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Anti-inflammatory effects of *Solanum procumbens* on a low dose complete Freund´s adjuvant-induced arthritis rat model

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ABSTRACT

Objective: To investigate the anti-inflammatory and analgesic effects of *Solanum procumbens* on complete Freund's adjuvant-induced arthritis rat models.

Methods: We isolated and identified five compounds in the ethanol-soluble *Solanum procumbens* extract (SP) with anti-inflammatory effects, including ursolic acid, β-sitosterol, hexadecanoic acid, *cis*-vaccenic acid, and vanillic acid. Additionally, we investigated the anti-inflammatory effects of SP on rheumatoid arthritis symptoms, including paw volumes, local temperatures, withdrawal latency, and mechanical withdrawal threshold at the hind paw and white blood cell (WBC) number from complete Freund's adjuvant-induced arthritis rat models.

Results: We have successfully established a complete Freund's adjuvant-induced arthritis rat model at a low dose (1 mg/mL). SP extract significantly reduced paw volumes (P<0.05), prolonged withdrawal latencies (P<0.05), decreased local temperature, and increased the mechanical withdrawal threshold (P<0.05), but only SP extract at the dose of 300 mg/kg significantly decreased WBC numbers.

Conclusions: SP extract could be a potential medication candidate with anti-inflammatory effects for arthritis, but it requires further investigation into the mechanism of the SP and its effectiveness on other models as well as clinical trials.

KEYWORDS: *Solanum procumbens*; Arthritis; Anti-inflammation; Complete Freund's adjuvant; Rats

Significance

Solanum procumbens is used widely as a traditional herb in Vietnam to treat low back pain and joint pain. This study isolated and identified five compounds including ursolic acid, β-sitosterol, hexadecanoic acid, *cis*-vaccenic acid, and vanillic acid, and shows the anti-inflammatory of *Solanum procumbens* fractionated extract on a complete Freund's adjuvant-induced arthritis rat model. Another remarkable result of this study is the success of using a low dose of complete Freund's adjuvant (1 mg/mL) to establish the arthritis model in rats.

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1. Introduction

Rheumatoid arthritis (RA) is a common disease and usually causes joint inflammation and pain in patients[1]. The painful joint could affect the patient's movement and cause weight gain and other health problems. Up to now, disease-modifying antirheumatic drugs, non-steroidal anti-inflammatory drugs, steroid hormones, *etc*., have been used for RA treatment[2]. Despite these drugs could reduce transient inflammation and disease progression, they do not significantly improve the outcome of diseases in the long term[3]. Furthermore, serious side effects such as gastrointestinal ulcers, cardiovascular adverse effects, and renal disturbances appear in patients who receive long-term treatment with non-steroidal anti-inflammatory drugs[4–6]. Additionally, 27% of patients have characteristics of hepatotoxicity, nephrotoxicity, anemia, and gastrointestinal disorders after using disease-modifying antirheumatic drugs[7]. Thus, it requires an alternative, effective, and safe drug for RA treatment.

Traditional herbal medicine has been used for RA treatment for a long time[8,9]. The mechanisms of conventional medications include alleviating pain, antiinflammation, immunoregulation, and modulating angiogenesis and osteoclasts[10,11]. It has been reported that the therapeutic effects of traditional herbal extracts are attributed to their ingredients of flavonoids, phenolic acids, alkaloids, and triterpenes[8,9]. Therefore, active components of different herbal extracts that are potential medications for RA treatment have been investigated[8,9]. *Solanum procumbens* (*S. procumbens*) belongs to the Solanaceae family and is often cultivated in some tropical countries, including China, Laos, Cambodia, and Vietnam. *S. procumbens* is used to treat hepatitis, cirrhosis, low back pain, and joint pain and it may be the potential medication for arthritis treatment, such as RA.

RA model by injection of complete Freund's adjuvant (CFA) into rodents, which display symptoms similar to human RA, has been used widely[12]. In this animal model, signs of acute inflammation and hyperalgesia around the injection sites are observed one hour after the injection and continue in the first week. From the 10th to 15th day, animals show inflammation and pro-nociceptive signs, especially in the contralateral paw. Hyperalgesia associated with CFA injection lasts for eight weeks after CFA injection[13,14]. In this study, we aimed to isolate and identify compounds in *S. procumbens*, then investigate *in vivo* effects on the RA model. The effects of *S. procumbens* extract (SP) were evaluated based on the inflammation, pro-nociceptive, and hyperalgesia signs.

