**Natural Product Sciences**
9(2) : 97-101 (2003)

# Sulfuretin, an Antinociceptive and Antiinflammatory Flavonoid from *Rhus verniciflua*

**Jongwon Choi,[1] Byung-Jae Yoon,[1] Yong Nam Han,[2] Sang Kook Lee,[3] Kyung-Tae Lee,[4] and Hee-Juhn Park[5]***

[1]*College of Pharmacy, Kyungsung University, Pusan 608-736, Korea*
[2]*Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*
[3]*College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea*
[4]*College of Pharmacy, Kyung-Hee University, Seoul 130-701, Korea*
[5]*Division of Applied Plant Sciences, Sangji University, Wonju 220-702, Korea*

**Abstract** – This study was undertaken to evaluate the antinociceptive and antiinflammatory effects of the heartwood extract of *Rhus verniciflua* (Anacardiaceae) and the two major components, sulfuretin and fustin. The MeOH extract, its EtOAc-soluble portion and sulfuretin showed significant antinociceptive activity in writhing and hot plate test assays and antiinflammaory effects in carrageenan-induced hind paw edema in rats. In particular, treatment of sulfuretin with 10 mg/kg dose (i.p.) reduced writhing frequency by 48.0% ($p<0.01$) compared to that of a control group. Further, the treatment of sulfuretin (5, 10 mg/kg, i.p.) for 7 days prevented the carrageenan-induced hind paw edema significantly ($p<0.01$). The antiinflammatory effect of sulfuretin was also confirmed by microscopic observation of mast cell numbers in muscle. In addition, sulfuretin suppressed the cyclooxygenase-2 (COX-2) activity ($IC_{50}$ = 28.7 μM) in lipopolysaccharide-activated macrophage cells. This result indicates that the inhibitory effect of sulfuretin on COX-2 may be one of the antinociceptive/antiinflammatory mechanism.
**Key words** – *Rhus verniciflua*, Anancardiaceae, sulfuretin, antinociceptive, anti-inflammatory, COX-2

## Introduction

The exudates which could be obtained from the stem bark of *Rhus verniciflua* Stokes has been mainly used for traditional paint in east Asian countries (Kim *et al.*, 1998). Stem bark of *R. verniciflua* contains very much amount of urushiols, which is polymerized to form lacquer films by the radical chain reaction (Hirota *et al.*, 1998) and elucidated to have anti-AIDS, strong antioxidant and immune-enhancing activities (Miller *et al.*, 1996). Since this plant part contains urushiols, the allergenic principle, it will be hard to develop as a medicinal drug. In contrast, the heartwood of this plant does not cause this type of allergenic action at all, implying that it contains no urushiols. This part of the plant has been used as a kind of beverages for tonics, cancer prevention, lingering intoxication and several kinds of inflammatory diseases.

We have elucidated that the flavonoid-containing extract and an ingredient sulfuretin showed potent antimutagenic/anti-lipid peroxidative effects (Park *et al.*, 2001). In that report we suggested that sulfuretin had dose-dependent antimutagenic action whereas fustin showed dose-independent action suggesting that the latter compound has a prooxidant role in the living system. Other flavonoids, garbanzol, fisetin and mollisacasidin were too minor to represent the bioactivity of the heartwood. Therefore, we studied the antinociceptive/anti-inflammatory roles of the main constituents, sulfuretin and fustin, flavonoid-containing extract and fraction. In this communication, we report that sulfuretin may be an active principle for therapeutic uses. Moreover, the promising anti-inflammatory sulfuretin could be emphasized since it has already been found to have antimutagenic action.

*Author for correspondence
Fax: 82-33-745-2413, E-mail: hjpark@mail.sangji.ac.kr

## Materials and Methods

**Plant material and preparation of test samples** – The heartwood of *Rhus verniciflua* stokes was collected in September 1999, on Chiak Mountain, Kangwon Province, Korea, and was identified by Prof. G. T. Kim (Division of Applied Plant Sciences, Sangji University, Wonju, Korea). A voucher specimen (#natchem-18) is deposited in the herbarium of Applied Plant Sciences, Sangji University, Wonju, Korea.

