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Flavonoid and saponin rich fractions of kiwi roots (*Actinidia arguta* (Sieb.et Zucc.) Planch) with antinociceptive and anti-inflammatory effects

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The roots of kiwi (*Actinidia arguta* (Sieb.et Zucc.) Planch) have medicinal uses as anti-tumour, antinociceptive and anti-inflammatory agents. We aimed to evaluate the antinociceptive and anti-inflammatory effects of the major fractions of kiwi roots. The ethanolic extract of the plant roots was partitioned using a liquid–liquid extraction procedure to give five major fractions. Following phytochemical screening of isolated fractions, the total extract and each fraction were evaluated for their antinociception and anti-inflammatory effects using acetic acid, hot plate test, formalin and carrageenan-induced paw edema tests, respectively. The results indicated that the total extract, ethyl acetate fraction (EAF) and n-butanol fraction (BF) exhibited significant inhibition of acetic acid-induced writhing, and both phases of the formalin-induced pain response increased in time of response to thermal stimulation in hot plate test and exhibited significant dose-related inhibition of carrageenan induced paw oedema volumes when compared with the control group. It can be concluded that the flavonoid and saponin content of kiwi roots can be responsible for antinociception and anti-inflammatory effects of the plant, respectively.

Key words: *Actinidia arguta*, anti-inflammatory, antinociceptive, flavonoid, saponin.

INTRODUCTION

Actinidia arguta (Sieb.et Zucc.) Planch belongs to Actinidia genus in the family of Actinidiaceae, which is a large deciduous vine. The genus *Actinidia* contains around 54 species. The plant is chiefly distributed in the mountains of South China. Some species are also found in Siberia, Japan, Indochina, Malaysia, Indonesia (Rosemary et al., 1994), New Zealand (Kyoung and Hong, 2009; Robert, 1983).

As known to all, the fruits of *A. arguta* (Sieb.et Zucc.) Planch (kiwi fruit) are edible, but interestingly, the roots of kiwi, which are called Tengligen usually, are used to treat tumors of the alimentary canal widely in traditional Chinese medicine, such as gastric cancer, esophagus cancer, liver cancer etc. (Song et al., 2001; Zhong et al., 2004; Liang et al., 2007). Pharmacological research indicated that the extract of Tengligen could inhibit the carcinoma of gastric cells (Wei et al., 2005; Li et al., 2004; Fu et al., 2011). Zhang et al. (2007) and Guo et al. (2011) reported that the extracts from Tengligen by n-butyl alcohol had good inhibitory effect on human carcinoma of esophagus cells (Eca-109). Sun et al. (2011) have reported that the extracts from Tengligen by ethyl acetate could induce the apoptosis of EC109 cell in a dose-and time-dependent manner *in vitro*. At the same time, the extracts could down regulate the expression of Bcl-2, up regulate the expression of Bax protein level and increase intercellular calcium which promotes apoptosis. Lou et al. (2009) notified that the fraction extracted by

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chloroform displayed potent activity against hepatocellular carcinoma with Bel-7402 cells and the results were confirmed in murine hepatocellular carcinoma H22 and human Bel-7402 xenograft. The results of recent study showed that the extracts could inhibit the proliferation of A549 cells during the G_0-G_1 period and significantly decrease the cell ratio of S stage (Wang et al., 2010).

To the best of our knowledge, there was no study on the evaluation of antinociceptive and anti-inflammatory effects of the major fractions of kiwi roots so far. Therefore, the present study was aimed to evaluate the antinociceptive and anti-inflammatory effects of fractions of ethanol extract of kiwi roots. The antinociceptive effect was examined against chemically and thermally induced nociceptive pain in mice, using the acetic acid, hot plate test and formalin methods. In addition, the anti-inflammatory effect was investigated by utilizing the carrageenan induced paw edema method in mice.

