Essential oil from leaves of *Cinnamomum osmophloeum* acts as a xanthine oxidase inhibitor and reduces the serum uric acid levels in oxonate-induced mice

S.Y. Wang^{a,*}, C.W. Yang^a, J.W. Liao^b, W.W. Zhen^a, F.H. Chu^c, S.T. Chang^{c,*},²

^aDepartment of Forestry, National Chung-Hsing University, Taichung 402, Taiwan

^bGraduate Institute of Veterinary Pathology, National Chung-Hsing University, Taichung 402, Taiwan

^cSchool of Forestry and Resource Conservation, National Taiwan University, Taipei 106, Taiwan

Abstract

The xanthine oxidase (XOD) inhibitory activity and anti-hyperuricemia effect in mice of *Cinnamomum osmophloeum*, which is an endemic tree in Taiwan, were evaluated in this study. The results demonstrated that the essential oil of *C. osmophloeum* leaves presented the strongest XOD inhibition activity ($IC_{50} = 16.3 \mu\text{g/ml}$); however, no significant XOD inhibition activities were found in ethanolic and hot water extracts. Furthermore, among the main compounds of essential oil, the cinnamaldehyde exhibited the potent XOD inhibition activity with an $IC_{50} = 8.4 \mu\text{g/ml}$. Besides, the reducing serum uric acid levels in oxonate-induced mice by cinnamaldehyde were further investigated. The hyperuricemic mice were oral administrated cinnamaldehyde at a dosage of 150 mg/kg, the uric acid value in serum was reduced from 5.25 ± 0.63 to 2.10 ± 0.04 mg/dl, the levels of serum uric acid in mice was lowered down by 84.48% as compared to the hyperuricemic control group. Based on the results obtained in this study, cinnamaldehyde may be a potential lead compound for developing the pharmaceutical for anti-hyperuricemia agent.

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Keywords: *Cinnamomum osmophloeum*; Cinnamaldehyde; Hyperuricemia; Xanthine oxidase inhibitor; Hyperuricemia; Goat treatment

Introduction

The Lauraceae family, in the order Laurales, consists of more than 45 genera with around 2000–2500 species (Lu et al., 2000). The genus *Cinnamomum* belongs to Lauraceae, which includes 250 species. Many plants of *Cinnamomum* have been applied as folk medicines for

their interesting bioactivities. *Cinnamomum osmophloeum* Kaneh. is an endemic tree of Taiwan. It grows in the natural hardwood forest at elevations between 400 and 1500 m. The most impressive character for *C. osmophloeum* is that chemical compositions of its leaves essential oil are similar to those of the famous *C. cassia* (Cheng et al., 2004). Up to date, many biological activities of *C. osmophloeum* leaf essential oil have been confirmed, including anti-fungal activity (Wang et al., 2005; Cheng et al., 2006), anti-bacterial activity (Chang et al., 2001), anti-termitic activity (Chang and Cheng, 2002), anti-mite activity (Chen et al., 2002), mosquito larvicidal activity (Cheng et al.,

*Corresponding author.

E-mail addresses: taiwanfir@dragon.nchu.edu.tw (S.Y. Wang), peter@ntu.edu.tw (S.T. Chang).

¹Tel.: +886 4 22840345x138.

²Tel.: +886 2 33664626.

2004), antiinflammatory activity (Chao et al., 2005; Fang et al., 2006). As regards to the bark of *C. cassia*, it is a very famous traditional medicine and widely used in Asia country for a long time. The extracts from the bark of *C. cassia* have been claimed for antiinflammation (Lee et al., 2002), decreased serum glucose, total cholesterol and platelet counts (Khan et al., 2003). It was also proved to be active against HIV-1 and HIV-2 (Premanathan et al., 2000). Moreover, according to the results reported by Kong and his co-worker, the methanolic extract from the twig of *C. cassia* possessed the potent inhibitory activity for xanthine oxidase among 122 traditional Chinese medicinal plants, which were selected according to the clinical efficacy and prescription frequency for the treatment of gout and other hyperuricemia-related disorders (Kong et al., 2000). Recently, Zhao et al. (2006) demonstrated that cassia oil significantly reduced serum and hepatic urate levels in the hyperuricemia mice caused by oxonate.

Overproduction or underexcretion of uric acid leads to hyperuricemia, which is present in 5–30% of the general population and serum to be increasing global. Hyperuricemia has been considered an important risk factor for gout (Shimoto et al., 2005). Xanthine oxidase (XOD) catalyses the oxidation of hypoxanthine and xanthine to uric acid (Ramallo et al., 2006). XOD inhibitors could block the biosynthesis of uric acid from purine in the body, which should be one of the therapeutic approaches for treating hyperuricemia (Unno et al., 2004; Kong et al., 2002). As regards to XOD inhibitors, allopurinol is the most commonly used in the past decades (Field et al., 1996). However, a number of side effects caused accompanied with employed the allopurinol e.g. hepatitis, nephropathy, allergic reaction and 6-mercaptopurine toxicity (Kong et al., 2000, 2002). Therefore, there is an urgent need and trend to develop the new XOD inhibitors from natural source.

Although there are some evidence indicated that both of the methanolic extract of *C. cassia* and cassia oil exhibited XOD inhibition activity (Kong et al., 2000; Zhao et al., 2006), the exactly active components in the methanolic extract and/or *C. cassia* oil for inhibition the XOD activity and reducing serum urate levels in animal are not clear. On the other hand, since the chemical composition of essential oil from *C. osmophloeum* leaves is similar to those of *C. cassia*, it seems quite likely that the essential oil of *C. osmophloeum* may possess the XOD inhibition activity and reducing the serum uric acid in animal. In this study, the XOD inhibition activity of extracts from *C. osmophloeum* was evaluated. Furthermore, the chemical composition of active extracts was also characterized. Meanwhile, the principals of XOD inhibition and serum uric acid reducing effect in mice were also investigated.