Dried heartwood (2 kg) of the stem of *R. verniciflua* was cut and extracted three times with MeOH under reflux and evaporated to give a viscous mass (280 g). This material was successively suspended in 3 L $H_2O$ and then partitioned

with each 3 L of $CHCl_3$, EtOAc and *n*-BuOH. Each layer was dried *in vacuo* to yield each of $CHCl_3$ fraction (68 g), EtOAc fraction (95 g) and *n*-BuOH fraction (70 g). A part of EtOAc fraction (20 g) was chromatographed over silica gel (600 g, 7×70 cm; Merck, Art 7734, Germany) with eluting solvent of $CHCl_3$-MeOH-$H_2O$ (73:27:10, lower phase, 5L) to give 5 fractions (fraction 1-5), for further isolation. Eluents were collected by 80 ml each to afford 70 fractions and each was checked under UV (254 and 365 nm). The fractions was showing similar patterns on TLC were grouped and evaporated on a rotary evaporator to give 5 fractions (fraction 1-5). Repeated column chromatography of fraction 3 (1,040-1,200 ml) were carried out over silica gel with eluting solvent of $CHCl_3$-MeOH (10:1) yielded orange-yellow needles (**1**, Rf 0.65, 74 mg) by recrystallization from MeOH. Fraction 5 (1,360-1,600 ml) containing impurities was subjected to ODS and sephadex LH-20 column chromatography to give compound **2** (Rf 0.53, 190 mg). The compounds **1** and **2** were identified as sulfuretin and fustin, respectively, by physicochemical and spectroscopic data. {**1**: Orange-yellow prisms (160 mg) from MeOH, mp 280-285° (dec.); EI-MS (70 eV) m/z: 270.3 ($M^+$, $[C_{15}H_{10}O_5]^+$). (Harborne *et al.*, 1994a); **2**: White needles (250 mg) from MeOH, mp 228-229°, $[\alpha]_D$ +28.3(c, 0.9 in 50% aqueous acetone); EI-MS (70 eV) m/z: 288.3 ($M^+$, $[C_{15}H_{12}O_6]^+$). (Harborne *et al.*, 1994b)}. Sulfuretin and fustin were used as test samples for animal experiment together with the MeOH extract and EtOAc fraction.

**Preparation of test sample solutions** – Test samples (MeOH extract, EtOAc fraction, sulfuretin and fustin) were first dissolved with 10% tween 80 and diluted by saline. The same volume of solvent only was administered for the normal group. The extract and fractions were orally administered with 150 and 250 mg/kg and the isolated compounds (sulfuretin and fustin) were intraperitoneally administered with 5 and 10 mg/kg based on the preliminary experiments.

**Animals** – Both 4 week-old ICR male mice and Sprague-Dawley male rats were purchased from Korean Experimental Animal Co. and adapted them in a constant condition (temperature: 20±2°C, dampness: 40-60%, light/dark cycle: 12 hr) for two weeks or more. Twenty-four hours before the experiment, only water was offered to the animals. Considering the variation of enzyme activity during one day, the animals were sacrificed at fixed time (10:00 A.M.-12:00 A.M.).

**Acetic acid writhing and hot plate method** – Test solution was orally or intraperitoneally administered 30 min before the experiment, and further 0.1 ml/10 g of 0.7% acetic acid-saline was injected intraperitoneally. 10 min after the injection, the frequency of writhing in mice was counted for 10 min (Whittle, 1949). Aminopyrine (100 mg/kg) was used as a positive control agent.

Hot plate made by Ugo Basile (Italy) was used for the measurement of antinociceptive effect by hot plate method. The response-time showing writhing syndrome was recorded.

**Induction and measurement of edema** – Each 0.1 ml of 1% carrageenan (Sigma Co., U.S.A.) was injected to right footprints of rats and edema was induced. The test solution (each extract: 150, 250 mg/kg p.o.; each compound: 5, 10 mg/kg) had been orally administered for 7 days. The effect was taken by plethysmometer (Ugo Basile, Italy) (Winter *et al.*, 1962). The inhibitory effect was calculated as follows: Inhibitory effect of edema (%)=(volume of control group-volume of treatment group/volume of control group)×100

**Histological examinatination under microscope** – For the histological examination, muscle tissues in the hind paw muscle of the rat with edema or sample treated were stood in 10% formalin for 24 h and washed and further followed by washing with formic citrate. This was formatted in paraffin and it was sliced to 6 μm thickness with microtome. After staining with hematoxylin-eosin solution, it was observed under optical microscope. Mast cells were counted using on five blocks under microscopes and calculated as mean±S.E.M..