MATERIALS AND METHODS

The roots of *A. arguta* (Sieb.et Zucc.) Planch was collected in Changbai Mountains, Jilin Province, China on March, 2012, and identified by Dr. Yue-Chun Sun, Life Science and Technology College, Heilongjiang Bayi Agricultural University, Daqing, China. Ibuprofen powder (Tianjin Shike Pharmaceutical Co. Ltd., China) and carrageenan (type I, Sigma Co., UK) were of pharmaceutical grade. The other chemicals and reagents were of analytical grade. An amount of 5 kg of dried kiwi roots was extracted with ethanol applying heat refluxing method. The obtained extract was evaporated under vacuum to give a viscose mass. Then, an amount of 525 g of the extract was suspended in 1000 ml of distilled water and was partitioned sequentially with cyclohexane (5 × 500 ml), chloroform (5 × 500 ml), ethyl acetate (5 × 500 ml), n-butanol (5 × 500 ml) at room temperature. Totally, six major fractions were collected and concentrated under vacuum and stored at -20°C until pharmacological tests.

Phytochemical screening

The total extract of the kiwi roots and each of the fractions were screened to investigate the presence of saponins, flavonoids, alkaloids and terpenoids.

Animals

Wistar rats (age, 8 to 12 weeks; weight, 180 to 200 g) and imprinting control region (ICR) mice (age, 2 to 3 weeks; weight, 18 to 22 g) of either sex were used for the experiments. Animals were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. and used once only. The animals were kept in standard laboratory conditions (relative humidity: 55 to 60%, room temperature 25 ± 2°C, 12 h light/dark cycle) and had free access to standard diet and water *ad libitum* during the experiment. The animals were acclimatized to the laboratory environment for a period of 7 days prior to performing the experiments.

Acetic acid induced writhing response

The acetic acid-induced writhing test was carried out using the reported technique (Pinheiro et al., 2010; Mariana et al., 2012). The mice were pre-treated with positive drug and test drugs for 3 days, half hour after final administration 0.6% acetic acid (0.1 ml/10 g) by intraperitoneal injection to mice. The number of writhing movements during the next 15 min was recorded. The number of writhes in each treated group was compared with control group which received only the saline. The inhibition rate of writhes was calculated thus:

$$[(Mean_{control} – Mean_{test}) / Mean_{control}] \times 100$$

In the writhing test, mice were randomly divided into eight groups. They are control group with i.g. isometrical physiological saline, the test drug groups at the dosage of 50 mg/kg of five major fractions and total extract (200 mg/kg) in the experiments with i.g. The mice of positive drug group were conducted with i.g. ibuprofen (50 mg/kg) to compare the results obtained.

Hot plate test

The method is an adaptation of that described by Eddy and Leimbach (1953) and Ramzi et al. (2012). In the test, female mice were placed in a 24 cm diameter glass cylinder on a heated metal plate maintained at 55 ± 1°C. Animals were habituated twice to the hot plate in advance. Response was defined as licking or biting of a paw, or jumping. The time in seconds between the placing of the animal on the platform and reaction was recorded as the response latency. The mice exhibiting latency time greater than 30 s or less than 5 s were excluded. Animals were divided into eight groups of ten mice each and pretreated with oral dose of total extract (200 mg/kg), 50 mg/kg of five major fractions, respectively. Ibuprofen (50 mg/kg) was used as the standard drug. Mice were tested at 30, 60, 90 and 120 min after oral administration of the extracts and ibuprofen.

Formalin induced nociception

The procedure was similar to the method described by Hunskaar and Hole (1987) with some modifications done by Gomes et al. (2007). Animals were divided into eight groups of ten mice each and pretreated with oral dose of five major fractions (50 mg/kg, respectively) and total extract (200 mg/kg). Ibuprofen (50 mg/kg) was used as the standard drug. The mice were pre-treated with positive drug and test drugs for 3 days, half hour after final administration 20 µl of 5% v/v formalin was injected subcutaneously into the right hind paw of mice. The time that animals spent on licking or biting responses of the injected paw was taken as an indicator of pain response. On the basis of the response pattern described by Mariana et al. (2012), responses were measured for 5 min after formalin injection (early phase, neurogenic pain response) and 15 to 30 min after formalin injection (later phase, inflammatory pain response).

