

## COMPARATIVE EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF VARIOUS MEDICINAL PARTS OF *CAPPARIS SPINOSA*: A CONSIDERATION OF ECOLOGICAL ENVIRONMENT AND RESOURCE CONSERVATION

Hong-Juan Liu<sup>1</sup>, Tao Yang<sup>1</sup>, Xue-Mei Cheng<sup>1,2\*</sup>, Chang-Hong Wang<sup>1,2\*</sup>

<sup>1</sup>The Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, The MOE Key Laboratory for Standardization of Chinese Medicines and The SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, 1200 Cailun Road, Zhangjiang Hi-Tech Park, Shanghai 201210, People's Republic of China.

<sup>2</sup>Shanghai R&D Centre for Standardization of Chinese Medicines, 199 Guoshoujing Road, Shanghai 201210, People's Republic of China.

### ABSTRACT

*Capparis spinosa* (CS) (Capparidaceae) is wide distribution in west or central Asia and spreading particularly across the Mediterranean basin. CS fruits, stem-leaves and roots are used in folk medicine due to their anti-inflammatory and analgesic effects. Excessive use of roots will lead to CS resources exhausting and environmental disruption. To evaluate the potency of the extracts from CS fruits, stem-leaves and roots on anti-inflammatory and analgesic activities is very urgent and important in consideration of ecology environment and resource conservation. The present results indicated that CS fruits and stem-leaves at three doses (3.86, 7.72 and 15.44 g/kg) showed significant inhibitions ( $P < 0.01$ ) on acetic acid-induced vascular permeability and xylene-induced ear edema with a dose dependent, CS roots, meanwhile, only at high dose or not. The CS stem-leaves and fruits produced a significant analgesic effects induced by acid-induced writhing response with a dose dependent ( $P < 0.01$ ) and prolonged the hot-plate latency of mice at high dose with a time dependent ( $P < 0.05$ ), but the CS roots only produce a weak response at high dose. The present study infers that CS stem-leaves and fruits demonstrated remarkable anti-inflammatory and analgesic activities. It is suggested to make extensive use of the aerial parts (stem-leaves and fruits) of CS instead of the roots.

**Keywords:** Analgesia, *Capparis spinosa*, Fruits, Health functional food, Inflammatory, Roots, Stem-leaves.

### INTRODUCTION

*Capparis spinosa* L. (CS), Capparaceae family, is a perennial plant originating from dry regions in west or central Asia and spreading particularly across the Mediterranean basin [1]. It is an economically valuable plant that has medicinal and aromatic properties. The young shoots, flower buds, fruits and seeds of *C. spinosa* are used for food [2]. Capers are the flower buds of the caper plant and have been used as a seasoning since ancient times. Spain, Morocco, Italy and Turkey are the leading world producers; it is estimated that the world production of capers is ~10,000 tons per annum [3,4].

Also, the fruits of *C. spinosa* have a great deal of oil, rich with vitamin E, and can be used as a new source of edible oils [5].

In China, CS widely distributes in Xinjiang Uygur Autonomous Region, extensive in Turpan basin, it also grows scattered in the western Gansu, Tibet and other desert areas of China. The stem-leaves, fruits, and roots of CS have been widely used for the treatment of rheumatoid arthritis and gout in folk medicine in Xinjiang [6,7]. The root bark of CS, listed in the Uygur Drug Standard of Ministry of Public Health [8], has been used as a traditional folk medicinal substance with a long history for

treatment of rheumatics, gout, and chorionitis in Xinjiang Uygur Autonomous Region, China [9]. In addition to its medicinal application, it also plays a much important role in defending wind and consolidating sand.

Modern pharmacological studies have indicated that CS also exhibited hepatoprotective, hypolipidemic, and antioxidant activities [7,10,12]. CS fruits, stem-leaves and roots are used in certain therapeutic treatments including pain, and edema by traditional Chinese physicians. CS fruits and flowering buds have been studied for its anti-inflammatory and analgesic activities as well as anti-arthritic activity [1,12]. However, the anti-inflammatory and analgesic effects of all the portions of CS have not been studied and compared. Hence, in order to achieve the purpose of resources and environmental protection, the major aim of the present study was to determine which parts of CS are the optimal medicament portions by comparing the anti-inflammatory and analgesic activities of various portions of CS, including fruits, stem-leave and roots.

