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In vivo Anti-Inflammatory and Antinociceptive Activities of the Extracts and Chemical Constituents of an Endemic Turkish Plant, *Salsola grandis*

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Abstract: *Salsola* is one of the largest and most important genera in the family Chenopodiaceae. *Salsola* species are used for their anti-inflammatory, antinociceptive, anthelmintic and antipruritic effects and also as diuretic in traditional medicines worldwide. The aim of the present study is to assess the activity potential of the extracts, subextracts and isolated compounds from the aerial parts of and *Salsola grandis*, an endemic species in Turkey in a scientific platform. The effects of the extracts, subextracts and isolated compounds were investigated using *in vivo* experimental models of inflammation and pain in mice, and also total phenolic content of the plant was determined. For the evaluation of the anti-inflammatory activity, carrageenan-induced paw edema and for the assessment of antinociceptive activity, *p*-benzoquinone-induced nociception tests in mice were employed. The crude ethanol extract of the plant was sequentially fractionated into five subextracts, namely *n*-hexane, CHCl₃, EtOAc, *n*-BuOH and remaining water subextracts. Further studies were carried out on the bioactive *n*-BuOH subextract. Through bioassay-guided fractionation and isolation procedures from active fraction, ten flavonoids, isorhamnetin-3-*O*-rutinoside (**1**), quercetin-3-*O*-rutinoside (**2**), quercetin-3-*O*-metylether (**3**), tiliroside (**4**), isorhamnetin-3-glucuronide (**5**), isorhamnetin-3-*O*-glucoside (**6**), quercetin-3-*O*-galactoside (**7**), quercetin-3-*O*-rhamnoside (**8**), quercetin (**9**) and manghaslin (**10**), and two oleanane-type saponins, momordin II b (**11**) and momordin II c (**12**), and one amino acid derivative compound, *N*-acetyltryptophan (**13**) were isolated, and their structures were established on the basis of spectroscopic techniques. The anti-inflammatory and antinociceptive activities of **1**, **4**, **6**, **7** and **12** were investigated firstly in this study, and **4** and **7** were found to have the most potent inhibitory activity in used models. The present study demonstrates the anti-inflammatory and antinociceptive activities in *in vivo* experimental models, chemical composition and total phenolic content of *S. grandis* for the first time.

Keywords: *Salsola grandis*; inflammation; nociception; flavonoids; saponins; *N*-acetyltryptophan. © 2015 ACG Publications. All rights reserved.

1. Introduction

Inflammatory diseases are among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of the natural sources for the discovery of novel bioactive compounds that might serve as leads for the development of potent drugs.

Salsola L. genus, from the family Chenopodiaceae, is represented by sixteen species in Turkish Flora. Among these species *S. grandis* Freitag, Vural & N. Adıgüzel is endemic to Turkey [1]. *Salsola*

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species were reported to be used for their analgesic, anti-inflammatory, astringent, antipuriritic, contraceptive, diuretic, anthelmintic and purgative properties in folk medicine worldwide [2].

The literature survey revealed that *Salsola* species have various constituents that provide several biological activities such as anti-inflammatory, analgesic, anti-hypertensive, antioxidant, hepatoprotective and tyrosinase inhibitory activity [2, 3]. The phytochemical analysis on *Salsola* species indicated that these species are rich in especially alkaloids such as salsolin and salsolidin, besides flavonoids and isoflavonoids [4-6]. *Salsola* genus also has lignans, saponins and coumarins [2, 7, 8].

Previously, *S. volkensii* Schweinf. & Asch. and *S. villosa* Delile were demonstrated to possess anti-inflammatory and analgesic effects [3]. However, there is no information regarding the phytochemical feature and bioactivity of *S. grandis*. Thus, in the present study, we aimed to evaluate the *in vivo* anti-inflammatory and antinociceptive activities of *S. grandis* as well as determine the active phytochemical constituents through bioassay-guided fractionation techniques and phenolic contents of the plant.

