

Original Article

# Pungent ginger components modulates human cytochrome P450 enzymes *in vitro*

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**Aim:** Ginger rhizome is used worldwide as a spicy flavor agent. This study was designed to explore the potential effects of pungent ginger components, 6-, 8-, and 10-gingerol, on human cytochrome P450 (CYP450) enzymes that are responsible for the metabolism of many prescription drugs.

**Methods:** The activities of human CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were analyzed using Vivid P450 assay kits. The mRNA expression of CYP3A4 in human hepatocellular carcinoma cell line HepG2 was measured using quantitative real-time PCR assay.

**Results:** All three gingerols potently inhibited CYP2C9 activity, exerted moderate inhibition on CYP2C19 and CYP3A4, and weak inhibition on CYP2D6. 8-Gingerol was the most potent in inhibition of P450 enzymes with IC<sub>50</sub> values of 6.8, 12.5, 8.7, and 42.7 μmol/L for CYP2C9, CYP2C19, CYP3A4, and CYP2D6, respectively. By comparing the effects of gingerols on CYP3A4 with three different fluorescent substrate probes, it was demonstrated that the inhibition of gingerols on CYP3A4 had no substrate-dependence. In HepG2 cells, 8-gingerol and 10-gingerol inhibited, but 6-gingerol induced mRNA expression of CYP3A4.

**Conclusion:** 6-, 8-, and 10-gingerol suppress human cytochrome P450 activity, while 8- and 10-gingerol inhibit CYP3A4 expression. The results may have an implication for the use of ginger or ginger products when combined with therapeutic drugs that are metabolized by cytochrome P450 enzymes.

**Keywords:** ginger rhizome; gingerol; cytochrome P450 enzyme system; CYP2C9; CYP2C19; CYP3A4; CYP2D6; dietary supplements; drug-diet interactions

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## Introduction

Ginger rhizome (*Zingiber officinale* Roscoe), commonly known as ginger, is used worldwide as a spicy flavor agent. It is a well-known dietary supplement for treating nausea<sup>[1]</sup> and motion sickness<sup>[2]</sup>. Ginger rhizomes are widely consumed in various forms: fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger), and in tea and beverages. In many countries, particularly those in Southeast Asia, fresh and processed ginger products have a long history of use as traditional medicine for treating a broad range of ailments, such as the common cold, inflammation, rheumatic disorder, and gastrointestinal discomforts<sup>[3]</sup>. Ginger contains 1.0% to 3.0% volatile oils and a number of pungent compounds<sup>[2]</sup>; 6-, 8-, and 10-gingerol (Figure 1A) are the major pungent constit-

uents of ginger that lead to the spicy taste<sup>[4]</sup>, and 6-gingerol is the most abundant one. The gingerols, which are often chemically differentiated by the length of their unbranched alkyl chains, account for the pharmacological activity of ginger<sup>[5]</sup>.

