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Full Length Research Paper

Anti-inflammation activity and chemical composition of flower essential oil from *Hedychium coronarium*

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Hedychium coronarium **Koen. (Family Zingiberaceae), popularly named butterfly ginger, is widely available in tropical and subtropical regions. It has been used in folk medicine for many conditions, such as contusion inflammation, anti-rheumatic and so on. In this study, chemical compositions and anti-inflammatory activity of this flowers' essential oil were investigated for the first time. Followed by GC–MS analysis, a total of 29 components were identified and the main constituents included *β-trans*-ocimenone (28.05%), linalool (18.52%), 1,8-cineole (11.35%), *α*-terpineol (7.11%), 10-epi-*γ*-eudesmol (6.06%), sabinene (4.59%) and terpinen-4-ol (3.17%). We measured the antioxidant activity of the essential oil *in vitro* (DPPH reduction assay and ferric reducing antioxidant power assay) and the anti-inflammatory activity *in vivo* (carrageenan-induced hind paw edema in rats). The oil (100 mg/kg *p.o.*) produced significant inhibition of paw oedema, but showed poor antioxidant activity (with the DPPH IC_{50} value of 1091.00 µg/ml and FRAP value of 0.22 µmol Fe^{2+}/mg). The results reveal that there is no direct correlation between anti-inflammatory effect and antioxidant activity of this essential oil.**

Key words: *Hedychium coronarium*, essential oil, anti-inflammation, antioxidant activity.

INTRODUCTION

The Zingiberaceae plant *Hedychium coronarium* Koen., which has many common names including butterfly ginger, butterfly lily, cinnamon jasmine, garland flower and ginger lily is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries and so on. The rhizome of *H. coronarium* ("Tuqianghuo"in Chinese) has been used for the treatment of headache, diabetes, contusion inflammation and sharp pain due to rheumatism in Chinese traditional medicine, while it is also used as a febrifuge, tonic, excitant and anti-rheumatic in the Ayurvedic system of traditional Indian medicine (Jain et al., 1995).

Studies on the chemical composition of the rhizome and flower of *H. coronarium* resulted in the isolation of several labdane-type diterpenes and farnesane-type sesquiterpenes (Itokawa et al., 1988a,b; Nakatani et al., 1994; Yoshikawa et al., 1994, 1998; Yamahara et al., 1995; Matsuda et al., 1998, 2001a,b,c,d, 2002a,b; Muraoka et al., 2001; Morikawa et al., 2002a; Shrotriya et al., 2007). Simultaneously, in the course of pharmacological studies of this natural medicine, it was reported to have analgesic and anti-inflammatory activities in animal model (Seikou et al., 2008) and cytotoxic activity on Chinese hamster V-79 cells (Itokawa et al., 1988a), showing inhibitory effects on the vascular permeability induced by acetic acid (AcOH) in mice and nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages, as well as the inhibition of the release of b-hexosaminidase from rat basophilic leukemia (RBL-2H3) cells (Matsuda et al., 2002b; Morikawa et al., 2002b) and representing a hepatoprotective effect on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes (Shrotriya et al., 2007; Yoshikawa et al., 2008). In addition, antimicrobial testing and gas chromatographic analysis of pure oxygenated monoterpenes and aroma chemicals with chiral and achiral columns were performed and published (Jirovetz et al., 2005; Schmidt et al., 2005). However, the chemical constituents as well as the pharmacological properties of *H. coronarium* flowers essential oil remained uncertain. The objective of the

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present study was to identify the main constituents of the essential oil from the flowers of *H. coronarium* and to investigate the antioxidant and anti-inflammatory activities for the first time.

MATERIALS AND METHODS

Plant materials and essential oil preparation

The fragrant white flowers held in dense spikes of *H. coronarium*, appeared in late spring through summer were collected in August 2008 from plants growing in natural stands in different locations of Hangzhou city, Zhejiang Province, People's Republic of China. The plants were selected at random. The samples were immediately placed in plastic bags and carried to the laboratory within a maximum of 6 h prior to the experiment. The botanical identity was confirmed by the Lab of Plant Systematic Evolution and Biodiversity, Zhejiang University. Voucher specimens were deposited in the Herbarium of Zhejiang University (ZJUH, H2008026), China.