2. Materials and Methods

2.1. Chemicals

In the extraction procedure, EtOH, *n*-hexane, CHCl$_3$, EtOAc and *n*-BuOH were of analytical grade and were purchased from Merck Co. Analytical TLC was performed on precoated Kieselgel 60 F$_{254}$ plates (Art. 5554, Merck). The plates sprayed with 1% vanillin-H$_2$SO$_4$ solution [vanillin (Boehringer Mannheim) and H$_2$SO$_4$ (Merck Co.)]. Folin-Ciocalteau's phenol reagent, gallic acid (purity >99%), *p*-benzoquinone (PBQ), carrageenan, acetylsalicylic acid (purity >99%) and indomethacin (purity >99%) were from Sigma-Aldrich.

2.2. Plant material

Aerial parts of *S. grandis* (Chenopodiaceae) were collected in flowering period from Nallıhan bird sanctuary, Ankara, Turkey, in July 2010, and identified by Prof. Dr. Mecit Vural from the Department of Botany, Faculty of Science, Gazi University. A voucher specimen (GUE 2641) is deposited at the Herbarium of Faculty of Pharmacy, Gazi University, Turkey.

2.3. Extraction, fractionation and isolation procedure

2.3.1. Preparation of the ethanol extract

Aerial parts of the plant were dried in shade at room temperature and, then powdered to a fine grade by using a laboratory scale mill. The powdered aerial parts (2270 g) were extracted with 96% EtOH (30 L) at room temperature and evaporated to dryness under reduced pressure below 40°C to yield "EtOH extract" (185.28 g).

2.3.2. Preparation of the subextracts of EtOH extract

127.54 grams of EtOH extract was dissolved in 200 mL of EtOH and extracted with *n*-hexane (8 × 300 mL) in a separatory funnel. Combined *n*-hexane extracts were evaporated under reduced pressure to yield "*n*-Hexane subextract" (21.71 g). EtOH was removed from the remaining extract and diluted with distilled H$_2$O to 150 mL and further fractionated by successive solvent extractions with CHCl$_3$ (5 × 250 mL), EtOAc (10 × 300 mL) and *n*-BuOH saturated with H$_2$O (13 × 300 mL). Each extract as well as remaining water phase after solvent extractions was evaporated to dryness under reduced pressure to yield "CHCl$_3$ subextract" (3.63 g), "EtOAc subextract" (3.74 g), "*n*-BuOH subextract" (17.76 g) and "R-H$_2$O subextract" (52.84 g), respectively.

2.3.3. Chromatographic separation and isolation of the active constituents

The *n*-BuOH subextract (16.67 g) was subjected to polyamide column chromatography (Polyamide 6, Fluka 02395) by using mobile phase system increasing MeOH from 0 to 100% to give 19 main fractions (Fr. 1-26) by TLC control.   Fraction 17 (482 mg) was subjected to column

chromatographic separation on silica gel (Kieselgel 60, 0.063-0.200 mm, Merck Art. No. 7734) using $CHCl_3$:MeOH:H_2O (80:20:2, 75:25:2.5, 70:30:3, 65:35:3.5, 60:40:4, 61:32:7) and MeOH as mobile phase systems to give 11 subfractions. Among the subfractions, Fr. 16-17, Fr. 18-19 and Fr. 20-22 (totally 26.9 mg); Fr. 26-29 and Fr. 31-37 (totally 26.9 mg) were further purified on a Sephadex LH 20 column by eluting with 100% MeOH to yield pure **1** (41.6 mg) and **2** (22.9 mg), respectively.