## MATERIALS AND METHODS

### Plant material and chemical reagents

The CS fruits, stem-leaves and roots were collected in July, 2007, from Turpan, Xinjiang Uygur Autonomous Region, China. The plant was identified by Prof. Chang-hong Wang and a voucher specimen was deposited at the Herbarium of the Shanghai R&D Centre for Standardization of Chinese Medicines, Shanghai, China. Aspirin was supplied by Shandong Xinhua Pharmaceutical Co., Ltd. Normal saline was supplied by Shanghai World best Treeful Pharmaceutical (Group) Co., Ltd. Evans blue, glacial acetic acid, and xylene were analytical grad reagent from market, Shanghai, China.

### Preparation and constituent's identification of individual extracts of CS

The powdered CS fruits, stem-leaves and roots (1.0 kg for each) were separately extracted successively three times with 8 L of 85% ethanol under reflux, and the combined extract were concentrated under vacuum at 50°C and then dried by lyophilization to afford fruits residue (209 g), stem-leaves residue (150 g), and roots residue (110 g), respectively. The extract yields from CS fruits, stem-leaves, and roots with respect to original dry plant material were 20.9, 15, and 11%, respectively.

A previous valuated HPLC method [18] was used to identify the constituents from individual extracts of CS fruits, stem-leaves, and roots.

### Drug administration

The ethanol extracts of CS fruits, stem-leaves, and roots suspended in water were orally administered at a dose of 3.86, 7.72, and 15.44 g/kg body weight (BW) with respect to original dry plant material. Aspirin dissolved in distilled water was orally administered at a dose of 0.08

g/kg BW. The plant extracts or aspirin was administered orally for three consecutive days daily. The animals of control group received normal saline as the same experimental handling as those of the test groups. Mice were given orally in a volume of 0.2 ml/10g BW with appropriate volumes of the dosing vehicle.

### Animals

All experiments were performed with either sex KM mice, weighing 18-22 g, obtained from the Experimental Animal Center, Shanghai University of Traditional Chinese Medicine, China. Animals were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2°C and 12-h light/dark cycle) with free access to standard laboratory food and water *ad libitum*. All animals were fasted for 12 h but with access to water prior to the administration of the test drugs. Animal welfare and experimental procedures were carried out in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals in China. Groups each with 10 animals were used in all tests.

### Xylene-induced ear edema

The xylene-induced ear edema test was performed according to the method of Hossein zadeh *et al* [19]. In the experiment, the tested extracts of CS fruits, stem-leaves, and roots were taken for three consecutive days. The tested samples including aspirin as a positive-control were given orally to the mice. At the third drug administration day, thirty minutes after oral administration, each animal received 20 µl of xylene on the anterior and posterior surfaces of the left ear lobe and the right ear was considered as control. The mice were sacrificed by cervical dislocation 20 minutes later and both ears were cut. Circular sections were taken using a cork borer with a diameter of 6 mm and weighed. The percentage of ear edema was calculated based on the weight increase of the left ear *versus* the right ear without treatment by xylene.

### Acetic acid-induced vascular permeability

According to the described method [20], at the third drug administration day, 1h after the oral administration of test samples (aspirin as positive-control drug), the female mice were intravenously injected with 0.1 ml/10g body weight of 0.5% Evans blue dye solution in normal saline, immediately followed by an intraperitoneal injection of 0.6% acetic acid 0.3 ml. The mice were sacrificed by cervical dislocation 20 minutes later, the viscera were exposed and the peritoneal exudates were collected by washing the peritoneum with 5 ml of normal saline, centrifuged at 4000 × g for 10 minutes and taking the supernatant for determination. The vascular permeability was expressed as the absorbance of the supernatant, which was read at 590 nm on Sunrise ELISA Analyzer (Tecan Company, Austria).

### Acetic acid-induced writhing response

The writhing test in mice was carried out using the reported technique [21]. At the third drug administration day, 1 hour after extracts and standard drug administration, mice were treated with a single intraperitoneal injection with 0.2 ml/10g body weight of 0.6% acetic acid. The number of writhing movements (contraction of abdominal muscles and stretching of hind limbs) were counted between 5 and 15 minutes after the acetic acid injection.

### Hot-plate test

Hot-plate assay was carried out according to the methods of Eddy and Leimback [22], Lanhers *et al* [23], and Williamson *et al* [24] by some modification. Mice were habituated twice to the hot-plate in advance. For testing, female mice were placed on a hot-plate maintained at  $55 \pm 0.5^\circ\text{C}$ . The time that elapsed until the occurrence of either a hind paw licking or a jump off the surface was recorded as the hot-plate latency. Mice with baseline latencies of  $<5\text{s}$  or  $>30\text{s}$  were eliminated from the study. After the determination of baseline response latencies, hot-plate response latencies were re-determined at 30, 60 and 120 minutes after oral administration of test drugs (aspirin as reference drug) at the third drug administration day. The time it took for the mice to respond to the thermal stimulus (indicated by paw licking or jumping) was noted as the response latency (in seconds). The mean response latency for each group was thus determined.