2. Materials and methods

2.1. Plant material

The whole plant of *S. procumbens* was collected at Tinh Bien District, An Giang Province, Southern Vietnam, in October 2016 and deposited ((DMC-9002) at the Department of Medicinal Chemistry, Faculty of Chemistry, VNUHCM-University of Science, Ho Chi Minh City, Vietnam. The plant was identified by Dr. Anh Tuan Dang-Le (VNUHCM-University of Science, Ho Chi Minh City, Vietnam).

2.2. Extraction and isolation

The whole dried plant of *S. procumbens* L. (4 kg) was processed using a Soxhlet extractor with *n*-hexane (10 L), EtOAc (10 L), and MeOH to yield soluble extracts of *n*-hexane (31.9 g), EtOAc (56.3 g), and MeOH (115.2 g), respectively. Furthermore, crude material plant (400 g) was also processed in a Soxhlet extractor with ethanol (4 L) to yield an ethanol-soluble extract (40 g) for the behavioral experiment.

The EtOAc-soluble extract was subjected to silica gel column chromatography with a MeOH-$CHCl_3$ gradient system (0%-80%) to yield 17 fractions: fraction 1 (7.3 g), fraction 2 (2.1 g), fraction 3 (2.8 g), fraction 4 (1.5 g), fraction 5 (0.4 g), fraction 6 (1.6 g), fraction 7 (1.8 g), fraction 8 (1.1 g), fraction 9 (2.4 mg), fraction 10 (1.7 g), fraction 11 (6.3 g), fraction 12 (4.6 g), fraction 13 (4.6 g), fraction 14 (4.3 g), fraction 15 (4.4 g), fraction 16 (5.3 g), and fraction 17 (2.4 g). Fraction 2 was chromatographed further using an acetone-*n*-hexane gradient system (0%-80%) to afford 12 sub-fractions (fraction 2.1: 136 mg; fraction 2.2: 186 mg; fraction 2.3: 185 mg; fraction 2.4: 147 mg; fraction 2.5: 125 mg; fraction 2.6: 367 mg; fraction 2.7: 212 mg; fraction 2.8: 49 mg; fraction 2.9: 162 mg; fraction 2.10: 120 mg; fraction 2.11: 66 mg; and fraction 2.12: 242 mg). Sub-fractions of 2.1 and 2.2 were subjected to silica gel column chromatography, eluted with $CHCl_3$-*n*-hexane gradient mixtures (0%-80%) to isolate Compound 3 (43.2 mg) and Compound 4 (35.7 mg), respectively. Fraction 3 was separated by silica gel column chromatography with a $CHCl_3$-*n*-hexane gradient system (0%-80%) to yield 8 sub-fractions (fraction 3.1: 68 mg; fraction 3.2: 508 mg; fraction 3.3: 928 mg; fraction 3.4: 197 mg; fraction 3.5: 160 mg; fraction 3.6: 367 mg; fraction 3.7: 260 mg; and fraction 3.8: 130 mg). Sub-fraction 3.3 was applied to silica gel column chromatography with an EtOAc-*n*-hexane gradient mixture (0%-50%) to afford Compound 2 (63.4 mg) and Compound 5 (26.2 mg). Sub-fraction 3.5 was again rechromatographed on silica gel column chromatography with an isopropanol-*n*-hexane gradient system (0%-50%) to obtain Compound 1 (36.8 mg). Then, ^{1}H- and ^{13}C-nuclear magnetic resonance spectra of Compounds 1-5 were analyzed to obtain their chemical shifts using a Bruker AVANCE III HD 500 MHz spectrometer operating at 500.20 and 125.79 MHz for ^{1}H- and ^{13}C-nuclei, respectively.