Flowers (0.2 kg) were cut into pieces and subjected to conventional hydrodistillation in a modified Clevenger-type apparatus for 3 h, cooled, centrifuged at 15,000 rpm for 10 min and the supernatants were extracted with diethyl ether. The ether phase was then dried over anhydrous sodium sulphate (Na_2SO_4) overnight, evaporated in vacuum at low temperature and reduced pressure by rotary evaporator and kept in a labeled, sterile screw-capped bottle at –20°C until use.

GC–MS analyses of essential oil

The GC-MS analysis was done in a HP-6890 Series GC System / HP-5973 Mass Selective Detector equipped with a HP-5ms fused silica capillary column with a (5% phenyl)-methylpolysiloxane stationary phase (30 m long × 0.25 mm i.d., film thickness 0.25 µm). The temperature of the column was programmed at 50°C for the first 2 min and was then increased 3°C per minute up to the isothermal at 250°C (being held for 30 min). The injector and detector temperatures were maintained at 250°C. The carrier gas was helium at a flow rate of 1.0 ml/min. The quadrupole mass spectrometer was scanned over the range 30–350 amu at 3 scan s^{-1}, with an ionizing voltage of 70 eV, ionic source temperature of 230°C, split ratio of 1:20 and an ionization current of 150 µA. The oil components were identified by retention times (Davies, 1990; Adams, 2001), retention indices, co-injection of the standard and compareson with a homologous series of National Institute of Standards and Technology (NIST) V.2.0 GC–MS library. Relative amount of the individual component was obtained electronically by FID peak-area normalization without the use of correction factors.

Experimental animals

Male Wistar rats weighing 200 ± 30 g were used for the evaluation of anti-inflammatory activity. Animals were obtained from the Central Animal House, Zhejiang Chinese Medical University, China. They were fed with standard pellet diet and water *ad libitum* and kept in the laboratory environment (23 ± 2°C under a 12 h dark/12 h light cycle) for seven days for acclimatization. Prior to the experiments, the animals were fasted overnight and weighed. All experiments were carried out in strict compliance with The Principle of Laboratory Animal Care (NIH, 1985).

Anti-inflammatory assay

To measure the anti-inflammatory activities of flower essential oil from *H. coronarium* under consideration, carrageenan-induced paw edema test was examined according to the method of Winter et al. (1962). Thirty minutes before application of the inflammatory agent, the animal groups were orally treated (*p.o.*) either with 1 ml of essential oil (100 mg/kg) previously emulsified in 3% Tween 80, or with 1 ml of indomethacin (10 mg/kg; pos. control) or an equal volume of 0.9% aq. NaCl (neg. control). Then male Wistar rats were briefly anesthetized with ethyl ether and injected subplantarly into the right hind paw with 0.1 mL of 1.0% carrageenan in isotonic saline. The left hind paw was injected with 0.1 mL of saline to be used as a control. Measurement of paw size was carried out by gently wrapping a piece of white cotton thread around the paw and measuring the circumference on a meter rule (Bamgbose and Noamesi, 1981; Olajide et al., 2000) at hourly intervals for 5 h after the stimulus.

Antioxidant activity assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay was estimated according to the procedure described by Cardador-Martinez et al. (2002) with a slight modification. The essential oil of *H. coronarium* was dissolved in EtOH at different concentrations. Different dilutions of the oil (4 ml) were added to 1 ml of 0.5 Mm DPPH (in EtOH), resulting in a final concentration of 0.1 mm DPPH. The mixture was shaken vigorously and left for 30 min in the dark at room temperature. The absorbance was measured at 517 nm. The scavenging capacity was calculated as follow.

$$\text{Scavenging capacity } \% = [1 - (A_{test}/A_{control})] \times 100$$

Where A_{test} was the absorbance of the test sample (DPPH solution plus antioxidant) and $A_{control}$ was the absorbance of the control (DPPH solution without test sample). BHT (butylated hydroxyltoluene) and ascorbic acids were used as positive references while EtOH as negative and each assay was performed in triplicates. IC_{50} value, which represented the concentration of the essential oil that caused 50% scavenging was calculated by linear regression module of Prism 5.0.