### Statistical analysis

All data obtained during the anti-inflammatory and analgesic tests were expressed as mean  $\pm$  standard errors (SEM), and analyzed by ANOVA one-way analysis of variance, followed by Dunnett's test. When the probability ( $P$ ) was less than 0.05, the difference was considered to be significant.

## RESULTS

The present work was taken to evaluate the various portions of CS (including 85% ethanol extracts of CS fruits, stem-leaves, and roots) as to the anti-inflammatory and analgesic activities in mice.

### Xylene-induced ear edema

It could be seen from Fig. 1, compared with control group, the extract groups of CS stem-leaves at dose of 1.16, and 2.32 g/kg showed significant activities with inhibition percentages of 31.19 and 34.66% ( $P < 0.05$ ,  $P < 0.01$ ), respectively. Meanwhile, groups treated with extracts from CS fruits and roots at dose of 3.23 and 1.70 g/kg showed inhibition percentages of 36.36 and 36.75% ( $P < 0.01$ ), and the aspirin group (positive control) showed inhibition percentage of 41.17% ( $P < 0.01$ ) at dose of 0.08 g/kg.

### Acetic acid-induced vascular permeability

It could be seen from Fig. 2, the extract of CS stem-leaves showed a significant suppression for acetic acid-induced vascular permeability at oral doses of 0.58, 1.16, 2.32 g/kg, compared with the control group ( $P < 0.01$ ). Aspirin also showed a clear inhibition of the inflammation induced by acetic acid compared with the control group ( $P < 0.01$ ). It also could be observed that CS fruits and CS stem-leaves at three doses showed significant inhibitions ( $P < 0.01$ ) with a dose dependent. Meanwhile, the groups treated with CS roots only at high dose (1.70 g/kg) showed significant inhibitory activities (38.7%) ( $P < 0.01$ ).

### Acetic acid-induced writhing response

It could be seen from Fig. 3, the CS stem-leaves produced a significant analgesic effect with inhibition activities of 43.9, 52.2 and 67.6% induced by acid-induced writhing response with a dose dependent at the dose of 0.58, 1.16 and 2.32 g/kg ( $P < 0.01$ ), respectively. Groups of CS fruits also produced significant inhibition activities of 27.0% ( $P < 0.05$ ), 49.3% ( $P < 0.01$ ) and 53.6% ( $P < 0.01$ ) induced by acid-induced writhing response, at a dose dependent of 0.81, 1.62 and 3.23 g/kg. However, the CS roots groups only at high dose of 1.70 g/kg showed significant inhibitions (63.3%,  $P < 0.01$ ) on acid-induced writhing response. As the positive control, aspirin group also showed a significant inhibition (66.5%,  $P < 0.01$ ) at dose of 0.08 g/kg on acid-induced induced writhing response.

### Hot-plate test

It could be seen from Fig. 4, as positive control, aspirin (0.08 g/kg) showed a clear protective effects on pain threshold induced by hot plate method with the response latency of  $23.71 \pm 5.78\text{ s}$  (30 min,  $P < 0.05$ ),  $25.66 \pm 4.94\text{ s}$  (60 min,  $P < 0.01$ ),  $29.21 \pm 15.95\text{ s}$  (120 min,  $P < 0.01$ ). Compared with the control group, CS fruits at high dose of 3.23 g/kg showed a significant inhibition suppression pain threshold with a time dependent induced by hot plate method at 30, 60 and 120 min ( $P < 0.01$ ). Meanwhile, CS fruits had protective effects on pain threshold induced by hot plate method only at 120 min at the middle dose of 1.62 g/kg. CS stem-leaves showed significant inhibition at the high dose (2.32 g/kg) in all tested points with a significant level of  $P < 0.05$ , at middle dose (1.16 g/kg) in 120 minute test point with the response latency of  $26.97 \pm 14.42\text{ s}$  ( $P < 0.05$ ), at lower dose (0.58 g/kg) in 60 minute test point with the response latency of  $22.45 \pm 9.54\text{ s}$  ( $P < 0.05$ ), respectively. But, the CS roots showed analgesic effect only at 120 min at the high dose of 1.70 g/kg with the hot-plate latency of  $28.32 \pm 13.26\text{ s}$  ( $P < 0.05$ ).