2.3. Animals

A total of 60 Wistar rats (body weights 200-230 g) were used to create the RA model. Animals were housed in a room with controlled temperature (24℃) and 12 h light/dark cycles. They were fed in individual cages with no limitation on food and water. Animals were randomly divided into 6 groups, including non-arthritis and vehicle treatment (Control, *n*=10), CFA-induced arthritis without treatment

(Arthritis, n=10), CFA-induced arthritis and treatment with Mobic 4 mg/kg (Standard, n=10), CFA-induced arthritis and treatment with SP 100 mg/kg (SP 100, n=10), CFA-induced arthritis and treatment with SP 200 mg/kg (SP 200, n=10), and CFA-induced arthritis and treatment with 300 mg/kg (SP 300, n=10).

2.4. Ethics statement

All experimental protocols involving animals were approved by the Animal Care and Use Committee of Vietnam Military Medical University, approval number: IACUC-1256/QĐ-HVQY, dated on 26th March 2021. All animals were cared in accordance with the Institutional Ethical Guidelines which follow the recommendation of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH publication No 86-23 revised 1985). All procedures complied with the institutional guideline for animal care and use at Vietnam Military Medical University.

2.5. Induction of animal arthritis models and treatments

Rats were anesthetized with ketamine (Sigma Aldrich) at 20 mg/kg. Based on previously reported papers, a rat model of arthritis was established[12,13]. A total of 50 μL CFA (1 mg/mL, Sigma Aldrich) was subcutaneously injected into the rats' plantar surface (*i.p.*) of the right hind paw. In the control group, the rats were injected with the same volume of saline. Ethanol-soluble extracts of SP (see above) dissolved in saline (0.2 mL/kg) were administrated orally and daily for 21 d, starting the same day after the CFA injection. In the standard and control groups, the animals received oral administration of similar volumes of Mobic or saline. The body weight of all rats was measured before, during the second week, and the fourth week after treatments.

2.6. Paw volumes

Volumes of the right hind paws at the injected site of all rats were measured using a water displacement plethysmometer (UGO BASIL, Italy) the day before the injection of CFA or saline into the hind paw and every three days for 24 d after injection. The paw volumes at the time point before arthritis induction was used as the control volume.

2.7. Heat avoidance test

Heat avoidance test measures avoidance thresholds in response to nociceptive thermal stimuli in rats[15]. Briefly, the animals were placed in a plastic chamber with a glass floor. They were allowed to habituate for 5 min before the behavior testing. Then, a light heat source was placed under the glass floor beneath the hind paw in the injected site. The intensity of the heat stimuli was adjusted to produce withdrawal response latencies of 6 s in naive animals. Withdrawal durations of the rats in all groups were recorded. Each animal was tested two times at 5-min intervals on each testing day. The mean value of the two measurements was used to analyze. The testing was conducted the day before the CEA injection into the paw and every three days for 24 d after the injection.

2.8. Local temperature of the injected sites

The animals were held by the hands of researchers in a room with controlled temperatures. The temperatures of the injected sites of the rats were measured using an infrared thermometer. The temperatures were measured on the day before the CEA injection day and every three days for 24 d after the injection.

2.9. Mechanical pain thresholds

Mechanical pain thresholds of the rats were measured using an Analgesy-Meter (UGO BASIL)[16]. The pressure force from a cone-shaped pusher with a rounded tip was applied to the inflammation-induced paw of the animals. The force was gradually increased to induce animals' paw withdrawal. The test was repeated three times on each testing day, and mean forces inducing paw withdrawal were calculated each day for animals on the day before the CEA injection day and every three days for 24 d after the injection.

2.10. White blood cell (WBC) counts

One week after the injection (Day 7), animals were anesthetized with ketamine (75 mg/kg, *i.m.*) and then 2 mL of blood was collected from the tail vein. Numbers of WBC were measured using an auto Hematology Analyzer (XN1000, Sysmex, Japan).

2.11. Statistical analysis

Data were reported as mean ±SD. Two-way ANOVA was used to analyze the statistics. Bonferroni tests were used for multiple comparations. P<0.05 was considered as the significant difference. Data analyses were performed using STATA software version 12.0.

3. Results

3.1. Compounds in the SP extract

The data of the ^{1}H- and ^{13}C-NMR chemical shifts for Compounds 1 and 2 in the $CDCl_3$ solution are shown in Table 1. The data for Compounds 3 and 4 in the $CDCl_3$ solution are shown in Table 2, while the data for Compound 5 in the CD_3COCD_3 solution are in Table 3.