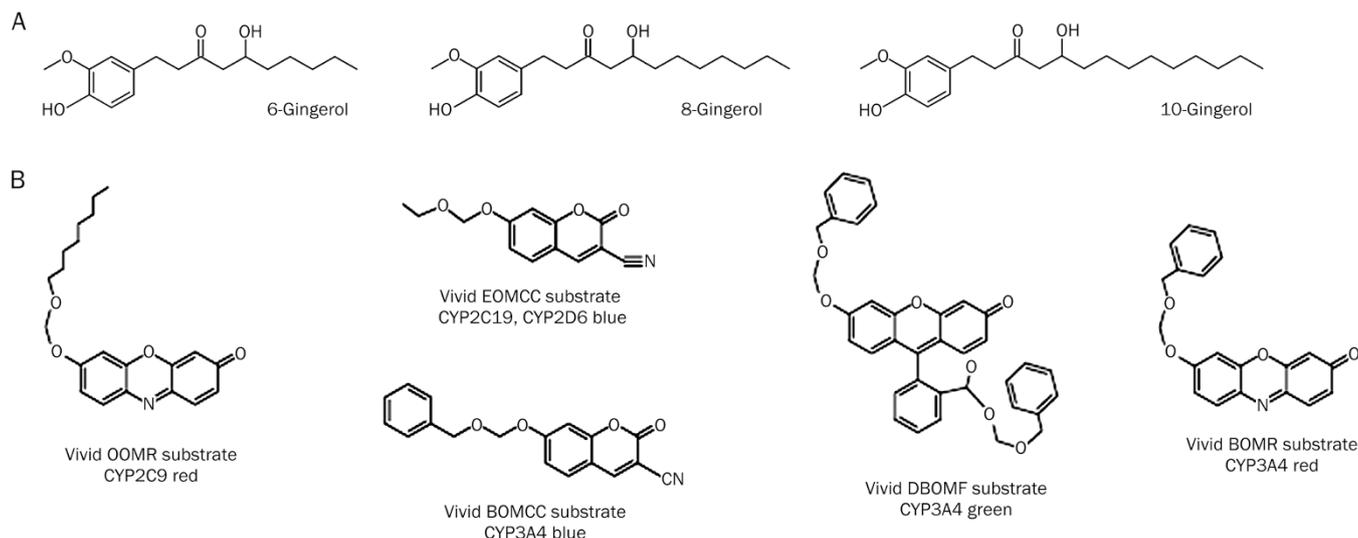
The pharmacological properties of gingerols include antioxidant, analgesic and anti-inflammatory<sup>[6]</sup>, antipyretic<sup>[7]</sup>, cardiotoxic<sup>[8]</sup>, and hypothermic<sup>[9]</sup> activities. More recent studies have suggested that gingerols exhibit preventive activity against cancers of the skin<sup>[10]</sup>, pancreas<sup>[11]</sup>, gastrointestinal tract<sup>[12]</sup>, colon<sup>[13]</sup>, and breast<sup>[14]</sup>. Ginger can also effectively prevent nausea induced by cancer treatment<sup>[15]</sup>. It has been reported that 6-, 8-, and 10-gingerol exist in ginger-containing dietary supplements, spices, food products and beverages<sup>[16]</sup>. Pharmacokinetic studies in humans have shown that after absorption and metabolism, 6-, 8-, and 10-gingerol are present in the serum as glucuronide and sulfate conjugates<sup>[17]</sup>.

There is an increasing interest in determining the importance of drug-drug, drug-nutrient, and drug-dietary supple-

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**Figure 1.** Structures of gingerols (A) and Vivid fluorescent probes (B).

ments interactions in basic, translational, and clinic research settings<sup>[18–20]</sup>. It is necessary to evaluate the potential influence of gingerols on human drug metabolism. The present study was designed to test the hypothesis that gingerols affect human P450 enzymes, and such effects may have an implication in clinical practice when ginger is combined with therapeutic drugs that are metabolized by P450s. In the present study, we investigated the effects of gingerols on several P450 enzymes that have been shown to have pharmacogenomic variations in humans, including CYP2C9, CYP2C19, CYP2D6, and CYP3A4. The potential influence of gingerols on the cellular transcription activity of CYP3A4, the most abundant CYP enzyme in the liver, was also examined.

## Materials and methods

### Chemicals and reagents

6-, 8-, and 10-gingerol were purchased from Phytomarker Ltd (Tianjin, China) with purities of 98.02%, 98.87%, and 99.41%, respectively, as analyzed by HPLC. Miconazole nitrate salt, quinidine, and ketoconazole were purchased from Sigma-Aldrich (St Louis, MO, USA). Sulfaphenazole was a gift from Ms Yuan-yuan DAI at the Cancer Hospital/Institute, Chinese Academy of Medical Sciences (Beijing, China). Chromatographic-grade methanol and acetonitrile were purchased from Tedia Company (Fairfield, OH, USA). Rifampicin (RIF) was purchased from AppliChem GmbH (Darmstadt, Germany).

### Determination of P450 activity with Vivid P450 assay kits

The inhibitory effects of gingerols on the catalytic activities of cDNA-expressed human P450 enzymes were determined using Vivid P450 screening kits (Vivid OOMR substrate CYP2C9 red, Vivid EOMCC substrate CYP2C19 blue, Vivid EOMCC substrate CYP2D6 blue, Vivid DBOMF substrate CYP3A4 green, Vivid BOMR substrate CYP3A4 red, and Vivid

BOMCC substrate CYP3A4 blue) (Figure 1B) according to the manufacturer's instructions (Invitrogen Corporation; Carlsbad, CA, USA). Each kit contained P450 reaction buffer, P450 BACULOSOMES reagent, a fluorescent substrate, a fluorescent standard, the regeneration system (333 mmol/L glucose-6-phosphate and 30 000 U/L glucose-6-phosphate dehydrogenase in 100 mmol/L potassium phosphate, pH 8.0), and 10 mmol/L NADP<sup>+</sup> in 100 mmol/L potassium phosphate, pH 8.0.