**Measurements of $PGE_2$ accumulation by COX-2 in cultured LPS-induced RAW 264.7 cells** – RAW 264.7 macrophage cells were maintained in DMEM supplemented with penicillin-streptomycin and 10% FBS at 37°C, 5% $CO_2$ in humidified air. For evaluating the inhibitory activity of test materials on COX-2, the cells were allowed to adhere for 4 hr in the presence of sulfuretin in 96-well culture plate, washed three times with media, and then incubated in the fresh medium with 1 μg/ml of LPS. Test materials were simultaneously added to each well. After an additional 16 hr incubation, the media were removed and analyzed by $PGE_2$ enzyme immunometric assay (EIA). $PGE_2$-EIA was performed according to Pradelles with the minor modification. In these assays, 100% activity is defined as the difference between $PGE_2$ accumulation in the absence and in the presence of LPS for 16 hr in triplicate determinations. % Inhibition was expressed as [1-($PGE_2$ level of sample / $PGE_2$ level of vehicle treated-control)]×100. For evaluating direct enzyme activity on COX-2, the cells were allowed to adhere for 4 hr in the presence of sulfuretin in 96-well culture plate, washed three times with media, and then incubated in the fresh medium with 1 μg/ml of LPS for 16 hr. After removing the medium, cells were treated with test materials for 15 min and then incubated with 10 uM arachidonic acid for 10 min, and the levels of PGE2 production were determined as mentioned above.

## Results and Discussion

The oral administration of the MeOH and EtOAc extract (150 mg/kg and 250 mg/kg) of *R. verniciflua* significantly inhibited the writhing numbers in mice induced by acetic acid, respectively, whereas $CHCl_3$-, BuOH- and $H_2O$ fraction showed no significant reduction (Table 1). In this experiment, oral administration of a positive control aminopyrine (100 mg/kg) exhibited a strong antinociceptive effect by 80.3%. Thus, these results suggest that the antinociceptive effect of the MeOH extract could be attributed to the components distributed in EtOAc fraction. In addition, the observed order of antinoiceptive effects was shown as follows in acetic acid-induced writhing test and hot plate test: Aminopyrine> sulfuretin>fustin>EtOAc ext.>MeOH ext.. Therefore, it was suggested that the flavonoids could be antinociceptive substances. In particular, sulfuretin with 10 mg/kg administration (i.p.) reduced the writhing frequency by 48.0% in writhing test and prolonged the action time by 247.3% in hot-plate assay test.

**Table 1.** Effect of *R. verniciflua* on the writhing syndrome induced by acetic acid in mice

| Group | Dose (mg/kg, p.o.) | Frequency Count/10 min | Inhibition (%) |
|---|---|---|---|
| Control | – | 49.3±3.23a | 0 |
| MeOH ext. | 150 | 38.4±2.43b* | 22.1 |
| | 250 | 34.7±3.92* | 29.6 |
| $CHCl_3$ fr. | 150 | 48.8±2.39 | 1.0 |
| | 250 | 50.7±3.11 | −2.8 |
| EtOAc fr. | 150 | 35.2±4.21** | 28.6 |
| | 250 | 27.8±2.17** | 43.6 |
| BuOH fr. | 150 | 47.2±4.11a | 4.3 |
| | 250 | 49.9±4.25a | −1.2 |
| $H_2O$ fr. | 150 | 49.9±4.25a | −1.2 |
| | 250 | 47.2±3.46a | 4.3 |
| Aminopyrine | 100 | 9.7±1.46*** | 80.3 |

Values represent means±S.E.M (n=10).
***p<0.001, **p<0.01, *p<0.05 compared with the control.

The inhibitory effects of the EtOAc fraction and the isolated flavonoids on the carrageenan-induced edema are summarized in Table 3. The EtOAc fraction was administered orally (150, 250 mg/kg), but sulfuretin and fustin were administered intraperitoneally (5, 10 mg/kg). All the test samples in Table 3 showed various significant effects in anti-edema test but weaker than indomethacin, which effects were observed in 60 min. Among the treatment groups, sulfuretin showed the lower swelling rate than fustin, which were also observed in 60 min.. It is well known that the carrageenan-

**Table 2.** Antinociceptive effect of sulfuretin and fustin isolated from the EtOAc fraction of the heartwood extract of *R. verniciflua* by acetic acid-induced writhing and hot-plate method in mice

| Group | Dose (mg/kg) | Frequency (Count/10 min) | Action time (sec) |
|---|---|---|---|
| Control | | 48.7±3.10 (0)[1)] | 26.4±4.23 (0)[2)] |
| Sulfuretin | 5 (i.p.) | 30.5±3.54* (37.3) | 60.8±3.55** (230.3) |
| | 10 (i.p.) | 25.3±2.43** (48.0) | 78.9±5.10** (298.9) |
| Fustin | 5 (i.p.) | 34.7±3.27* (28.7) | 55.8±4.19** (211.4) |
| | 10 (i.p.) | 29.2±2.96* (40.0) | 65.3±4.27** (247.3) |
| Aminopyrine | 100 (p.o.) | 10.8±1.24*** (77.8) | 153.3±10.23*** (580.7) |

[1)]% inhibition of writhing frequency.
[2)]% prolongation of action time; Values represent means±S.E.M. (n=10).
***p<0.001, **p<0.01, *p<0.05. Values in the parentheses are % antinociceptive effect to control group.