Carrageenan induced paw edema

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory effect (Winter et al., 1962; Zhu et al., 2012; Ali et al., 2012). Rats were orally treated with the saline, five major fractions (50 mg/kg, respectively), total extract (200 mg/kg) and ibuprofen (50 mg/kg), 30 min prior to injection of 1% carrageenan (0.1 ml) in the right hind paw sub-plantar region of each mouse. Hind paw volumes were measured using the plethysmograph at 0.5, 1, 2 and 3 h intervals after injection of the phlogistic agent. Percent of inhibition was calculated according to the hind paw volumes.

Table 1. The result of phytochemical screening of total extract and separated fractions from Kiwi roots.

Sample	Saponin	Flavonoid	Alkaloid	Terpenoids
Total extract	+++	+++	-	++
CYF[a]	-	-	-	++
CHF[b]	-	-	-	-
EAF[c]	+	+++	-	-
BF[d]	+++	+	-	-
Aqueous fraction	+	+	-	-

[a]Cyclohexane fraction; [b]Chloroform fraction; [c]Ethyl acetate fraction; [d]n-butanol fraction; +++: high content; ++: medium content; +: low content; -: no content (content was evaluated as the sediment or the intensity of color).

Table 2. The antinociceptive effect of total extract and major fractions separated from kiwi roots in acetic acid-induced nociception.

Group	Dose (mg/kg)	Number of writhing (per 15 min)	Inhibition (%)
Control	-	56.27±2.03	-
Ibuprofen	50	10.48***±2.46	81.37***
CYF[a]	50	54.12±2.49	3.82
CHF[b]	50	52.27±2.12	7.11
EAF[c]	50	12.59***±2.26	77.63***
BF[d]	50	16.41***±2.57	70.84***
Aqueous fraction	50	53.43±2.64	5.47
Total extract	200	11.73***±2.87	79.15***

[a]Cyclohexane fraction; [b]Chloroform fraction; [c]Ethyl acetate fraction; [d]n-butanol fraction; *$p < 0.05$ significantly different from control; **$p < 0.01$ significantly different from control and ***$p < 0.001$ significantly different from control.

Statistical analysis

The experimental data was expressed as mean ± standard error of mean (SEM). The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey's t-test. The differences with $p < 0.01$ were considered statistically significant, $p < 0.001$ were considered highly significant.

RESULTS

Fractionation and phytochemical screening

The yield of extraction was measured about 42.7%, from which five major fractions were separated. As shown in Table 1, in phytochemical screening, the fractions of ethyl acetate and n-butanol exhibited strong positive reaction for flavonoids and saponins, respectively.

Effect of kiwi roots on acetic acid induced writhing

In the acetic acid-induced writhing mice (Table 2), the total extract, ethyl acetate fraction (EAF), n-butanol fraction (BF), at the doses used, exhibited a significant analgesic effect after oral administration in mice submitted to acetic acid-induced writhing when compared with control group ($p < 0.001$). In addition, the highest analgesic activity observed with the total extract (200 mg/kg) was lower than the analgesic activity of ibuprofen (50 mg/kg). The maximal inhibition of the nociceptive response (81.37%) was achieved by the ibuprofen at a dose of 50 mg/kg. However, the results presented in Table 2 showed that no significant analgesic effects were generated at all doses of CYF (Cyclohexane fraction), CHF (Chloroform fraction) and aqueous fraction.

