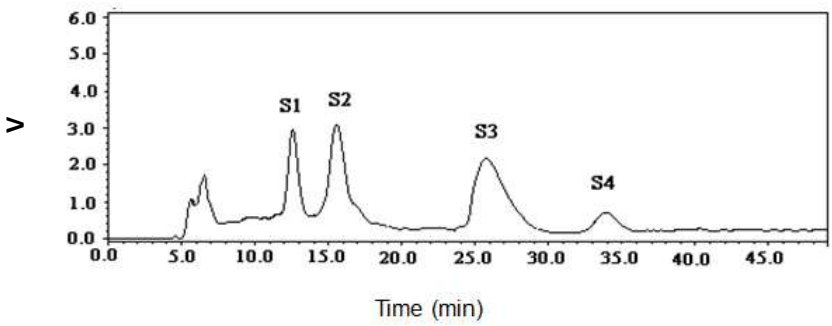

Figure 2. HPLC–UV chromatogram of EECB.

Assay for anti-inflammatory activity

In accordance with the method (Winter et al., 1962), the EECB was checked out of anti-inflammatory activity on carrageenan (CAS:9000-07-1, Sigma-C1013, United States)-induced paw edema. Male Wistar rats were randomly assigned to five groups of eight rats: Group 1 (G1): Negative control, received 0.5 ml/kg, p.o. of distilled water; Group 2 (G2): Positive control, received 30 mg/kg, p.o. of luteolin; Group 3 (G3): Low dose, received 100 mg/kg, p.o. of EECB; Group 4 (G4): Middle dose, received 200 mg/kg, p.o. of EECB; and Group 5 (G5): High dose, received 300 mg/kg, p.o. of EECB.

The experiment was carried out using an electric plethysmometer 7140 (Ugo Basile, Italy). One hour after administration, 2.5% carrageenan (0.05 ml) was injected subcutaneously into the plantar surface of the rat's left hind paw. The volume of paw was measured at 0 h (before carrageenan injection) and 1, 3, 5, and 10 h later.

In accordance with the method (Meng et al., 2003), rats were randomly grouped into five groups of eight rats as in the previous experiment.

Half an hour after administration, 0.1 ml of fresh egg-albumin was injected subcutaneously into the left hind paw of each animal in all groups. Prior to and 60 min after albumin injection and at every 60 min up to 300 min, the volume of paw edema of each rat was measured using an electric plethysmometer 7140 (Ugo Basile, Italy).

Acute toxicity

The EECB in 300, 500, 800, 1200, and 3000 mg/kg doses was administered to rats (male and female) orally (p.o.), which were assigned to six groups of eight rats. The control group received p.o. distilled water (10 ml/kg). All animals were observed for 72 h after drug administration (Wang et al., 2016).

Statistical analyses

The observations were expressed as mean ± standard error (SE), statistical analyses were done by using Student's *t*-test. $P < 0.05$ is considered as significant.

RESULTS

Isolation and identification of EECB

Form Figure 2, there are mainly four compounds (S1-S4). Compounds S1-S4 were identified by different spectroscopic techniques and by comparison with those reported in the literature (Wang and Wang, 2007; Liu et al., 2007; Deng et al., 2005; Ren and Yang, 2001).

Apigenin-7-*O*-*β*-*D*-glucopyranoside (S1): Yellow powder; ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 6.80 (s, H-3), 6.45 (d, *J* = 2.0 Hz, H-6), 6.84 (d, *J* = 2.0 Hz, H-8), 7.97 (d, *J* = 8.0 Hz, H-2'), 6.95 (d, *J* = 8.0 Hz, H-3'), 6.95 (d, *J* = 8.0 Hz, H-5')7.97 (d, *J* = 8.0 Hz, H-6')12.9 (s, 5-OH), 4.47 (d, *J* = 7.5 Hz, H-1''), 2.97 (dd, *J* = 7.5, 6.5 Hz, H-2''), 3.17 (dd, *J* = 7.5, 6.5 Hz, H-3''), 3.01 (m, H-4''), 3.14 (m, H-5''), 3.68 (dd, *J* = 12.0, 2.0 Hz, H-6''a), 3.42 (dd, *J* = 12.0, 2.0 Hz, H-6''b). ^{13}C-NMR (DMSO-d_6, 500 MHz) *δ*: 164.2 (q, C-2), 103.2 (t, C-3), 182.1 (q, C-4), 163.1 (q, C-5), 99.6 (t, C-6), 161.5 (q, C-7), 94.9 (t, C-8), 161.2 (q, C-9), 105.4 (q, C-10), 121.1 (q, C-1'), 128.8 (t, C-2'), 116.1 (t, C-3'), 157.1(q, C-4'), 116.1 (t, C-5'), 128.8 (t, C-6'), 100.0 (t, C-1''), 73.2 (t, C-2''), 77.1 (t, C-3''), 69.6 (t, C-4''), 76.5 (t, C-5''), 60.7 (s, C-6'').