Figure 1. Effect of ethanol extracts from CS fruits, CS stem-leaves, and CS root on xylene-induced ear edema in mice after oral administration at dose of 3.86, 7.72, and 15.44 g/kg body weight with respect to original dry plant material for three consecutive days daily. Values were expressed as mean  $\pm$  standard errors (SEM). \*:  $P < 0.05$  and \*\*:  $P < 0.01$  are compared with the control group (ANOVA followed Dunn's tests). †: Dose in bracket refers to extract

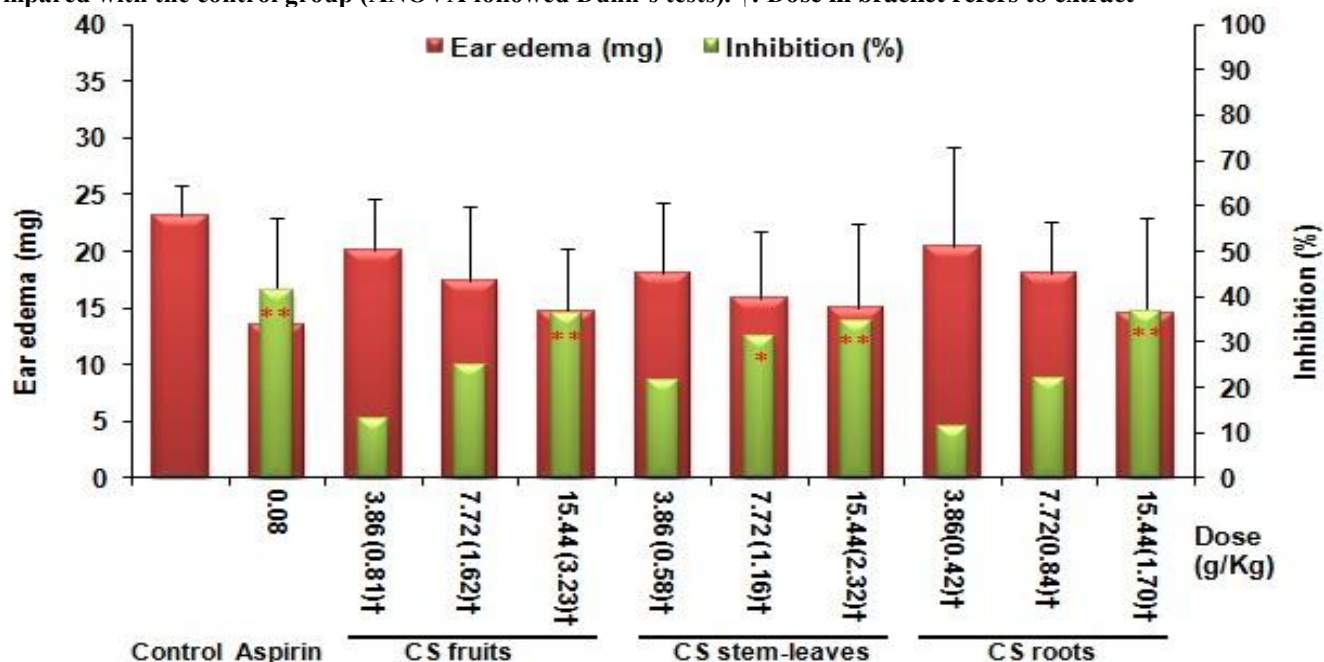


Figure 2. Effect of ethanol extracts from CS fruits, CS stem-leaves, and CS root on acetic acid-induced vascular permeability in mice after oral administration at dose of 3.86, 7.72, and 15.44 g/kg body weight with respect to original dry plant material for three consecutive days daily. Values were expressed as mean  $\pm$  standard errors (SEM). \*:  $P < 0.05$  and \*\*:  $P < 0.01$  are compared with the control group (ANOVA followed Dunn's tests). †: Dose in bracket refers to extract