Effect of kiwi roots on the nociception in hot plate test in mice

In the hot plate test, the results presented in Figure 1 show that the total extract, EAF, BF, ibuprofen produced a significant increase in the response time in the heated plate experiment from 30 to 120 min, respectively ($p < 0.001$). At the same time, the antinociceptive response observed with the total extract, EAF, BF was considerably more pronounced than that obtained with ibuprofen at all the tested doses from 60 to 120 min. Nevertheless, the analgesic activity observed with the total extract, EAF, BF was lower than the analgesic activity of ibuprofen from 0 to 30 min.

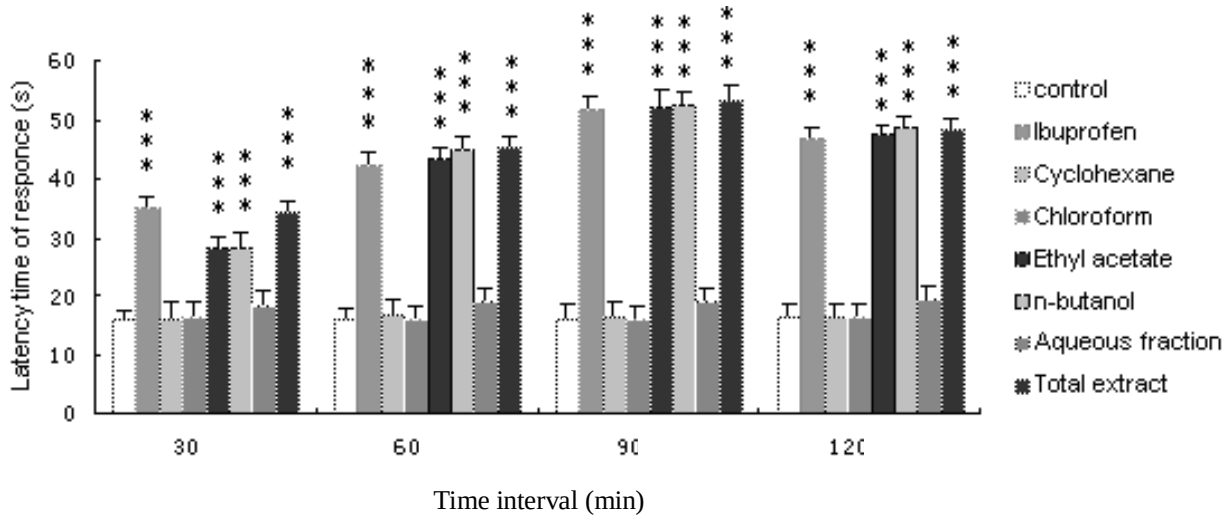

Figure 1. The antinociceptive effect of total extract and major fractions separated from kiwi roots in hot plate test. The effect has been calculated on the basis of latency time of response. Each point is the mean ± SEM of ten animals. *p < 0.05 ,**p < 0.01 ,***p < 0.001 in comparison to normal saline group.