Based on the analysis of the NMR spectroscopic data and comparison with previous literature, these compounds were identified as ursolic acid (Compound **1**) (9), β-sitosterol (Compound **2**) (10), hexadecanoic acid (Compound **3**) (9), *cis*-vaccenic acid (Compound **4**) (11), and vanillic acid (Compound **5**) (12) (Figure 1).

Table 1. ^{1}H- and ^{13}C-NMR spectroscopic data for ursolic acid and β-sitosterol in $CDCl_3$.

Position	Ursolic acid		β-Sitosterol	
	δH	δC	δH	δC
1		38.6		37.4
2		27.2		31.9
3	3.16 (dd, 9.7 & 5.8)	79.1		72.0
4		38.8		42.5
5		55.3		140.9
6		18.3	5.35 (d, 5.3)	121.9
7		33.0		32.1
8		39.5		32.1
9		47.6		50.3
10		37.0		36.7
11		23.3		21.3
12	5.20 (t, 3.2)	125.8		39.9
13		138.0		42.5
14		42.0		56.9
15		29.4		26.3
16		24.2		28.4
17		47.9		56.2
18		52.7	1.01 (s)	12.0
19		39.1	0.69 (s)	18.9
20		38.9		36.3
21		30.6	0.92 (d, 6.5)	19.2
22		36.7		34.1
23	0.93 (s)	28.2		26.3
24	0.73 (s)	15.5		46.0
25	0.87 (s)	15.6		29.4
26	0.76 (s)	17.1	0.83 (d, 6.6)	20.0
27	1.04 (s)	23.6	0.81 (d, 6.9)	19.6
28		181.1		23.3
29	0.81 (d, 6.4)	17.0	0.85 (t, 7.3)	12.1
30	0.90 (d, 6.2)	21.2		

Table 2. ^{1}H- and ^{13}C-NMR spectroscopic data for hexadecanoic acid and *cis*-vaccenic acid in $CDCl_3$.

Position	Hexadecanoic acid		*cis*-Vaccenic acid	
	δH	δC	δH	δC
1		179.2		179.9
2	2.35 (t, 7.5)	33.7	2.33 (t, 7.5)	34.2
3	1.63 (p, 7.5)	32.1	1.61 (p, 7.5)	32.1
4		29.9		29.8
5		29.8		29.8
6		29.7	5.35 (d, 5.3)	29.7
7		29.6		29.6
8		29.5		29.5
9		29.3		29.3
10		29.3	2.02 (m)	29.3
11		29.2	5.34 (m)	130.2
12		29.2	5.34 (m)	129.9
13		29.2	2.02 (m)	29.4
14		24.8		29.2
15		22.8		29.2
16	0.88 (t, 7.0)	14.3		24.8
17				22.8
18			0.87 (t, 7.0)	14.2

Table 3. ^{1}H- and ^{13}C-NMR spectroscopic data for vanillic acid in CD_3COCD_3.

Position	Vanillic acid	
	δH	δC
1		124.9
2	7.56 (d, 1.8)	113.7
3		152.1
4		148.2
5	6.91 (d, 8.2)	115.6
6	7.59 (dd, 8.2 & 1.8)	123.1
1-COOH		167.5
3-OCH_3	3.90 s	56.5