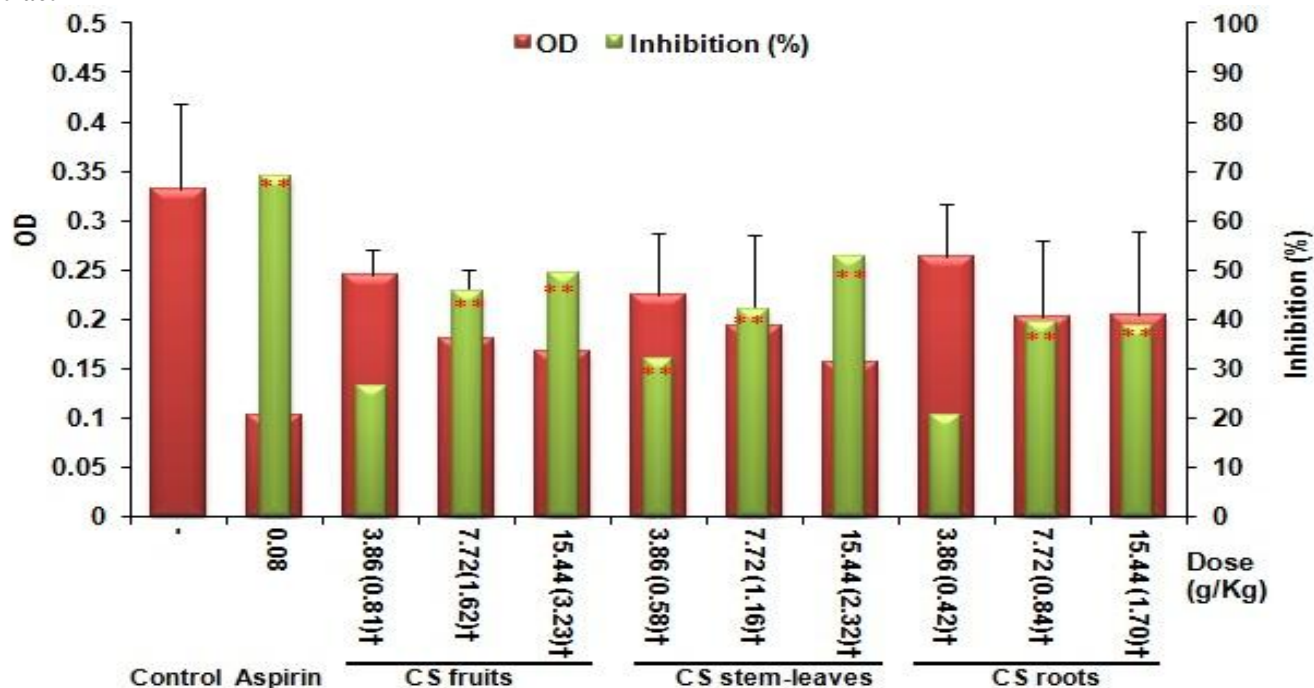


Figure 3. Effect of ethanol extracts from CS fruits, CS stem-leaves, and CS root on acetic acid-induced writhing response in mice after oral administration at dose of 3.86, 7.72, and 15.44 g/kg body weight with respect to original dry plant material for three consecutive days daily. Values were expressed as mean ± standard errors (SEM). \*: P < 0.05 and \*\*: P < 0.01 are compared with the control group (ANOVA followed Dunn's tests). †: Dose in bracket refers to extract.