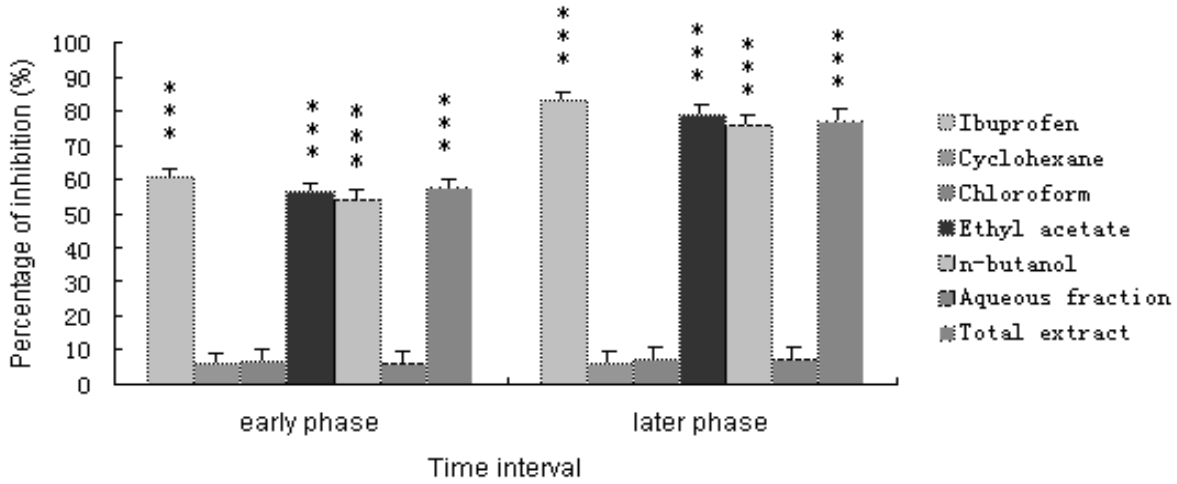

Figure 2. The antinociceptive effect of total extract and major fractions separated from kiwi roots in formalin-induced nociception. The effect has been calculated on the basis of percentage of pain inhibition. Each point is the mean ± SEM of ten animals. *p < 0.05 ,**p < 0.01 ,***p < 0.001 in comparison to normal saline group.

Effect of kiwi roots on formalin induced pain

As indicated in Figure 2, treatment with total extract (200 mg/kg), EAF (50 mg/kg), BF (50 mg/kg) and ibuprofen (50 mg/kg) significantly increased the percentage of pain inhibition in early phase and later phase of formalin test (p < 0.001). Moreover, in both early and late phases of experiment, the percentage of pain inhibition of total extract, EAF and BF was as good as ibuprofen. However, as shown in Figure 2, the percentage of pain inhibition of total extract, EAF, BF, and ibuprofen was more in the later phase than in the early phase.

Carrageenan induced paw edema

In carrageenan-induced hind paw edema experiment, as shown in Table 3, the examined samples demonstrated a significant anti-inflammatory activity at all tested doses in comparison with control group 3 h after carrageenan administration (p < 0.001). Amongst the examined samples, the total extract (200 mg/kg), EAF (50 mg/kg), BF (50 mg/kg) and ibuprofen (50 mg/kg) could significantly induce a reduction in paw edema from 1 to 3 h of experiment in animals (p < 0.05, p < 0.01, p < 0.001). The total extract exhibited the highest inhibition of paw edema

Table 3. The antinociceptive effect of total extract and major fractions separated from kiwi roots in carrageenan-induced hind paw edema.

Group	Percent of inhibition (%)			
	0.5 h	1 h	2 h	3 h
Ibuprofen (50 mg/kg)	8.21±1.68	43*.73±2.51	71.79**±2.49	96.61***±2.16
CYF[a] (50 mg/kg)	3.15±2.62	19.82±2.85	36.82±2.57	45.07*±3.93
CHF[b] (50 mg/kg)	4.41±2.31	16.58±2.03	45.73*±2.71	49.79*±2.17
EAF[c] (50 mg/kg)	16.57±1.87	46.45*±1.73	78.96**±2.63	95.31***±2.82
BF[d] (50 mg/kg)	17.15±2.69	44.82*±2.36	74.89**8±2.27	93.63***±2.93
AF[e] (50 mg/kg)	11.47±2.52	19.87±2.93	45.25*±3.09	54.46*±2.97
Total extract (200 mg/kg)	15.36±1.82	42.76*±2.09	79.74**±2.94	97.62***±1.88

[a]Cyclohexane fraction; [b]Chloroform fraction; [c]Ethyl acetate fraction; [d]n-butanol fraction; [e]Aqueous fraction; *p < 0.05 significantly different from control; **p < 0.01 significantly different from control; ***p < 0.001 significantly different from control.

at 3 h in comparison with the control group (p < 0.001).

DISCUSSION

This study investigated the antinociceptive and anti-inflammatory effects of kiwi roots in three analgesic models: acetic acid-induced writhing model, hot plate test and formalin-induced licking model for assessing antinociceptive effect as well as carrageenan-induced hind paw edema model for assessing anti-inflammatory.