Fr. 24-26 (356 mg) was subfractionated by silica gel column chromatography eluted with $CHCl_3$:MeOH:H_2O mobile phase system in increasing polarity (0:20:1, 80:20:2, 70:30:3, 65:35:3.5, 60:40:4, 61:32:7) to give 14 subfractions. Compound **3** (12.3 mg) was isolated from the subfraction, Fr.$_{3-4}$. Subfractions 5, 6 and 7 were subjected to Sephadex LH 20 column by eluting with aceton:MeOH (1:1) to obtain the compound **4** (34.4 mg). Subfractions 26-31 were applied to Sephadex LH 20 column by eluting with MeOH to give **5** (25.4 mg).

Fr. 20 (235.8 mg) was subfractionated on a Silica gel column chromatography. Among 6 subfractions, Fr.$_{4-5}$ and Fr.$_{6-9}$ yielded **6** (37.4 mg). On the other hand, Fr.$_{28-31}$ and Fr.$_{32}$ (totally 40.6 mg) were submitted to MPLC on reversed phase silica gel (LiChrosorb C_{18} 40-63 μm; 36 x 460 mm) using stepwise MeOH:H_2O gradient (60-100%, 35 mL/fraction) to give 29 fractions. The compound **11** (12.8 mg) was isolated from the subfractions namely, Fr.$_{10-12}$, Fr.$_{13-15}$ and Fr.$_{16-23}$.

Fr. 21-22 (547 mg) was further purified on a silica gel column eluting with $CHCl_3$:MeOH:H_2O (90:10:1, 80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 61:32:7) to give 7 main subfractions (Fr.$_{1-52}$). Subfraction 14-22 (110.4 mg) was then subjected to silica gel column with the solvent EtOAc:MeOH:H_2O (100:10:5) to yield 6 subfractions. The compounds **7** (54.7 mg) and **8** (3.6 mg) were isolated from subfraction 2, and subfractions 5, 6, respectively. Otherwise, among main subfractions, subfraction 3-8 (84.1 mg) was purified by silica gel column chromatography eluted with EtOAC:MeOH (100:10) to give 10 subfractions and compound **9** (17.4 mg) was isolated from subfractions 1 and 2.

Fr. 11-12 (321 mg) was purified by Sephadex LH 20 column by eluting with MeOH to give 5 fractions (Fr.$_{1-11}$). Subfractions 5 and 6 (84.6 mg) were submitted to silica gel column eluting with $CHCl_3$:MeOH:H_2O (90:10:1, 87:13:1, 84:16:1, 80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 61:32:7) to yield 13 subfractions (Fr.$_{1-67}$). Compound **10** (7 mg) was isolated from subfractions 59-62.

Fr. 19 (314 mg) was subfractionated by silica gel column chromatography eluted with $CHCl_3$:MeOH:H_2O (80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 61:32:7) to give 5 subfractions (Fr.$_{1-34}$). Subfractions 14-20 and 21-24 (totally 76.4 mg) were applied to MPLC on reversed phase silica gel using stepwise MeOH:H_2O gradient (50-100%, 35 mL/fraction) to give 9 subfractions (Fr.$_{1-40}$) and subfraction Fr. 30-32 yielded pure **12** (12.5 mg).

Fr. 18 (412 mg) was applied to silica gel column with the solvent system $CHCl_3$:MeOH:H_2O (80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 61:32:7) to give 5 subfractions (Fr.$_{1-42}$). Subfractions Fr. 16-18, Fr. 19-21, Fr. 22 and Fr. 23-26 (totally 103.8 mg) was separated by C_{18} MPLC (50-100% MeOH: H_2O) to afford **12** (32 mg).

Fr. 23 (169 mg) and Fr. 24-26 (356 mg) were separately further purified on a silica gel column with the solvent system $CHCl_3$:MeOH:H_2O (80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 61:32:7). Some of the obtained same subfractions were combined (247.3 mg) and subjected to C_{18} MPLC column (50-100% MeOH: H_2O, 35 mL/fraction) to afford **13** (36.6 mg).