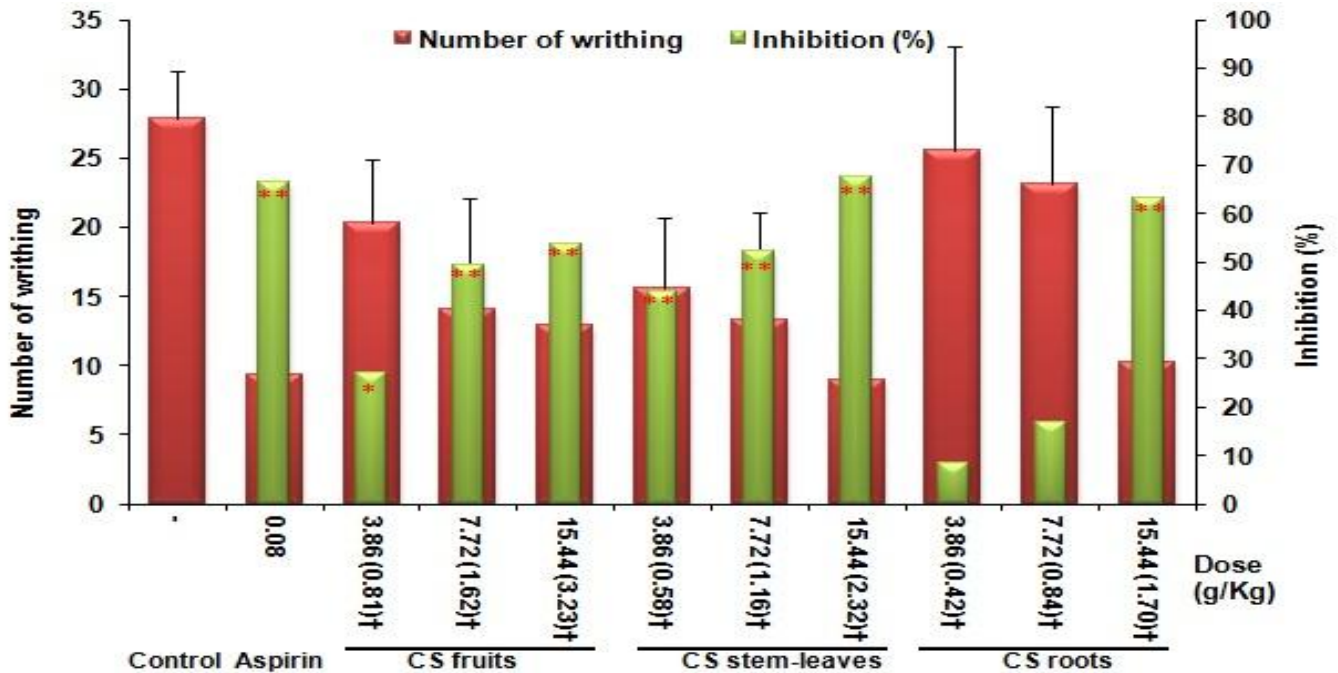
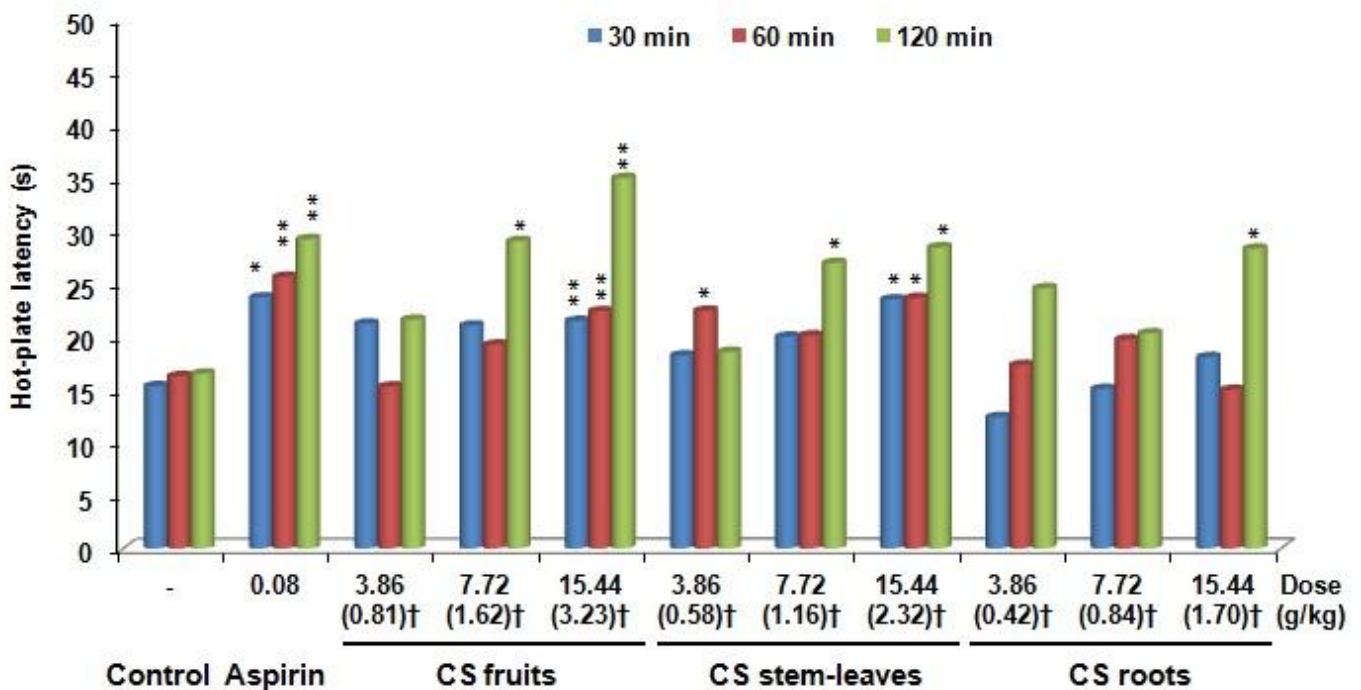


Figure 4. Effect of ethanol extracts from CS fruits, CS stem-leaves, and CS root on analgesic effect in hot-plate test in mice after oral administration at dose of 3.86, 7.72, and 15.44 g/kg body weight with respect to original dry plant material for three consecutive days daily. Values were expressed as mean ± standard errors (SEM). \*: P < 0.05 and \*\*: P < 0.01 are compared with the control group (ANOVA followed Dunn's tests). †: Dose in bracket refers to extract



## DISCUSSION

Traditional medicine has a long history of serving people all over the world. Medicinal plant is an important element of indigenous medical systems in many regions of our planet [25]. CS is a traditional herbal medicine which has been used for the treatment of some kinds of inflammatory diseases in Xinjiang Uygur Autonomous Region, China [6,7]. In present studies, it is first to compare with the anti-inflammatory and analgesic activities of various portions of CS.