**Table 3.** Anti-inflammatory effect of the *Rhus verniciflua* heartwood extracts on carrageenan edema of the hind paw in rats

| Group | Dose mg/kg | Swelling percent | | | |
|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 120 min |
| Control | – | 21.3 ± 3.99 | 25.7 ± 4.11 | 31.6 ± 3.10 | 40.3 ± 3.17 |
| MeOH ext. | 150 (p.o.) | 20.8 ± 23.8 | 23.8 ± 3.37 | 30.8 ± 2.84 | 38.2 ± 2.30 |
| | 250 (p.o.) | 21.7 ± 3.90 | 20.4 ± 2.93 | 25.2 ± 2.65 | 27.8 ± 2.30* |
| EtOAc ext. | 150 (p.o.) | 18.9 ± 4.57 | 22.8 ± 1.40 | 20.3 ± 2.11* | 26.8 ± 2.18* |
| | 250 (p.o.) | 22.5 ± 4.13 | 18.5 ± 2.43* | 20.9 ± 2.30* | 21.0 ± 2.17* |
| Sulfuretin | 5 (i.p.) | 23.6 ± 2.84 | 20.8 ± 1.87 | 20.7 ± 3.50* | 25.8 ± 2.30* |
| | 10 (i.p.) | 21.4 ± 3.10 | 15.2 ± 1.63* | 17.6 ± 2.17** | 20.3 ± 3.00** |
| Fustin | 5 (i.p.) | 20.8 ± 3.36 | 21.4 ± 3.99 | 21.8 ± 2.80* | 29.3 ± 3.12* |
| | 10 (i.p.) | 19.4 ± 2.99 | 20.8 ± 1.87 | 18.5 ± 3.11** | 25.8 ± 4.00* |
| Indomethacin | 100 (p.o.) | 18.9 ± 1.34 | 13.3 ± 1.57** | 11.4 ± 2.00*** | 10.4 ± 1.89*** |

Values represent means ± S.E.M. (n=10). ***p<0.001, **p<0.01, *p<0.05 compared with the control.

**Table 4.** Effect of the *Rhus verniciflua* heartwood extracts and its components on the degree of infiltrated mast cell on carrageenan-induced rats

| Group | Dose mg/kg, p.o. | Concenration of mast cell | % inhibition |
|---|---|---|---|
| Control | – | 70±4.79 | 0 |
| MeOH ext. | 150 (p.o.) | 66.5±3.72 | 5 |
| | 250 (p.o.) | 60.5±4.00* | 15 |
| EtOAc ext. | 150 (p.o.) | 58±4.27* | 17 |
| | 250 (p.o.) | 52±2.36* | 29 |
| Sulfuretin | 5 (i.p.) | 30.5±2.84** | 56 |
| | 10 (i.p.) | 16±1.97*** | 77 |
| Fustin | 5 (i.p.) | 48±3.00* | 31 |
| | 10 (i.p.) | 34±2.11* | 51 |
| Indomethacin | 100 (p.o.) | 9.30±1.43*** | 88 |

Values represent means ± S.E.M. (n=5). ***p<0.001, **p<0.01, *p<0.05 compared with the control.

induced edema could be observed as two phases (phase I and II edema). The phase I edema caused by histamine and serotonin release appears immediately after the carrageenan-injection and reaches maximum edema at 30 min and then disappeared (Crunkhorn P *et al.*, 1971). However, Phase II edema that could be caused by the involvement of kinins, proteases and prostaglandins begins to appear at about 1 h after the injection. Since sulfuretin showed significant decrease the edema by 1 h after the injection, the anti-inflammatory response by sulfuretin was presumed to depend on anti-Phase II edema (Lewis AJ *et al.*, 1975; Moncada *et al.*, 1975). When the infiltrated mast concentration in muscle was calculated, each inhibitory rate was comparable to the corresponding anti-edema effects. Sulfuretin most significantly inhibited the appearance of mast cell number and fustin was next (Table 4). The infiltrated mast cells in the treatment groups were significantly decreased when compared with control group. In particular, the reduction rate of infiltrated mast cells in 10 mg/kg sulfuretin-treated group was shown by 77%, which indicates the potent antiinflammaory effect of sulfuretin.