2.3.4 Structure elucidation of the compounds

Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) and Mass Spectral (MS), DEPT, ^{1}H-^{1}H COSY, 2D HMQC and HMBC techniques were used for the structure elucidation of the compounds. NMR spectra were recorded on a Varian Mercury 400 FT-NMR spectrometer and 600 MHz Bruker DMX-600 spectrometer instrument (400 MHz and 600 MHz for ^{1}H-NMR, and 100 MHz and 150 MHz for ^{13}C NMR) and using CD_3OD as solvent. The Mass spectra were performed using a Waters ZQ Micromass LC-MS spectrometer by using ESI(+) method.

2.4. Determination of total phenolic content of the extract and subextracts

Total phenol content of the ethanol extract and its subextracts of *S. grandis* were determined by using the Folin-Ciocalteu technique [9]. Sample (50 μL) was mixed with 250 μL of undiluted Folin-Ciocalteu reagent. After 1 min, 750 μL of 20% (w/v) aqueous Na_2CO_3 was added, and the volume was made up to 5.0 mL with H_2O. After 2 h incubation at 25ºC, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The total phenolics were determined as gallic acid equivalents (mg gallic acid/g extract), and the values are presented as means of triplicate analyses.

2.5. Biological activity tests
2.5.1. Test animals

Male Swiss albino mice (20-25 g) were purchased from Kobay Experimental Animals Laboratory, Ankara/Turkey. The animals left for two days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access of water. A minimum of six animals were used in each group. The study was permitted by the Institutional Animal Ethics Committee (Gazi University Ethical Council Project Number: G.U.ET-05.004; Approval date: April 2011). Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals.

2.5.2. Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or ASA (100 mg/kg) in 0.5% CMC, used as reference drug.

2.5.3. p-Benzoquinone-induced abdominal constriction test in mice

p-Benzoquinone-induced abdominal constriction test was performed on mice for the determination of antinociceptive activity as described earlier [10]. After 60 min of oral administration of a test sample, the mice were intraperitonally injected with 2.5% (w/v) PBQ solution (0.1 mL/10 g body weight) in distilled water, whereas control animals received an appropriate volume of dosing vehicle. The mice were placed individually for observation and the total number of the abdominal contractions (writhing movements) were counted for the following 15 min, starting 5 min after the PBQ injection. The data represents the average of the total number of writhes observed. Antinociceptive activity was expressed as the percentage change from writhing controls. ASA (100 mg/kg) served as the reference drug in this test.

2.5.4. Carrageenan-induced hind paw edema

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity [11]. After 60 min of oral administration of either test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of carrageenan (0.5 mg/25 μL) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, saline solutions (25 μL) were injected into that of the left hind paw. Paw edema was measured at 90 min interval during 6 h after induction of inflammation. Indomethacin (10 mg/kg) was used as the reference drug. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of a control group and analyzed by using statistical methods.

2.5.5. Acute toxicity

Animals employed in the carragenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

2.5.6. *Gastric-ulcerogenic side effect*

After the antinociceptive activity experiment mice were sacrificed and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings on the gastric mucosa.

2.5.7. *Statistical analysis*

All data obtained from animal experiments were expressed as mean S.E.M. Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p < 0.05$ was considered to be significant [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$].

3. Results and Discussion

In case of an injury, inflammatory mediators are released interacting with nociceptors and facilitating the transmission of pain signals through the nervous system [12]. In the present study, anti-inflammatory and antinociceptive activities of *S. grandis* were investigated by carrageenan-induced paw edema and *p*-benzoquinone-induced nociception models, respectively, through bioassay-guided fractionation and isolation procedures.

For the preliminary activity assessment, EtOH extract from the aerial parts of *S. grandis* was prepared and tested in *in vivo* anti-inflammatory and analgesic activity models. Moreover, EtOH extract and its subextracts were investigated for the total phenolic content by using a spectrophotometric method; Folin-Ciocalteau [9]. The results were presented in Table 1. The phenolic content of the EtOH extract and its subextracts were determined between 18.60 ± 1.65 and 56.10 ± 1.34 mg/g.