Orally administration of grades doses of ethanol extract from CS fruits in mice give a medial lethal dose (LD<sub>50</sub>) value of 154.26 g/kg (in proportion to CS fruits)[17]. This finding probably suggested that the plant extract is relatively safe in mice. Three doses equivalently to one-tenth, one-twentieth and one-fortieth of the LD<sub>50</sub> value were adopted as the test doses for CS fruits, stem-leaves and roots in present studies.

Xylene-induced ear edema and acetic acid-induced vascular permeability in mice reflected the edematization during the early stages of acute inflammation [26]. Experimental evidence obtained in the present studies indicated that CS stem-leaves possessed better anti-inflammatory effects than the other CS fruits and root. In xylene-induced ear edema in mice, CS stem-leaves at the middle dose (1.16 g/kg) and high dose (2.32 g/kg) showed significant inhibition activities ( $P < 0.05 - 0.01$ ) with the inhibition percentages of 31.19 and 34.66%, respectively. While the inhibition activity of aspirin, positive control group, was 41.17%. In acetic acid-induced vascular permeability test in mice, CS stem-leaves groups at low (0.58 g/kg), middle (1.16 g/kg) and high doses (2.32 g/kg) showed a gentle inhibition ( $P < 0.01$ ).

Williamson *et al.* [24] and Koster *et al.* [27] postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for evaluation of peripherally and centrally acting analgesic drugs. By observing the analgesic responses in acetic acid-induced writhing test and in hot-plate latency test in mice, it was

found that the analgesic effects of CS stem-leaves and fruits were stronger than that of CS roots in the two pain models used. CS stem-leaves and CS fruits at the low (0.58, 0.81 g/kg), the middle (1.16, 1.62 g/kg) and high doses (2.32, 3.23 g/kg) showed a gentle inhibition on acetic acid-induced writhing response in mice. CS stem-leaves and CS fruits at the middle (1.16, 1.62 g/kg) and high doses (2.32, 3.23 g/kg) showed a protective activity in hot-plate latency test. These results indicated that the CS stem-leaves and CS fruits both could produce central but also peripheral analgesic action. The results from present study indicated the weakest effects on anti-inflammatory and anti-nociceptive activities of CS roots. These results strongly indicated that the excessive excavation and use of CS roots should be banned, for the sake of protection of the plant resources and ecological environment.

## CONCLUSION

In conclusion, CS has anti-inflammatory and analgesic activities and the fruits and stem-leaves showed better anti-inflammatory and analgesic potency than the roots. So, it is intensively suggested that the CS fruits and stem-leaves could be used extensively in clinic and the excessive excavation and use of CS roots should be banned. Although the anti-inflammatory and analgesic effects of CS fruits, stem and roots were studied, further investigation is required to elucidate the exact mechanism which underlies these effects.

## ACKNOWLEDGMENTS

This work was supported by research grant from the Foundation of Shanghai Municipal Education Commission (No.05CZ31 awarded to Prof. Chang-hong Wang).

## DECLARATION OF INTEREST

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

## REFERENCES

1. Trombetta D, Occhiuto F, Perri D, Puglia C, Santagati NA, de Pasquale A. Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds. *Phytother Res*, 19, 2005,19-23.
2. Rodrigo M, Lazaro M, Alvarruiz A, Giner V, Composition of capers (*Capparis spinosa*), influence of cultivar, size, and harvest date. *J Food Sci*, 57, 1992, 1152-1154.
3. Giuffrida D, Salvo F, Ziino M, Toscano G, Dugo G. Initial investigation some chemical constituents of capers (*Capparis spinosa* L.) from the island of salina. *Ital J Food Sci*, 14, 2002, 25-33.
4. Matthaus B, Ozcan M. Glucosinolates and fatty acid, sterol, and tocopherol composition of seed oils from *Capparis spinosa* Var. *Spinosa* and *Capparis ovata* Desf. Var. *canescens* (Coss.) Heywood. *J Agric Food Chem*, 53, 2005, 7136-7141.
5. Khanfar MA, Sabri SS, Zarga M H, Zeller KP. The chemical constituents of *Capparis spinosa* of Jordanian origin. *Nat Prod Res*, 17, 2003,9-14.
6. Yang T, Wang CH, Chou GX, Wu T, Cheng XM, Wang ZT. New alkaloids from *Capparis spinosa*, Structure and X-ray crystallographic analysis. *Food Chem*, 123, 2010, 705-710.
7. Yang T, Wang CH, Liu HJ, Chou GX, Cheng XM, Wang ZT. A new antioxidant compound from *Capparis spinosa* L. *Pharm Biol*, 48, 2010, 589-594.