Luteolin-7-O-*β*-D-glucopyranoside (S2): Yellow powder; ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 6.43 (d, *J* = 2.0 Hz, H-6), 6.76 (d, *J* = 2.0 Hz, H-8), 6.78 (s, 3-H), 7.42 (d, *J* = 2.0 H-2'), 6.89 (d, *J* = 8.0 Hz， H-5'), 7.45 (dd, *J* = 8.0, 2.0 Hz, H-6'), 12.3 (s, 5-OH), 5.02 (d, *J* = 7.0 Hz, H-1''), 3.01 (dd, *J* = 7.0, 6.0 Hz, H-2''), 3.19 (dd, *J* = 7.0, 6.0 Hz, H-3''), 3.04 (m, H-4''), 3.12 (m, H-5''), 3.61 (dd, *J* = 11.5, 2.0 Hz, H-6''a), 3.40 (dd, *J* = 11.5, 2.0 Hz, H-6''b). ^{13}C-NMR (DMSO-d_6, 500 MHz) *δ*: 164.7 (q, C-2), 103.4 (t, C-3), 182.1 (q, C-4), 163.1(q, C-5), 99.7 (t, C-6), 161.3 (q, C-7), 94.9 (t, C-8), 157.1 (q, C-9), 105.5 (q, C-10), 121.6 (q, C-1'), 113.8 (t, C-2'), 145.9 (q, C-3'), 150.1(q, C-4'), 116.2 (t, C-5'), 119.4 (t, C-6'), 100.0 (t, C-1''), 73.3 (t, C-2''), 76.6 (t, C-3''), 69.7 (t, C-4''), 77.3 (t, C-5''), 60.8 (s, C-6'').

α-Adenosine (S3): White needles crystals; ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 8.33 (s, H-2), 8.10 (s, H-8), 7.31(d, NH_2), 5.85 (d, *J* = 4.5 Hz, H- 1'), 4.61 (dd, *J* = 10. 0, 6.5 Hz, H-2'), 4.13 (m, H-3'), 3.95(m, H-4'), 3.66 (m, H-5'a), 3.55 (m, H-5'b). ^{13}C-NMR (DMSO-d_6, 500 MHz) *δ*: 152.6 (t, C-2), 149.3 (q, C-4), 119.7 (q, C-5), 156.3 (q, C-6), 140.2 (t, C-8), 88.2 (t, C-1'), 73.7 (t, C-2'), 71.2 (t, C-

Table 1. Anti-inflammatory effects of the different doses of EECB on carrageenan-induced hind paw edema in rats (n = 8).

Group	Dose (p.o., mg/kg)	Volume of edema (ml) by hour			
		1	3	5	10
G1		1.16 ± 0.17	1.79 ± 0.20	2.43 ± 0.25	2.67 ± 0.28
G2	30	1.20 ± 0.21	1.63 ± 0.17*	1.77 ± 0.23**	1.83 ± 0.21***
G3	100	1.18 ± 0.41	1.76 ± 0.21	2.31 ± 0.19*	2.11 ± 0.18**
G4	200	1.20 ± 0.24	1.52 ± 0.24*	2.04 ± 0.18**	1.51 ± 0.14***
G5	300	0.92 ± 0.09*	1.43 ± 0.17**	1.52 ± 0.30***	1.80 ± 0.22***

*p < 0.05 compared with negative control; **p < 0.01 compared with negative control; ***p < 0.001 compared with negative control.

Table 2. Anti-inflammatory effects of the different doses of EECB on albumin-induced -induced hind paw edema in rats (n = 8).