Table 1. Total phenolic content of the extract and subextracts of *Salsola grandis*.

Extracts & Subextracts	Total phenolics (mg gallic acid/g extract ± S.E.M.)
EtOH extract	29.61 ± 1.21
n-Hexane subextract	27.56 ± 0.67
$CHCl_3$ subextract	56.10 ± 1.34
EtOAc subextract	40.66 ± 0.56
n-BuOH subextract	45.70 ± 0.89
R-H_2O subextract	18.60 ± 1.65

As shown in Table 2, EtOH extract showed a significant anti-inflammatory activity (between 31.5 and 35.3%). Bioassay-guided fractionation procedures were then conducted on this extract. The EtOH extract was submitted to first step fractionation process through subsequent solvent extractions in increasing polarity. Each subextract was administered to experimental animals. Among the subextracts obtained through successive solvent extractions, *n*-hexane and $CHCl_3$ subextracts were found totally inactive, and EtOAc and remaining water (R-H_2O) subextracts did not show any consistent and noteworthy effect. The inhibitory effect of *n*-BuOH subextract on carrageenan-induced paw edema was found to be 27.8-32.9%. On the other hand, 37.6% inhibition was detected for *n*-BuOH subextract in *p*-benzoquinone-induced nociception model (Table 3). Further studies were conducted on the *n*-BuOH subextract.

Table 2. Anti-inflammatory effect of the isolated flavonoids from *Salsola grandis* on carrageenan-induced paw edema in mice.

Test compounds	Dose mg/kg	Swelling thickness ± S.E.M. ($\times 10^{-2}$ mm) (inhibitory %)			
		90 min	180 min	270 min	360 min
Control		41.2 ± 4.3	46.1 ± 4.0	50.2 ± 3.9	54.4 ± 4.5
EtOH extract	100	40.6 ± 3.2 (1.5)	31.6 ± 2.9 **(31.5)****	32.5 ± 3.0 **(35.3)****	47.2 ± 4.3 (13.2)
n-Hexane subextract	100	42.7 ± 4.2	48.1 ± 3.4	51.1 ± 4.6	57.2 ± 3.8
CHCl$_3$ subextract	100	43.5 ± 4.8	49.4 ± 4.4	49.0 ± 3.5	55.3 ± 3.9
EtOAc subextract	100	35.7 ± 2.9 (13.3)	37.2 ± 2.8 (19.3)	42.5 ± 3.1 (15.3)	50.3 ± 3.1 (7.5)
n-BuOH subextract	100	32.0 ± 3.3 (22.3)	33.3 ± 2.5 **(27.8)***	33.7 ± 3.9 **(32.9)****	48.2 ± 4.4 (11.4)
R-H$_2$O subextract	100	38.5 ± 4.2 (6.6)	47.2 ± 4.0	52.6 ± 4.9	56.5 ± 4.7
1	100	42.4 ± 3.7	44.5 ± 3.1 (3.5)	48.1 ± 3.6 (4.2)	55.3 ± 4.0
4	100	30.1 ± 3.0 **(26.9)****	34.5 ± 2.6 **(25.2)****	40.8 ± 2.1 (20.7)	43.6 ± 2.7 (19.9)
6	100	37.6 ± 3.4 (8.7)	39.3 ± 3.5 (14.8)	44.8 ± 3.9 (10.8)	50.1 ± 3.1 (7.9)
7	100	31.9 ± 2.8 (22.6)	32.6 ± 2.9 **(29.3)****	34.3 ± 2.5 **(31.7)****	40.0 ± 2.7 **(26.5)****
12	100	39.1 ± 4.4 (5.1)	44.8 ± 3.6 (2.9)	53.2 ± 4.8	56.9 ± 4.6
Indomethacin	10	30.9 ± 3.2 **(25.0)***	31.4 ± 2.7 **(31.9)****	30.8 ± 2.3 **(38.6)*****	33.9 ± 2.4 **(37.7)*****

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ significant from the control values.