The reducing power of the oil was investigated using Ferric Reducing Antioxidant Power (FRAP) assay according to the method of Yen and Chen (1995) with a modification. FRAP reagent was freshly prepared by mixing 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml 1% potassium ferricyanide ($K_3Fe(CN)_6$). The oil (50-400 mg/l) in EtOH (1 ml) was mixed with FRAP reagent stirred thoroughly and incubated at 50°C for 30 min. Afterwards, 10% trichloroacetic acid (2.5 ml) was added followed by centrifugating at 3000 rpm for 10 min. Finally, the supernatant solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. Then the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of the essential oil was compared with those of BHT and ascorbic acid as positive controls. Calibration curve of ferrous sulfate (100 - 1000 mmol/L) was used and the results were expressed in µmol Fe^{2+}/mg dry weight extract.

Statistical analysis

The results are expressed as means ± S.E.M. Statistical analyses were carried out through ANOVA. Results with $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The compositions of the yellow-colored essential oil of *H.*

Table 1. Main components (%) of the flower essential oil of *H. coronarium.*

Compound [a]	Content (%) [b]
α-Thujene	0.14
α-Pinene	1.37
Camphene	0.12
Sabinene	4.59
β-Pinene	2.01
β-myrcene	0.35
α-Phellandrene	0.20
α-Terpinene	0.36
Limonene	2.02
1,8-Cineole	11.35
β-*trans*-Ocimenone	28.05
γ-Terpinene	2.21
Linalool	18.52
Fenchyl alcohol	0.13
Borneol	1.13
Terpinen-4-ol	3.17
α-Terpineol	7.11
Geraniol	1.03
β-Phellandrene-8-ol	0.13
Carvacrol	0.37
α-Gurjunene	0.18
β-Cedrene	0.41
(E)- β-Farnesene	2.28
ρ-Menthen-9-al	0.78
Nerolidol	1.25
Unknown	1.10
10-epi-γ-Eudesmol	6.06
Patchoulane	0.61
Isocyclocitral	0.33
β-Eudesmol	0.28

[a] Constituents listed in order of their elution from a HP-5ms capillary column.
[b] Compounds in the mixtures in percentages less than 0.05% are not presented.

coronarium flowers with characteristic odor, were listed in the order of their elution from the column (Table 1), showing a predominance of monoterpenes and sesquiterpenes. There were 30 constituents, 29 of which were identified, representing approximately 97.64% of the oil. According to the results obtained from GC–MS and GC–FID analyses, the major constituents in flower essential oil were quantified to be β-*trans*-ocimenone (28.05%), linalool (18.52%), 1,8-cineole (11.35%), α-terpineol (7.11%), 10-epi-γ-eudesmol (6.06%), sabinene (4.59%) and terpinen-4-ol (3.17%) and the remaining constituents were present in lower amount (< 3%). The result was similar with the published data on the rhizome essential oil of *H. coronarium* (Beena et al., 2007). Additionally, myrcene, limonene, camphene and terpinene were present in many *Hedychium* species (Sushil et al., 2008).

The genus *Hedychium* was well known as a group of medicinal plants. The essential oils can be extracted from leaves, flowers and rhizomes of these plants. It was well documented that *Hedychium* species'oils have many medicinal efficacies, including cercaricidal properties (Warren and Peters, 1968), molluscicidal activity (Saleh et al., 1982), potent inhibitory action (Kumar et al., 2000), antimicrobial activities (Medeiros et al., 2003; Gopanraj et al., 2005; Bisht et al., 2006; Joy et al., 2007), *in vitro* pediculicidal activity (Jadhav et al., 2007), anti-inflammatory and analgesic effects (Crunkhon and Meacock, 1971).

As shown in Table 2, intradermal injection of carrageenan caused a local inflammatory response that reached maximum edema intensity 3 h after administration of the phlogistic agent in all experimental groups. The oil at doses of 100 mg/kg (*p.o.*) significantly inhibited paw edema 1-5 h after carrageenan administration comparing with the negative control group and did not induce lethality or any other signs of toxicity. The time course of edema development in carrageenan-induced paw edema model in rats was generally represented by a biphasic curve. The first phase, which last for 3 h following carrageenan injection was due to the release of histamine, serotonin and bradykinin. The second phase was due to the release of prostaglandins, protease and lysosome (Shrotriya et al., 2007). The data obtained from the present study indicated that the essential oil of *H. coronarium* produced a pronounced anti-inflammatory effect in both phases of carrageenan-induced oedema.