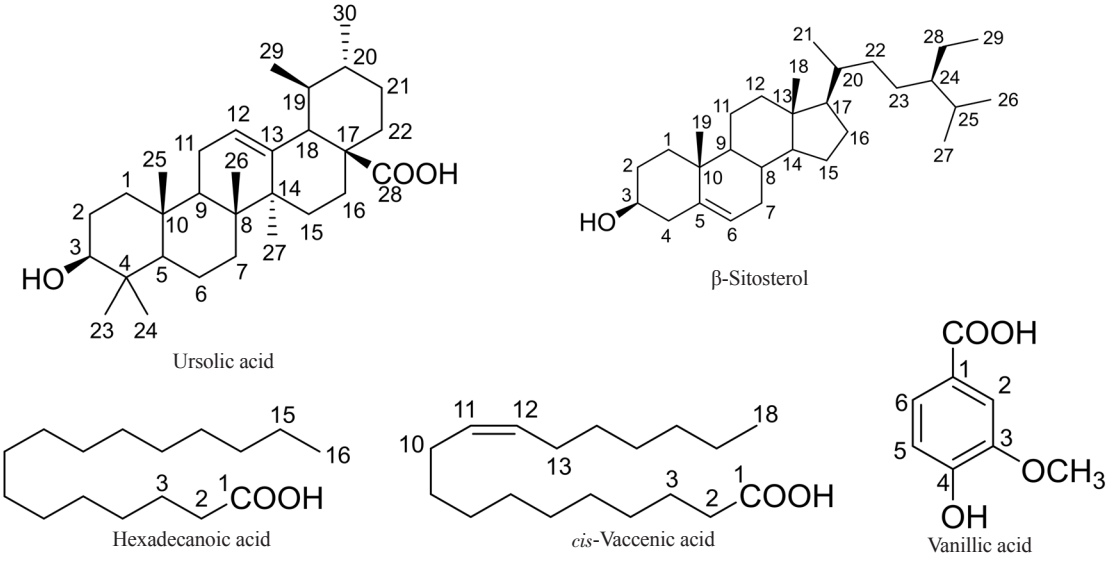

Figure 1. Chemical structures of the 5 compounds isolated from *Solanum procumbens* extract (SP). Chemical structure of ursolic acid, β-sitosterol, hexadecanoic acid, *cis*-vaccenic acid, and vanillic acid.

3.2. Body weights

The comparisons indicated that mean body weights at the 2nd week and 4th week after treatments were significantly higher than those before in all groups (P<0.01 and P<0.001). Furthermore, there were no significant differences in mean body weights between groups before treatments. In the 2nd week after treatments, mean body weights in SP 300 were significantly higher than in the control group (P<0.05). In the 4th week after treatments, the mean body weight in the arthritis group was significantly lower than in the control group (P<0.05). In contrast, mean body weights in SP 200 and SP 300 groups were significantly higher than those in the arthritis group (P<0.01 and P<0.001) (Table 4).

3.3. Paw volumes

Two-way ANOVA indicated that there was a significant interaction between the group and day [$F_{(40, 540)}$=6.375, P<0.001]. Furthermore, there were significant main effects of group [$F_{(5, 540)}$=91.416, P<0.001] and day [$F_{(8, 540)}$=93.777, P<0.001]. *Post hoc* comparisons indicated that paw volumes were significantly increased in the arthritis group compared to the control group from day 3 to day 21 (P<0.05) (Figure 2A). The paw volumes were significantly decreased in all three RA groups treated with SP (P<0.05) compared to the arthritis group from day 6 to day 21. Additionally, paw volumes of the SP 200 and SP 300 groups were smaller than those of the SP 100 group from day 6 to day 18 (P<0.05).

Table 4. Effects of *Solanum procumbens* extract (SP) extract on percentage changes in body weights (%).

Groups	2nd week	4th week
Control	10.4±4.9	22.3±9.6
Arthritis	2.3±1.9$^{\#}$	6.3±3.1$^{\#}$
SP 100	8.7±6.1	16.8±4.4
SP 200	8.7±3.8	17.2±8.4**
SP 300	11.5±7.4$^{\#}$	20.5±8.0**
Standard	12.0±5.2$^{\#}$	22.2±5.7***

Arthritis: complete Freund's adjuvant (CFA)-induced arthritis without treatment; Standard: CFA-induced arthritis and treatment with Mobic 4 mg/kg; SP 100: CFA-induced arthritis and treatment with SP 100 mg/kg; SP 200: CFA-induced arthritis and treatment with SP 200 mg/kg; SP 300: CFA-induced arthritis and treatment with SP 300 mg/kg. $^{\#}P$<0.05 compared with the control group; $^{**}P$<0.01, $^{***}P$<0.001 compared with the arthritis group.