Table 3. Antinociceptive effect of the isolated flavonoids from *Salsola grandis* on *p*-benzoquinone-induced nociception in mice.

| Test compounds	Dose mg/kg	Number of writhes ± S.E.M.	Inhibitory %	Gastric lesion[a]
Control	-	51.6 ± 5.1	-	0/6
EtOH extract	100	29.3 ± 3.4	**43.2*****	0/6
n-Hexane subextract	100	46.2 ± 3.9	10.5	0/6
CHCl$_3$ subextract	100	43.9 ± 4.0	14.9	0/6
EtOAc subextract	100	41.1 ± 3.6	20.3	0/6
n-BuOH subextract	100	32.2 ± 4.1	**37.6****	0/6
R-H$_2$O subextract	100	43.8 ± 4.4	15.1	0/6
1	100	42.1 ± 5.4	18.4	0/6
4	100	35.8 ± 2.9	**30.6****	0/6
6	100	40.8 ± 4.3	20.9	0/6
7	100	31.6 ± 3.5	**38.8****	0/6
12	100	46.6 ± 3.1	9.7	0/6
ASA	100	24.3 ± 2.0	**52.9*****	5/6

[a]: Number of mice with gastric lesions or bleeding. **: $p < 0.01$; ***: $p < 0.001$ significant from the control values.

After several chromatographic fractionations by using silica gel, Sephadex LH 20 column chromatography and MPLC techniques, ten flavonols, two oleanane-type saponins and one amino acid derivative compound were isolated from *n*-BuOH extract and their chemical structures (Figure 1) were elucidated through spectroscopic analysis techniques. The structures of the isolated flavonoids were

identified as isorhamnetin-3-*O*-rutinoside (**1**), quercetin-3-*O*-rutinoside (**2**), quercetin-3-*O*-metylether (**3**), tiliroside (**4**), isorhamnetin-3-glucuronide (**5**), isorhamnetin-3-*O*-glucoside (**6**), quercetin-3-*O*-galactoside (**7**), quercetin-3-*O*-rhamnoside (**8**), quercetin (**9**) and manghaslin (**10**) by spectroscopic data which were also compared with the data obtained from published in related literatures [6, 13-17]. Among them, **3**, **4, 5**, **7**, **8** and **10** were the new compounds for the genus *Salsola*. Moreover, in the present study, the saponin type compounds, momordin II b (**11**) and momordin II c (**12**) were isolated from the genus *Salsola* for the first time. Their structures were identified by comparison with the reported spectral data in the literature [18-21]. Furthermore, *N*-acetyltryptophan (**13**) as amino acid derivative compound was also isolated from the *n*-BuOH fraction of the EtOH extract of *S. grandis*. Among them, **1**, **2**, **6**, **9**, and **13** were isolated for the first time from *S. grandis*. Previously, **13** was isolated from *S. collina* Pall., which is widely distributed in Northeastern and Southwestern of China [21]. In this study, the structure of **13**, the spectral data of which have not been fully reported previously, was also confirmed by using extensive two-dimensional NMR methods (Table 4) and LC-MS analysis. The mass spectrum showed the major ion peak at *m/z* 247 and 269 which were assigned to $[M+H]^+$ and $[M+Na]^+$, respectively. The molecular formula of **13** was established as $C_{13}H_{14}N_2O_3$ by LC-MS analysis.

In the previous studies, anti-inflammatory activities of **2**, **3**, **5**, **8** and **9** were investigated [23-25]. The isolated compounds **10**, **11** and **13** were not in enough amounts to test their biological activities. Therefore, activities of **1**, **4**, **6**, **7** and **12** which have not been investigated before, were evaluated in the present study. Previously, Sala et al. (2003) reported the anti-inflammatory effect of **4** on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ear and phospholipase A_2-induced paw edema models [26]. In our study, the anti-inflammatory and antinociceptive effects of the same compound were investigated in different *in vivo* experimental models.