In brief, the assays were performed in Costar black-wall 96-well plates with ultra-thin clear bottoms (Corning Inc, Corning, NY, USA) in kinetic assay mode. Stock solutions (10 mmol/L) of gingerols in methanol were prepared and diluted to various concentrations (250, 50, 10, 5, and 1 μmol/L). In each well, 40 μL of the test compound in solution, solvent or a positive inhibitor control was incubated with 50 μL of pre-mixture (containing BACULOSOMES reagent, the regeneration system and reaction buffer) or 50 μL of reaction buffer only as a background control at room temperature (25 °C) for 20 min. The reaction was initiated by the addition of 10 μL/well of a mixture of substrate and NADP<sup>+</sup> with a respective concentration of the Vivid substrate (Table 1). The plates were read immediately for fluorescence changes every 30 s at 37 °C for 30 min using a FlexStation II384 fluorometric plate reader (Molecular Devices; Sunnyvale, CA, USA) with appropriate excitation and emission wavelengths for each P450 enzyme (Table 1). The final methanol volume in the reactions was ≤1%. The positive control compounds were sulfaphenazole (for CYP2C9), miconazole nitrate salt (for CYP2C19), quinidine (for CYP2D6), and ketoconazole (for CYP3A4); the final inhibitor concentrations of the inhibitors were chosen according to the Vivid P450 screening kit protocol to produce an inhibition of ≥90%.

### Cell culture and treatment

The human hepatocellular carcinoma cell line HepG2 was cul-

**Table 1.** Parameters of the enzymatic reactions used to determine the activities of P450 enzymes.

Vivid assay	Substrate concentration (μmol/L)	Wavelengths (nm)		CYP450 concentration (nmol/L)	Linear range of RFU~t curve (min) <sup>a</sup>
		Excitation	Emission		
CYP2C9 red	2	530	585	10	0~30
CYP2C19 blue	10	409	460	5	0~30
CYP2D6 blue	10	409	460	10	0~30
CYP3A4 green	2	485	530	5	0~5.5
CYP3A4 red	3	530	585	5	0~10
CYP3A4 blue	10	409	460	5	0~20

<sup>a</sup> The linear range was determined by visual inspection; parameters for substrate concentration; wavelength and CYP450 concentration were provided by the kit manufacturer.

tured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 units/mL penicillin and streptomycin at 37°C with 5% CO<sub>2</sub>. For the CYP3A4 expression study, 5×10<sup>5</sup> cells per well were seeded in 6-well plates. After 24 h of incubation, the cells were treated with the tested compounds at a final concentration of 10 μmol/L for 48 h. All compounds were dissolved in sterile DMSO to make 50 mmol/L stock solutions and kept at -20°C until use; 1% DMSO was used as a vehicle control.

#### Quantitative real-time PCR analysis

Total RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. First-strand cDNA was synthesized using a ReverTra Ace qPCR RT Kit (Toyobo, Japan) with 1 μg of total RNA. Quantitative real-time PCR for CYP3A4 was performed using SYBR Green fluorescent dye methodology as described previously<sup>[21]</sup>.

#### Statistical analysis

The quantitative data were presented as the mean±SD from at least three independent experiments. All the statistical analyses were conducted with Excel software. The statistical significance of differences between the control and treated groups was examined using Student's *t* test. A *P* value <0.05 was considered a statistically significant difference.

## Results

### The three pungent ginger components inhibit CYP450 activities *in vitro*

The parameters used to determine the activities of the P450 enzymes are summarized in Table 1. A solvent control (methanol) was included in the assays; 1% methanol showed no detectable inhibitory effect against Vivid green, red or blue metabolism by CYP3A4 but had minimal inhibitory activity against Vivid blue metabolism by CYP2C19 (Table 2). However, 1% methanol inhibited Vivid CYP2C9 red metabolism by up to 40.6% and CYP2D6 blue metabolism by 27.8%. When the final concentration of methanol was reduced to 0.5% or 0.1%, the inhibition of P450s activities was either negligible or within an acceptable range (Table 2).