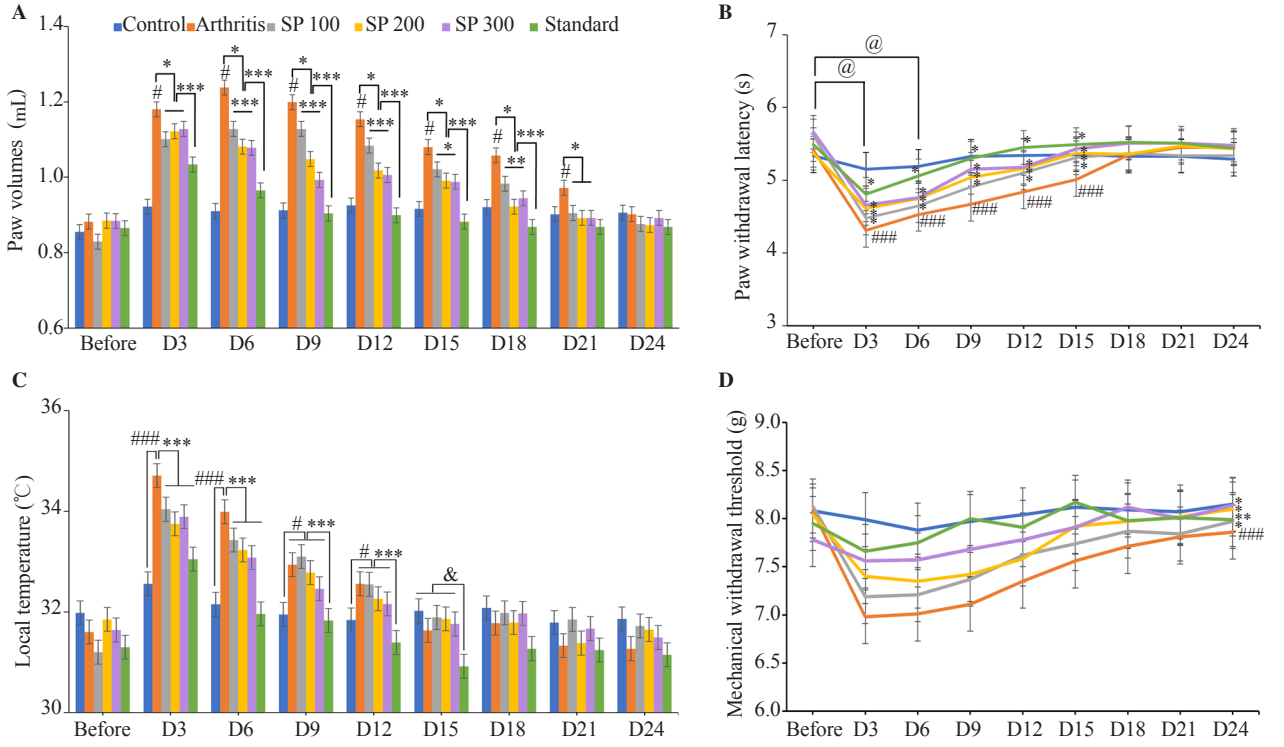

Figure 2. Effects of SP extract on paw volumes (A), heat paw withdrawal latency (B), local temperature at the complete Freund's adjuvant (CFA) injection sites (C), and mechanical withdrawal threshold (D). Arthritis: CFA-induced arthritis without treatment; Standard: CFA-induced arthritis and treatment with Mobic 4 mg/kg; SP 100: CFA-induced arthritis and treatment with SP 100 mg/kg; SP 200: CFA-induced arthritis and treatment with SP 200 mg/kg; SP 300: CFA-induced arthritis and treatment with SP 300 mg/kg. $^{@}P$<0.001 compared with that before treatment; $^{\#}P$<0.05, $^{\#\#\#}P$<0.001 compared with the control group; $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 compared with the arthritis group; $^{\&}P$<0.05 compared with the standard group. Before: the day before the injection of CFA or saline into the hind paw.

3.4. Heat avoidance latencies

Considering that inflammatory tissues are more sensitive to heat stimuli, thus heat avoidance latencies were performed on the animals' paws at the injected sites. Data showed that there was no significant interaction between the group and day [$F_{(40, 540)}$=0.421, P>0.05]. However, there were significant main effects of group [$F_{(5, 540)}$=2.656, P<0.05] and day [$F_{(8, 540)}$=9.581, P<0.001]. The withdrawal latencies were significantly shorter on day 3 and day 6 than before treatments in all groups (P<0.001). The mean value of withdrawal latencies was significantly lower in the arthritis group than in the control group from day 3 to 15 (P<0.05) and SP treatment significantly prolonged withdrawal latencies (Figure 2B). There was no difference in withdrawal latency between the control and SP treatment groups.