The acetic acid-induced writhing reaction in mice has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents, and is described as a typical model for visceral inflammatory pain (Dickenson and Besson, 1997). The hot plate test, which utilizes thermal stimulus to induce pain, is frequently used to evaluate centrally mediated antinociceptive effect (Su et al., 2011). The acetic acid-induced writhes and hot plate test methods have been regarded as useful techniques of evaluating the peripherally and centrally acting analgesic drugs, respectively (Eddy and Leimback, 1953; Koster et al., 1959). For this reason, the acetic acid-induced writhes in mice and hot plate test were selected to observe the analgesic response in this study. In order to explore the pathway of analgesic activity of extract of kiwi roots, ibuprofen was selected as the reference drug. The results indicated that the total extract, EAF and BF exhibited a significant analgesic effect which significantly inhibited the number of writhes and increased in time of response (latency) to chemical stimulation and thermal stimulation in comparison with the control group (p < 0.001) (Table 2 and Figure 1). In a word, the results of the study show that at all dose levels used, the total extract, EAF and BF significantly reduced acetic acid-induced writhes which suggests that its analgesic effects could be peripherally mediated. The increase in the reaction time, by the extract to the thermal stimulus in the hot plate test indicates that the total extract, EAF and BF also possess a central analgesic effect.

The formalin-induced nociception is a well-described model for evaluating the mechanism of pain and analgesia (Hunskaar and Hole, 1987). The study has shown that the total extract, EAF and BF can inhibit both phases of formalin-induced pain with a more potent effect on the later than the early phase (Figure 2). Considering the inhibitory property of the total extract, EAF and BF on the formalin test, we might suggest that the analgesic activity of the extract could be dependent on central and peripheral sites of action. The conclusion is in good agreement with the conclusion reported by Shibata et al. (1989). Taken together, the ability of the total extract, EAF and BF to suppress pain perception might be mediated via peripheral and central pathways of pain perception.

The carrageenan test is highly sensitive to non-steroidal anti-inflammatory drugs and has long been accepted as a useful phlogistic tool for investigating new anti-inflammatory drugs (Just et al., 1998). Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents (Willoughby and DiRosa, 1972) and therefore has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa et al., 1995). The results obtained show that the extract possesses anti-inflammatory activity (Table 3).

Previous investigations have reported that saponins, flavonoids, phenylpropanoids, quinones and steroids compounds have been separated and structurally identified from kiwi roots, among them are ursolic acid, oleanolic acid, succinic acid, quercetin, β-sitosterol, isoscopoletin, aesculetin, fraxetin, esculetin, umbelliferone, vanillic acid, protocatechuic acid, vanillic acid 4-O-β-D-glucopyranoside, carotenoids, lutein, and 5, 7-dihydroxychromone, etc. Flavonoids, saponins, tannins, phenolic compounds, and glycosides have all been associated with various degrees of anti-inflammatory and analgesic activities (Garcia et al., 1973; Hosseinzadeh and

Younesi, 2002; Wang et al., 2008; Thirugnanasambantham et al., 2007; Viana et al., 1998; Chang and Case, 2005; Yutaka et al., 1992; Yutaka et al., 1994; Lai and Xu, 2007; Lahlou et al., 2001; Cassano et al., 2006; Xu et al., 2010; Anne-Marie et al., 2002; Fu et al., 2010; Qian et al., 1999). The result indicated that the mechanism of antinociceptive and anti-inflammatory effect of extract of kiwi roots may be related to flavonoids and saponins.

However, the pharmacological studies have focused mainly on crude extracts, and many of the constituents responsible for different pharmacological activities remain unknown. Therefore, the antinociceptive and anti-inflammatory effects observed in this study are perhaps due to the activity(ies) of one or a combination of some of the identified classes of compounds. More studies are needed to prove clinical efficacy and reveal the exact mechanism of action.

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