The anti-inflammatory activities of **4** and **7** were almost as potent as that of indomethacin (10 mg/kg) which exerts 25.0-37.7% anti-inflammatory activity (Table 2). These compounds also displayed antinociceptive activities with the values of 30.6 and 38.8% respectively at 100 mg/kg dose, but not as potent as acetylsalicylic acid (ASA). Other flavonol derivative compounds **1**, **6** and saponin derivative compound **12** showed neither anti-inflammatory nor antinociceptive activities (Table 3), indicating that methoxy group in the 3' position (R_1) of the flavonoid structure decreased the activity.
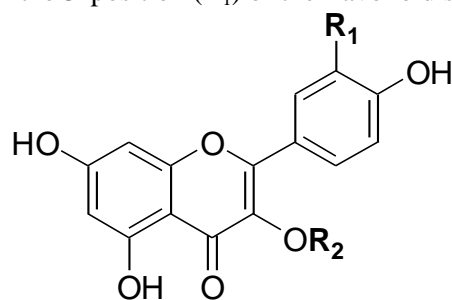
	Flavonols	R_1	R_2
1	Isorhamnetin-3-*O*-rutinoside	OCH_3	6-*O*-α-L-Rhamnopyranosyl-β-D-glucopyranose
2	Quercetin-3-*O*-rutinoside	OH	6-*O*-α-L-Rhamnopyranosyl-β-D-glucopyranose
3	Quercetin-3-*O*-metylether	OH	CH_3
4	Tiliroside	H	4-*O*-*E*-Coumaroyl-β-D-glucopyranose
5	Isorhamnetin-3-glucuronide	OCH_3	β-D-Glucuronopyranose
6	Isorhamnetin-3-*O*-glucoside	OCH_3	β-D-Glucopyranose
7	Quercetin-3-*O*-galactoside	OH	β-D-Galactopyranose
8	Quercetin-3-*O*-rhamnoside	OH	α-L-Rhamnopyranose
9	Quercetin	OH	H
10	Manghaslin	OH	2,6-di-*O*-α-L-Rhamnopyranosyl-β-D-glucopyranose
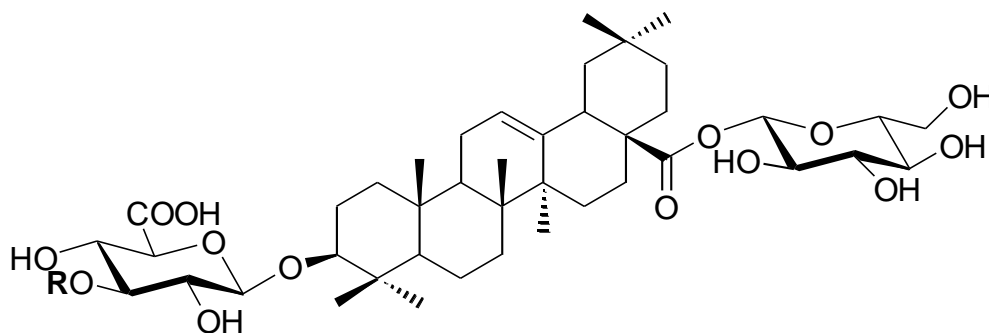
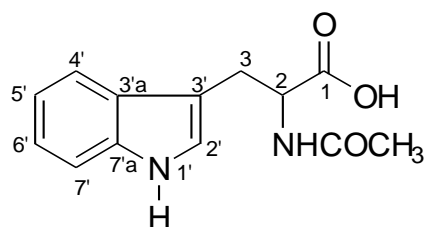|	Saponins	R
11	Momordin II b	H
12	Momordin II c	β-D-Xylopyranose

13 *N*-Acetyltryptophan

Figure 1. Chemical structures of the isolated compounds **1-13.**

Table 4. NMR data of *N*-Acetyltryptophan (**13**) (400 MHz, CD$_3$OD).