3.5. Local temperature of the hind paws

Two-way ANOVA showed that there was a significant interaction between the group and day [$F_{(40, 540)}$=2.639, P<0.001]. Furthermore, there were significant main effects of group [$F_{(5, 540)}$=16.797, P<0.001] and day [$F_{(8, 540)}$=60.089, P<0.001]. After CFA injection, the local temperature at the injection sites was significantly higher in the arthritis group than in the control group on days 3-9 (P<0.05), and SP treatment lowered temperature significantly on days 3 and day 6 (P<0.001). On days 9 and 12, the temperatures of the SP treatment groups were significantly higher than the control group (P<0.05) (Figure 2C).

3.6. Mechanical paw withdrawal thresholds

Two-way ANOVA indicated that there was no significant interaction in withdrawal thresholds between the group and day [$F_{(40, 540)}$=0.365, P>0.05]. However, there were significant main effects of day [$F_{(8, 540)}$=5.145, P<0.001] and group [$F_{(5, 540)}$=4.886, P<0.001]. Moreover, the mean of withdrawal thresholds in the arthritis group at all time points was significantly lower than in the control group (P<0.001), and SP treatment significantly increased the threshold (P<0.05) (Figure 2D).

3.7. Number of WBC

Data showed that there was a significant main effect of the group [$F_{(5, 59)}$=5.936, P<0.001). Additionally, the WBC number was higher in the arthritis group than in the control group (P<0.01), indicating inflammation in the RA rat models. Treatments with SP (300 mg/kg) significantly decreased WBC numbers compared to the arthritis group (P<0.05) (Figure 3).

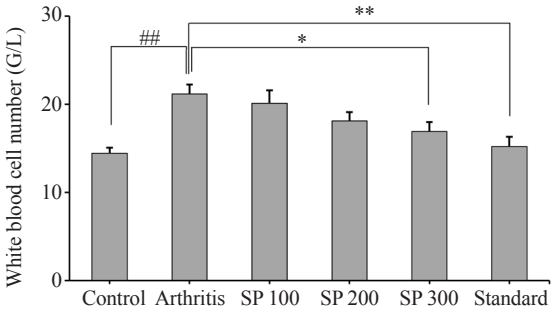

Figure 3. Effects of SP extract on white blood cell number. $^{\#\#}P$<0.01 compared with the control group; $^{*}P$<0.05, $^{**}P$<0.01 compared with the arthritis group.

4. Discussion

In the present study, we detected five main compounds in SP, including ursolic acid (Compound **1**), β-sitosterol (Compound **2**), hexadecanoic acid (Compound **3**), *cis*-vaccenic acid (Compound **4**), and vanillic acid (Compound **5**). Previous studies reported that these compounds have anti-inflammatory effects. For example, ursolic acid, which is included in various vegetables, could suppress acute and chronic inflammation in arthritis models of rodents[17]. Furthermore, ursolic acid decreased the expression of various pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and inducible nitric oxide synthase (an oxidative stress marker), in a mouse arthritis model through suppression of Th17 and B cell differentiation[18]. Additionally, β-sitosterol, which is found in various vegetable oils and nuts, was reported to attenuate inflammatory signaling related to TNF-α and nuclear factor-kappa beta (NF-κB) in human aortic endothelial cells[19,20] and the expression of inflammatory mediators (IL-6, inducible nitric oxide synthase, TNF-α, and cyclooxygenase-2) by inhibiting lipopolysaccharide-induced activation of ERK, p38 and NF-κB pathways in lipopolysaccharide-stimulated murine microglia[21]. Moreover, hexadecanoic acid (palmitic acid), a fatty acid observed naturally in animals and plants, was suggested to have anti-inflammatory activity by inhibiting phospholipase A_2[22]. Finally, *cis*-vaccenic acid had anti-inflammatory effects[23], and vanillic acid reduced the expression of pro-inflammatory cytokines (IL-1β, IL-2, IL-4, IL-6, IL-12, IL-13) by inhibiting NF-κB signaling pathways in human osteoarthritic chondrocytes[24]. Thus, the SP extract may have anti-inflammatory effects due to these components.