8. Pharmacopoeia Commission. Ministry of Public Health, Standard of Uygur drug, Drug Standard of Ministry of Health of the Peoples Republic China. Xinjiang, Technology and Public Health Press. 1998, 80.
9. Yang T, Liu YQ, Wang CH, Wang ZT. Advances on investigation of chemical constituents, pharmacological activities and clinical applications of *Capparis spinosa*. *Chin J Chin Mater Med*, 33, 2008,2453-2458.
10. Eddouks M, Lemhadri A, Michel JB. Caraway and caper, Potential anti-hyperglycaemic plants in diabetic rats. *J Ethnopharmacol*, 94, 2004, 143-148.
11. Gadgoli C, Mishra SH. Antihepatotoxic activity of *p*-methoxy benzoic acid from *Capparis spinosa*. *J Ethnopharmacol*, 66, 1999, 187-192.
12. Matthauss B, Ozcan M. Glucosinolate composition of young shoots and flower buds of capers (*Capparis* species) growing wild in Turkey. *J Agric Food Chem*, 50, 2002, 7323-7325.
13. Yang T, Yu FS, Wang CH, Wang ZT. Anti-inflammatory and analgesic effects of ethanol extract and isolated fraction from fruits of *Capparis spinosa*. *ActaUnivTrad Med Sin Pharm Shanghai*, 23, 2009,38-41.
14. Ageel AM, Parmar NS, Nossa JS. 1986. Anti-inflammatory activity of some Saudi Arabian medicinal plants. *Agents Action*, 17, 383-384.
15. Al-said MS, Abdeisatir EA Khalifa SI, el-Feraly FS. Isolation and identification of an anti-inflammatory principle from *Capparis spinosa*. *Phamazie*, 43, 1988, 640-641.
16. Zhou H, Jian R, Kang J, Huang X, Li Y, Zhuang C, Yang F, Zhang L, Fan X, Wu T, Wu X. Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J Agric Food Chem*, 58, 2010, 12717-12721.
17. Feng XL, Lu JC, Xin HL, Zhang L, Wang YL, Tang KX. Anti-arthritic active fraction of *Capparis spinosa* L. fruits and its chemical constituents. *YakugakuZasshi*, 131, 2011, 423-429.
18. Liu HJ, Yang T, Cheng XM, Wang CH, Wang ZT. Determination of rutin and kaempferol-3-O-rutside in different medicament portions of *Capparis spinosa* L. by RP-HPLC. *Shanghai J TCM*, 44(9), 2010, 260-263
19. Hosseinzadeh H, Haddadkhodaparast MH, Arash AR. Antinociceptive, anti-inflammatory and acute toxicity effects of *Salvia leriifolia* Benth seed extract in mice and rats. *Phytother Res*, 17, 2003, 422-425.
20. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesic. *Br J PharmacolChemother*, 22, 1964, 246-253.
21. Ahmed F, Shahid IZ, Biswas UK, Roy BA, Das AK, Choudhuri MSK. Anti-inflammatory, analgesic, and neuropharmacological activities of *Clerodendron viscosum*. *Pharm Biol*, 45, 2007, 587-593.
22. Eddy ND, Leimback D. Synthetic analgesics, II. Dithyienylbutenyl-amines and dithyienylbutylamines. *J PharmacolExpTher*, 3, 1953, 544-547.
23. Lanhers MC, Fleurentin J, Mortier F, Vinche A, Younos C. Anti-inflammatory and analgesic effects of an aqueous extract of *Harpagophytum procumbens*. *Planta Med*, 58, 1992, 117-123.
24. Williamson EM, Okpako DT, Evans FJ. *Pharmacological methods in phytotherapy research*. Vol.1 selection, preparation and pharmacological evaluation of plant materials, John Wiley, Chichester, 1996, 184-186.
25. Kong JM, Goh NK, Chia LS, Chia TF. Recent advances in traditional plant drugs and orchids. *ActaPharmacol Sin*, 24, 2003, 7-21.
26. Vogel HG, Vogel WH. Drug discovery and evaluation. Pharmacological assays. Springer, Berlin, 1997, 402-403.
27. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. *Fed proc*, 18, 1959, 412.