All three gingerols potently inhibited CYP2C9 activity, had moderate inhibitory effects on CYP2C19 and CYP3A4 activities, and had weak inhibitory effects on CYP2D6 activity (Table 2). Among the three compounds, 8-gingerol had the greatest inhibition against all four P450 enzymes, with IC<sub>50</sub> values of 6.8 μmol/L for CYP2C9, 12.5 μmol/L for CYP2C19, 8.7 μmol/L for CYP3A4, and 42.7 μmol/L for CYP2D6.

### Gingerol inhibition of CYP3A4 has no substrate-selective difference

Because of the potential influence of substrate selection on

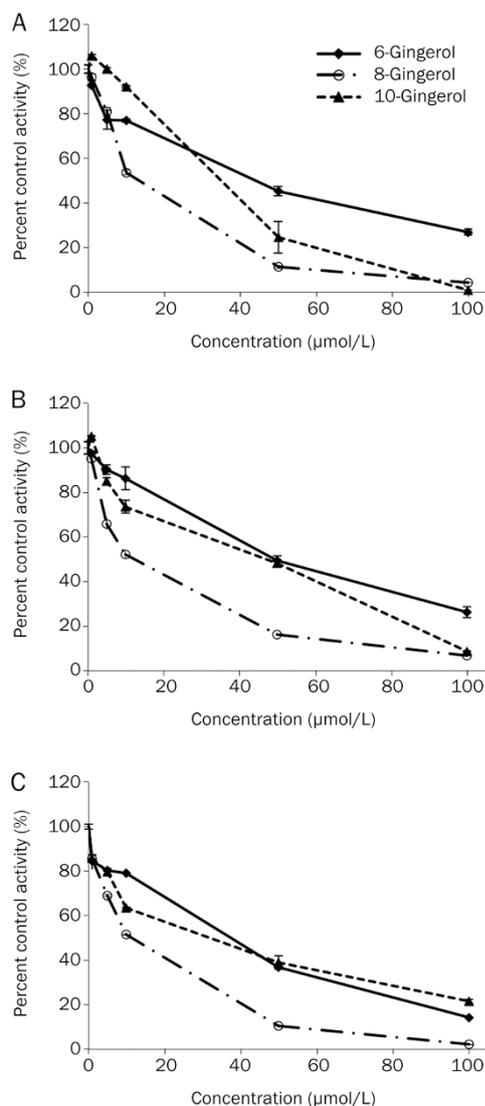
**Table 2.** IC<sub>50</sub> values of the gingerols against the activity of human P450 enzymes *in vitro*.

Test compounds	IC <sub>50</sub> (μmol/L) (% inhibition at 50 μmol/L) <sup>a</sup>					
	CYP2C9 red <sup>b</sup>	CYP2C19 blue	CYP2D6 blue	CYP3A4 green	CYP3A4 red	CYP3A4 blue
6-Gingerol	12.1	30.7	>50 (5.8%)	32.0	42.9	21.8
8-Gingerol	6.8	12.5	42.7	11.6	10.5	8.7
10-Gingerol	16.3	13.6	>50 (46.1%)	21.8	24.9	20.8

<sup>a</sup> The percent inhibition of gingerols against the P450 enzymes is shown when its IC<sub>50</sub> value is greater than the maximum concentration assayed.

<sup>b</sup> The maximum concentration of gingerols evaluated for their effects on CYP2C9 and CYP2D6 was 50 μmol/L due to the marked solvent effect of 1% methanol on these P450 enzymes (inhibition by 40.6% and 27.8%, respectively). When the concentration of methanol was decreased to 0.5%, the solvent effects were acceptable for these two enzymes (13.5% and 16.2%, respectively). 1% methanol had no inhibition against CYP3A4 and had an acceptable inhibitory effect on CYP2C19 (9.8%).