C/H Atom	δ_H [mult., J (Hz)]	δ_C	DEPT	HMBC (C→H)
1	-	177.7	C	H-3a,b, H-2
2	4.33 †	56.6	CH	H-3a,b
3a	3.23 dd (15.0, 4.2)	29.0	CH$_2$	H-2
3b	2.99 dd (15.0, 7.4)			
1'	-	-	-	-
2'	7.07 s	124.3	CH	H-3a,b
3'	-	112.6	C	H-2', H-4', H-3a,b
4'	7.52 brd (8.2)	119.7	CH	H-6'
5'	6.88 dd (t) (8.2)	119.3	CH	H-7'
6'	6.96 dd (t) (8.2)	121.8	CH	H-4'
7'	7.22 brd (8.2)	112.1	CH	H-2', H-5'
3'a	-	129.3	C	H-3a,b, H-5', H-2', H-7'
7'a	-	137.3	C	H-6', H-2', H-4'
2-NH<u>C</u>OCH$_3$	-	170.4	C	H-2, 2-NHCOC<u>H</u>$_3$
2-NHCO<u>C</u>H$_3$	2.53 s	40.8	CH$_3$	-

In carrageenan-induced paw edema model, histamine and serotonin released in the early phase (90-180 min), whereas prostaglandins, proteases and lysosomes are activated in the second phase (270-360 min) of the inflammation [27]. The results of the present study revealed that EtOH extract

and its *n*-BuOH subextract had significant inhibitory effect on both phases. On the other hand, tiliroside showed inhibitory activity on the first phase, while quercetin-3-*O*-galactoside demonstrated its activity mainly in the second phase.

Flavonoids have diverse therapeutic uses due to their important roles on biological systems [28]. The anti-inflammatory and antinociceptive activities of various flavonoid derivatives were previously reported by several authors [29-31]. It has been shown that flavonoids have capacity to inhibit cyclooxygenase and 5-lipoxygenase pathways which provide a reduction in the release of arachidonic acid and the formation of inflammatory metabolites [32]. According to the *in vitro* studies, the flavonoids possess inhibitory effect on the nitric oxide production and expression of iNOS [33].

Especially quercetin was demonstrated to have many beneficial effects on the treatment of cardiovascular problems, cancer, ulcer, inflammation and nociception. Antinociceptive activity of quercetin was hypothesized to be acting through the modulation of opioidergic mechanism in diabetic neuropathic pain [29]. Additionaly, it was suggested that quercetin blocks both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade [28]. Actually this data was supported by the conclusion that quercetin is an effective inhibitor of phospholipase A_2 which catalyzes the hydrolysis of phospholipids to release arachidonic acid as the precursor of the inflammatory response [34]. On the other hand, the kaempferol glycosides, tiliroside and astragalin, possess potent inhibitory effect on TNF-α production [30]. In the present study, tiliroside and quercetin-3-*O*-galactoside were found to have significant anti-inflammatory and antinociceptive effects. Similarly, in the previous studies, quercetin-3-*O*-galactoside was found to be the compound of the active plant extracts with anti-inflammatory activity [35]. Therefore, results of the present study have confirmed the outcome of the previous researches.

In conclusion, we provided the first evidence for the anti-inflammatory and antinociceptive activities of *S. grandis* in experimental models of inflammatory and nociceptive pain. According to the results of this study, tiliroside and quercetin-3-*O*-galactoside were isolated as bioactive compounds from *S. grandis* through biassay-guided procedures. However, further biological activity studies should be conducted using different test models of inflammation and nociception for the clarification of the mechanism(s) of action.

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