In order to examine the *in vitro* anti-oxidant effects of the essential oil, two different experimental models were chosen. The first was based on the free-radical-scavenging capacity of the stable DPPH radical, the second was based on the reduction of Fe^{3+} to Fe^{2+}, followed by reaction with $K_3[Fe(CN)_6]$, under formation of the colored complex Prussian Blue ($Fe_4[Fe(CN)_6]_3$).

The DPPH radical scavenging activities of the known antioxidative substances (BHT and ascorbic acid) and the oil studied in this paper are shown in Table 3. The result showed a dose-dependent inhibition in all the samples, but the scavenging capacity of the essential oil was significantly lower compared with that of standards. Concentration at which the oil decreased DPPH radical by 50% (IC_{50} values) was 1091.00 µg/ml. Correspondingly, IC_{50} values for BHT and ascorbic acid, used as standards, were 44.07 and 4.62 µg/ml, respectively. A similar trend was observed in the second test (Table. 3). The reducing power of a sample was related to its electron transfer ability and might, therefore, served as an indicator of its potential antioxidant activity. The reducing powers of the essential oil, BHT and ascorbic acid increased with the concentration. The oil exhibited sinificantly lower activity (0.22 µmol Fe^{2+}/mg). Whereas, the FRAP values of BHT and ascorbic acids, were 1.38 and 7.85 µmol Fe^{2+}/mg, respectively.

Table 2. Anti-inflammatory activity of the flower essential oil of *H. coronarium* on carrageenan-induced rat paw oedema over 5 h.

Treatment	Paw sizes [a] (cm)					
	0 h	1 h	2 h	3 h	4 h	5 h
Control (0.9% NaCl)	1.99 ± 0.04	2.51 ± 0.01	2.91 ± 0.01	3.02 ± 0.02	2.77 ± 0.01	2.68 ± 0.01
Essential oil (100 mg/kg)	1.98 ± 0.02	2.41 ± 0.03*	2.52 ± 0.02**	2.58 ± 0.02**	2.41 ± 0.02**	2.31 ± 0.02**
Indomethacin (10mg/kg)	2.05 ± 0.03	2.40 ± 0.01**	2.50 ± 0.01**	2.51 ± 0.02**	2.39 ± 0.02**	2.31 ± 0.01**

[a] Data were expressed as mean ± S.E.M. for six animals in each group; Indomethacin was used as positive control, 0.9% NaCl was used as negative control.
* $p < 0.05$, ** $p < 0.01$ compared with negative control treated group.

Table 3. Antioxidant activities (scavenging capacity and reducing power) of flower essential oil of *H. coronarium*.

Extract/control	DPPH radical scavenging activityIC_{50} (µg/ml)	FRAP value (µmol Fe^{2+}/mg dry weight extract)
Essential oil	1091.00 ± 75.61	0.22 ± 0.02
BHT	44.07 ± 4.58	1.38 ± 0.06
Ascorbic acid	4.62 ± 0.33	7.85 ± 0.22

Values are expressed as mean ± S.E.M.,
Values are significant at $p < 0.05$.

The inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although, it is a defense mechanism that helps the body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can be induced, maintained or aggravate many diseases (Sosa et al., 2002). The results of the above studies concerning the DPPH radical-scavenging and reducing power of the flower essential oil of *H. coronarium* indicated that there was no direct correlation between anti-inflammatory effect (significant decrease in rat-paw edema) and antioxidant activity (poor antioxidant capacity, which confirmed by earlier report (Zeng et al., 2008). Moreover, the carrageenan-induced paw edema model in rats was known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert et al., 1994). Taken into account that indomethacin was a representative blocker of cyclooxygenase activity and that both indomethacin and the tested essential oil inhibited the inflammatory process similarly, an alternative anti-inflammatory mechanism of this oil might be COX-1 and/or COX-2 inhibition (Esteves et al., 2005).

This preliminary study provides support for the popular use of *H. coronarium* in folk medicine for some inflammatory ailments. To find out the key elements responsible for the significant anti-inflammatory activity and to clarify the mechanisms involved, further investigations are warranted.

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