Group	Dose (p.o., mg/kg)	Volume of edema (ml) by hour			
		1	2	3	4
G1	-	1.33 ± 0.20	1.57 ± 0.33	1.89 ± 0.25	1.75 ± 0.31
G2	30	1.27 ± 0.15	1.26 ± 0.17*	1.42 ± 0.33**	1.08 ± 0.27**
G3	100	1.22 ± 0.31	1.42 ± 0.20	1.75 ± 0.24	1.46 ± 0.21*
G4	200	1.20 ± 0.18	1.38 ± 0.21	1.59 ± 0.12*	1.16 ± 0.23**
G5	300	1.09 ± 0.22*	1.05 ± 0.18**	1.29 ± 0.13***	1.03 ± 0.18**

*p < 0.05 compared with negative control; **p < 0.01 compared with negative control; ***p < 0.001 compared with negative control

3'), 86.3 (t, C-4'), 62. 2 (s, C-5').

Uridine (S4): White needles crystals; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 8.29 (s, NH), 5.61 (d, J = 7.8 Hz, H-5), 7.86 (d, J = 7.8 Hz, H-6), 5.36 (d, J = 7.5 Hz, H-1'), 4.41 (dd, J = 10. 0, 6.5 Hz, H-2'), 4.20 (m, H-3'), 3.75 (m, H-4'), 3.60 (m, H-5'a), 3.51 (m, H- 5'b). ^{13}C-NMR (DMSO-d6, 500MHz) δ: 150.5 (q, C-2), 163.0 (q, C-4), 101.3 (t, C-5), 140.3 (t, C-6), 87.6 (t, C-1'), 69.9 (t, C-2'), 73.5 (t, C-3'), 84.8 (t, C-4'), 60.8 (t, C-5').

Acute toxicity

The results of acute toxicity test showed that there is no mortality and LD_{50} values are more than 3000 mg/kg.

Anti-inflammatory activity

The results of anti-inflammatory activities of the EECB with carrageenan and egg-albumin in rats are shown in Tables 1 and 2.

As is shown in Table 1, the EECB had significantly anti-inflammatory effect at 100, 200 and 300 mg/kg observable to 10 h ($p < 0.01$), 5 h ($p < 0.01$) and 3 h ($p < 0.01$), respectively. The results (Table 2) showed that the EECB caused a dose dependent and significant inhibition of increase in paw edema.

DISCUSSION

The experimental study disclosed that the EECB was provided with significant anti-inflammatory activity at a dose of 300 mg/kg in experimental animals. On the carrageenan-induced paw edema experiment, at the doses used the EECB (300 mg/kg) after 5 h ($p < 0.001$) showed significant anti-inflammatory effects. Moreover, on the egg-albumin-induced inflammation experiment, at the doses used the EtOAc extract (300 mg/kg) after 3 h ($p < 0.001$) possessed significant anti-inflammatory effects. The results were more important than that of luteolin (standard drug).

According to the HPLC isolation of the EECB, there are two major classes of compounds, flavonoids and alkaloids. Flavonoids (apigenin-7-*O*-*β*-*D*-glucopyranoside and luteolin-7-O-*β*-D-glucopyranoside) involved in the late phase of acute inflammation and pain perception (Morimoto et al., 1988). Furthermore, alkaloids including adenosine and uridine were isolated from many plants possessing the significant pharmacological activities including anti-inflammatory, analgesic, antibacterial and anticancer effect (Bai et al., 2013; Meng et al., 2003). Furthermore, different phytochemicals produces have

been found to have a broad range of activities, which may help in protection against chronic diseases (Rahman et al., 2012). These compounds are known to be biologically active. Alkaloids are one of the largest groups of phytochemicals in plants, which have amazing effects on humans and have led to the development of powerful pain killer medications (Da Silva et al., 2010). It should be noted that the anti-inflammatory activities of many plants have been attributed to their alkaloids or flavonoid contents (Bai et al., 2013; Meng et al., 2003; Morimoto et al., 1988). Accordingly, the existence of flavonoids and alkaloids may be a contributing factor to the EECB's anti-inflammatory activity.

The pathogenesis of fever involves several mediators or multi process. Inhibition of any of these mediators may lead to antipyresis (El-Shenawy et al., 2002). The reliable nature of the anti-inflammatory mechanisms of flavonoids and alkaloids from EECB has not been clarified, but the present study's results were confirmed from the popular use of this plant in the treatment of inflammatory diseases. These studies are valuable for identifying lead compounds for anti-inflammatory drugs, bearing in mind the side-effects of synthetic and chemical medicines. In addition, human studies are needed to demonstrate the efficacy and safety of EECB in the long-term management of potential anti-inflammatory agents in everyday clinical practice.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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