The most typical *in vitro* assays for the antiinflammation mainly depend on the measurement of the inhibitory effects on inducible nitric oxide synthase, cyclooxigeanses, tumour necrosis factor-α, prosataglandins and related biochemical parameters. Sulfuretin, the active compount, was tested for the measurement of cyclooxigenase-2 in LPS-activated macrophage cell and the result was shown in Fig. 2., which $IC_{50}$ value was calculated to be 28.7 μM. This result indicated that the suppressive activity of sulfuretin on COX-2 plays an important role in antiinflammative and antinociceptive action. Since the inhibition of COX-2 activity decrease the prosataglandin $E_2$ formation, it contributes to the reduction in pain in addition to in inflammation. It was suggested that

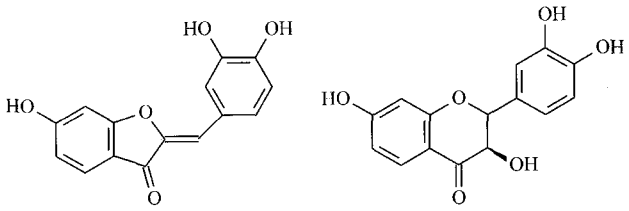


**Fig. 1.** Structure of sulfuretin and fusin isolated from *R. verniciflua.*

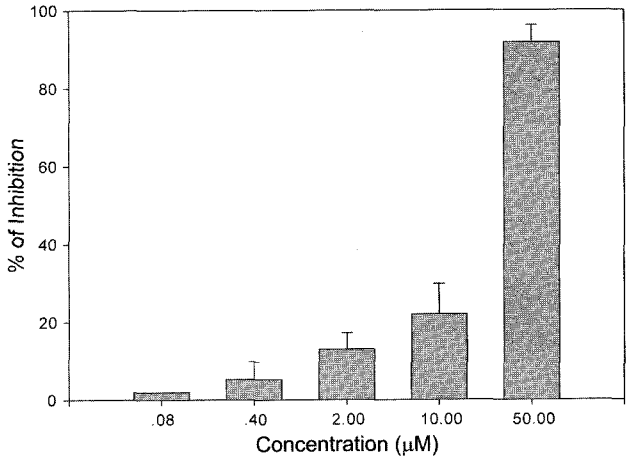


**Fig. 2.** Inhibitory effect of sulfuretin on LPS-induced COX-2 activity ($IC_{50}$=28.7 μM)

antiinflammatory effect *in vivo* and COX-2 inhibitory effect of sulfuretin might contribute to Phase II-edema inhibition.

## Acknowledgements

This work was supported by the Basic Research Program of the Korea Science & Engineering Foundation (R01-2000-00146).

## References

Crunkhorn P., Meacock S. C., Mediators of the inflammation induced in rat paw by carrageenin, British Journal of Pharmacology, **42**, 392-402 (1971).

Harborne, J. B., Dictionary of Natural Products Vol. 10. Chapman & Hall, New York, p. 5716 (1994a).

Harborne, J. B.,. Dictionary of Natural Products Vol. 9. Chapman & Hall, New York, p. 5455 (1994b).

Hirota, S., Matsumoto, H., Sakurai, T., Kitagawa, T., Yamaguchi, O., Observation of Cu-$N_3$-stretching bands for mono-axide adduct of *Rhus vernicifera* laccase. Biochemical and Biophysical Research Communication, **243**, 435-437 (1998).

Kim, M. J., Kim, G. T., Choi, T. B., Hyun, J. O., Effects of climatic factors and tapping date on yield and quality of lactree (*Rhus*

*verniciflua*) sap. Korean Journal of Plant Resources, **11**, 70-79 (1998).

Lewis, A. J., Nelson, D. J., Sugrue, M. F., 1975. On the ability of prostaglandin E1, and arachidonic acid to modulate experimentally induced oedema in the rat paw, British Journal of Pharmacology, **55**, 51-56 (1975).

Miller, W. C., Thielman, N. M., Swai, N., Cegielski, J. P., Shao, J., Ting, D., Mlalasi J., Manyenga D., Lallinger, G. J., Delayed-type hypersensitivity testing in Tanzanian adults with HIV infection. Journal of Acquired Immune Deficiency, **12**, 303-308 (1996).

Moncada, S,. Ferreira, S. H., Vane, J. R., Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in dog knee joint, European Journal of Pharmacology, **31**, 250-260 (1975).

Whittle, B. A., The use of change in capillary permeability to distinguish between narcotic and anagesic. British Journal of Pharmacology, **22**, 246-460 (1949).

(Accepted June 2, 2003)