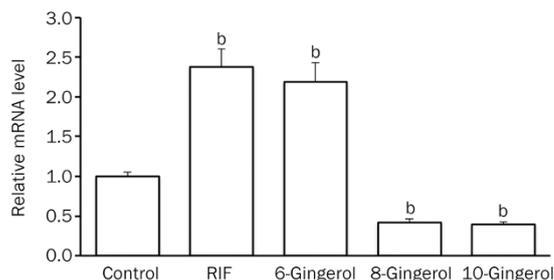
evaluating the effects of gingerols on the catalytic activity of CYP3A4, three fluorescent probes, Vivid DBOMF CYP3A4 green, Vivid BOMR CYP3A4 red and Vivid BOMCC CYP3A4 blue (Figure 1B), were used. The assay conditions are summarized in Table 1. The solvent effects of methanol (1%) on CYP3A4 green, red and blue metabolisms were negligible. For all the tested gingerols, the inhibition profiles determined with the green, red and blue substrates were essentially the same. No significant difference in the inhibitory potency against the three substrates was found for the same gingerol (Table 2). All three compounds inhibited CYP3A4 in a dose-dependent manner, with the potency decreasing in the following order: 8-gingerol > 10-gingerol > 6-gingerol (Figure 2A–2C).



**Figure 2.** Effects of the gingerols on the Vivid CYP3A4 green assay (A), Vivid CYP3A4 red assay (B) and Vivid CYP3A4 blue assay (C). Mean $\pm$ SD of a percentage ratio of CYP3A4 reaction velocities to the velocity with no inhibitor present.

### 8- and 10-gingerol inhibit but 6-gingerol induces CYP3A4 mRNA expression in HepG2 cells

The effects of the three gingerols on CYP3A4 mRNA expression were different. RIF (the positive control) induced CYP3A4 expression by 138% $\pm$ 29%. In HepG2 cells, 8- and 10-gingerol decreased the expression of CYP3A4 by 58% $\pm$ 7% and 61% $\pm$ 4%, respectively, but 6-gingerol increased the CYP3A4 mRNA level by 119% $\pm$ 31% (Figure 3).



**Figure 3.** Effects of the gingerols on CYP3A4 mRNA expression in HepG2 cells. After normalization to  $\beta$ -actin levels, the results from three independent assays were averaged and the relative expression levels compared to the vehicle control were reported (mean $\pm$ SD). RIF, Rifampicin. Differences were significant when compared to the vehicle control group (<sup>b</sup> $P$ <0.05).

### Discussion

Although microsomal assays are commonly used to evaluate the activity of P450 enzymes, fluorescent methods for determining P450 activity allow for high-throughput screening and more reproducible results<sup>[21–25]</sup>. Bell *et al* found that nearly 75% of marketed drugs yielded acceptable correlations between fluorogenic and HLM+LC-MS/MS assays for CYPs 3A4, 2D6, and 2C9, which supports the use of fluorogenic assays for rapid early risk binning based on IC<sub>50</sub> values<sup>[26]</sup>. In the present study, fluorescent probes were used to study the effects of three pungent constituents of ginger on P450-mediated drug metabolism. The correlation between fluorescence assays and LC-MS/MS methods was confirmed in our preliminary study. In the current study, 6-, 8-, and 10-gingerol substantially inhibited the four major P450 enzymes, with the exception that the inhibitory effects of gingerols against CYP2D6 were relatively weak. 6-, 8-, and 10-gingerol all exhibited strong inhibition against CYP2C9, with IC<sub>50</sub> values of 12.1, 6.8, and 16.3  $\mu$ mol/L, respectively. These values may have clinical implications. For example, 3.6 $\pm$ 1.1  $\mu$ mol/L of 6-gingerol, 0.84 $\pm$ 0.47  $\mu$ mol/L of 8-gingerol and 1.3 $\pm$ 0.5  $\mu$ mol/L of 10-gingerol were detected in human plasma 60 min after the ingestion of 2.0 g of ginger extract<sup>[27]</sup>. Compared to the corresponding IC<sub>50</sub> values, a relatively high exposure of ginger extract may cause drug-diet interactions through P450 inhibition; such interactions may have an important implication in the clinical use of gingerols in combination with pharmacotherapy.