Common signs of arthritis patients are redness, swelling, heat, and sometimes pain and loss of locomotor functions, especially in acute and sub-acute arthritis[25]. Herein, we have successfully established arthritis animal models with a low dose of CFA (1 mg/mL) injected into the plantar surface of the right hind paw of rats. The acute inflammation symptoms were presented as the increase in paw volumes, the local temperature at arthritis sites, and WBC numbers,

and the decrease of heat avoidance thresholds and mechanical withdrawal thresholds. Previous studies indicated the necessity of dose optimizing and the route of administration to establish a CFA-induced arthritis model in rats[13,26]. It is worth noting that the lower dosage of the drug used to produce a similar presentation of arthritis complications, the better for the animals. In a study by Noh *et al*.[13], they used the dose of 5 mg/mL, which they considered as the low dose but could not induce arthritis complications in rats by the same route of administration. The amount we used in this study was five times more minor, but the arthritis complications were presented and lasted until day 18, similar to the higher dose of 10 mg/mL in the reports of Noh[13] and others[27,28].

Furthermore, the SP extracts ameliorated CFA-induced arthritis-related symptoms in the rat model. Remarkably, SP extract decreased the paw volumes and increased mechanical withdrawal thresholds. Since the paw volume reflects a swelling in the joint, the SP extract helps to reduce the paw volume suggesting that this SP extract could reduce the inflammation. Previous studies reported that leukosis was observed in CFA-induced arthritis in animals and human rheumatoid arthritis[29]. Using the SP extract decreased the number of WBC to the normal value of the control. Interestingly, after four weeks, the bodyweight change in the SP treatment was insignificant compared to the control group. Meanwhile, the arthritis group showed the lowest body weight gained. The body weight change following time is an indicator of health status. This result means that treatment with SP extract could reduce RA rats' pain and maintain health status as the control. Our results were consistent with previous studies using herbal products, for example, green tea[30], curcumin[30], resveratrol[31], and other natural products, such as *Boswellia serrata*[32], *Rosa canina*[33], that have potent anti-inflammatory and analgesic effects in rat models of arthritis. It has been suggested that medical herbs have protective effects on RA symptoms by inhibiting signaling pathways involved in regulating inflammatory mediators, including mitogen-activated protein kinase, nitric oxide, NF-κB, IL-1, IL-6, TNF-α, cyclooxygenase, and lipoxygenase[34].

In the present study, there are some limitations. Firstly, the anti-inflammatory effects of SP extract were not assessed at cellular and molecular levels to reveal the mechanism of this plant. Secondly, histological images of local injection of CFA and concentrations of serum cytokines should be determined. Thirdly, acute inflammation effects induced by a single dose of CFA might be reduced after 24 d. Longer anti-inflammatory effects of SP extract should be followed to check about the other disorders after using this product. Nevertheless, these limitations have not affected results and conclusions of the present study. These limitations will be addressed in further work.

In conclusion, this study isolated and identified five compounds including ursolic acid, β-sitosterol, hexadecanoic acid, *cis*-vaccenic acid, and vanillic acid, and showed the anti-inflammatory effects of SP extracts on a CFA-induced RA rat model. The highest SP dose of 300 mg/kg has a more significant impact on reducing inflammation symptoms. The results suggest that SP extract could be a potential anti-inflammation medication. Further work should be done to investigate these compounds for treating arthritis diseases in humans.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

XHD performed experiments, data acquisition, data analysis, and manuscript writing. TNN performed experiments, data acquisition, and data analysis. TCD collected experimental data and participated in revising the manuscript. TTMN, TNN, VNTD, HTL, XHN, GLN, DKH and HPD contributed to the experimental design, and data analysis, and participated in completing the relevant experiments. XHD, VMC, and VQL contributed to the design of the study and wrote the original manuscript. XHD, VMC, and VQL confirmed the authenticity of all raw data and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

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