CYP2C9 represents approximately 20% of the hepatic CYP

content in liver microsomes<sup>[28]</sup>. CYP2C9 is expressed at the highest level of the CYP2C family. Pharmacogenomic variations of CYP2C9 have been well documented. This isoform is involved in the metabolism of approximately 10% of all drugs, including nonsteroidal antiinflammatory drugs, oral antidiabetics, antiinfectives, hypnotics, antiepileptics, oral anticoagulants, such as warfarin, sulfonylureas, psychotropics, and angiotensin-2 antagonists<sup>[29, 30]</sup>. It also metabolizes endogenous substrates, such as arachidonic acid and linolenic acid. CYP2C9 inhibition is involved in increases in the plasma concentration and toxicity of concomitant substrate drugs of CYP2C9, especially those with a narrow therapeutic index (eg, warfarin and phenytoin)<sup>[31]</sup>. Therefore, these factors should be taken into consideration when ginger is consumed.

The gingerols had significant inhibitory effects on CYP3A4. The CYP3A family contributes to approximately 50% of the total cytochrome P450 activity in the adult human liver and is involved in the metabolism of approximately 60% of therapeutic agents as well as the activation of toxic and carcinogenic substances<sup>[32]</sup>. CYP3A4 is the dominant CYP3A family enzyme expressed in the human liver and gastrointestinal tract<sup>[33]</sup>. Pharmacogenomic variations of CYP3A4 in humans have been indicated in many drug metabolism and drug-drug interactions. Because the effects on CYP3A4 might be substrate-dependent, as we found previously for the ginsenosides<sup>[34]</sup>, three fluorescent probes, Vivid CYP3A4 green, Vivid CYP3A4, red and Vivid CYP3A4 blue, were used to determine the effects of gingerols on CYP3A4. There was no significant difference in the inhibitory potency of these compounds.

In the present study, we also determined the effects of gingerols on CYP3A4 expression. The HepG2 cell line was employed in this study because it is a suitable surrogate of primary human hepatocytes to determine changes in CYP3A4 expression in the human body<sup>[35]</sup>. Based on the *in vitro* data, it appeared that 6-gingerol was a potent inhibitor of CYP3A4, and 8- and 10-gingerol also inhibited CYP3A4 transcription. Thus, attention should be paid when gingerols, especially 8- and 10-gingerol, are co-administered with other drugs, including herbal medicines that are metabolized by CYP3A4. 6-Gingerol potently induced CYP3A4 mRNA (similar to RIF) at a low concentration (10  $\mu\text{mol/L}$ ). At 10  $\mu\text{mol/L}$ , 6-gingerol weakly inhibited CYP3A4 (approximately 20%). This finding indicates that 6-gingerol may mainly influence the transcription of CYP3A4 at a low concentration, which is usually the case *in vivo*. Thus, complex interactions between ginger and substrates of CYP3A4 can be deduced, and further studies are needed.

The result also suggested a structure-activity relationship (SAR) for the effects of gingerols on most of the P450 enzymes examined (Table 2). These gingerols are analogs and differ only in the length of the alkyl chain linked to the 4-hydroxy-3-methoxyphenyl scaffold (Figure 1A). 8-Gingerol, which has a moderate length of the aliphatic chain, showed the strongest inhibition against P450 enzymes. It appears that the moderate length of the alkyl chain allowed 8-gingerol to interact with P450s in an effective manner; however, further investigations

are needed to confirm the molecular and structural basis of this finding.

In summary, our results indicate that the major pungent components of ginger can inhibit major human cytochrome P450 enzymes, which are responsible for the metabolism of most prescription drugs. Furthermore, the differential effects of gingerols on CYP3A4 gene expression may have complex consequences on the metabolism of different drugs. These findings highlight the importance of the safe use of ginger, especially in combination with clinically used drugs that utilize P450 enzymes as major metabolizing enzymes. This study may also serve as an example of drug-dietary supplement interactions in drug therapy.

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### Author contribution

Hui WANG directed the project, and along with Mian LI, defined the scope of the research; Mian LI and Pei-zhan CHEN designed methods and experiments and carried out the laboratory experiments; Mian LI analyzed the data, interpreted the results and drafted the manuscript; Qing-xi YUE and Jing-quan LI performed laboratory experiments and collected data; Rui-ai CHU and Wei ZHANG revised the manuscript; and Hui WANG reviewed the manuscript.

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