Material and methods

Plant material

Leaves of *C. osmophloeum* were collected in June 2006 from the Da-Pin-Ting of Taiwan Sugar Farm located in Nantou County in central Taiwan. The species were identified by Prof. Y.-H., Tseng (Department of Forestry, National Chung-Hsing University), and voucher specimens were deposited at the Herbarium of Department of Forestry, National Chung-Hsing University.

Extracts and essential oil preparation

The extracts of *C. osmophloeum* were prepared by the following extractive procedures. A total of 100 g fresh leaves were extracted separately twice with 500 ml of 70% ethanol at ambient temperature for 7 days. After combining two batches of extracts, the extract was concentrated to yield the ethanolic extract (EtOH-Ext). The hot water extract (HW-Ext) was obtained by refluxing 100 g fresh leaves with 500 ml double distilled water for 2 h and then lyophilized into powder. On the other hand, fresh leaves (200 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h, followed by determination of oil contents. Leaf essential oil (EO) was stored in airtight sample vial prior to analysis by gas chromatography–mass spectrometry (GC–MS) and bioactivity evaluation.

GC–MS analyses

A HP G1800A GC/MS instrument was used with a DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W scientific). The column temperature was held at 40 °C for 1 min, then increased at 4 °C/min to 260 °C and held for 4 min. The temperatures of injector and ion source (EI of 70 eV) were 250 and 260 °C, respectively. The carrier gas was He at a flow rate of 1 ml/min, and the split ratio of 1:50 was performed. The MS scan range was *m/z* 45–425. The Wiley GC–MS library (V. 7.00) was searched and authentic standards were used for compounds identification. The GC peak areas were used for quantification without individual response factors.

In vitro inhibitory activity of *C. osmophloeum* extracts on xanthine oxidase

The inhibitory effect on XOD was determined spectrophotometrically by following the increase in the absorbance at 295 nm (Kong et al., 2000). The reaction mixture consisted of 400 µl of 200 mM sodium pyrophosphate buffer (pH 7.5), 200 µl of 0.6 mM xanthine, 20 µl of sample solution dissolved in distilled water or

dimethylsulfoxide (DMSO), and 200 μ l xanthine oxidase (0.1 U). DMSO was used for the samples not dissolvable in distilled water; the final concentration of DMSO in the assay was 1%. The absorption increments at UV absorbance at 295 nm indicated the formation of uric acid. All determinations were performed in triplicate. For the EtOH-Ext, HW-Ext, and EO, the dosages for XOD inhibitory activity assays were examined at concentrations of 100, 50, 25, 10, 5, 1 μ g/ml, respectively. On the other hand, the dosages for pure compounds and allopurinol assay were 100, 50, 25, 10, 5, 3, 2, 1 μ g/ml. The inhibitory activity of XOD was assessed as inhibitory (%) = $(1 - b/a) \times 100$, where “a” is the change in absorbance per min without the sample, and “b” is the change in absorbance per min with the sample.

Hypouricemic effects of cinnamaldehyde on potassium oxonate-induced hyperuricemia in mice

Three-week-old male ICR mice (25–28 g) were purchased from BioLASCO Co. (Taiwan) and each 10 mice in one group were housed in a plastic cage. Mice were allowed 1 week to adapt environment before test. They were housed in the conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($55 \pm 5\%$), lighting (06:00–18:00 h) with roden diet (LabDiet[®] 5001 Rodent diet, Purina Mills LLC, ST. Lous, Mo, USA) and water *ad libitum*. The animals were transferred to the laboratory at least 1 h before the potassium oxonate-induced hyperuricemia experiment. Potassium oxonate (PO) is an uricase inhibitor (Stavric et al., 1995), which was used to induce hyperuricemia in ICR mice. The method used to examine the hypouricemic effects of cinnamaldehyde was followed by the methods reported previously (Zhao et al., 2006; Unno et al., 2004; Kong et al., 2002) with slight modifications. Briefly, the mice were divided into four groups ($n = 10$). Besides “normal group” (mice without treated with PO), the other three groups of mice, i.e. “PO”, “PO + CA”, and “PO + Allopurinol”, were injected intraperitoneally with PO at a dosage of 250 mg/kg 1 h before drug administration to increase the serum urate level. After 1 h, the mice in the “PO + CA” group were oral administrated 150 mg/kg cinnamaldehyde; in “PO + Allopurinol” group were oral administrated 10 mg/kg allopurinol; in “PO” group were administrated saline only. Two hours after PO-induced action, whole blood samples were collected from mice. The blood was allowed to clot for 1 h at ambient temperature and then centrifuged at 3000 rpm for 5 min to obtain the serum. The serum was stored at -20°C until assayed. The uric acid level was determined by the phosphotungstic acid method, as described elsewhere (Carroll et al., 1971).

Statistical analysis

Data are expressed as means $\pm$ SE. Statistical comparisons of the results were made using analysis of variance (ANOVA). Significant differences ($*p < 0.05$ and $**p < 0.01$) between the control (untreated) and treated cells were analyzed by Dunnett’s test.

Results and discussion

Yields and XOD inhibitory activity of extracts from *C. osmophloeum* leaves

The yields of extracts from *C. osmophloeum* using different preparation methods were shown in Table 1. The yields of ethanolic extracts (EtOH-Ext), hot water extracts (Hot-Ext), and hydrodistillation (EO) were $7.5 \pm 0.1\%$, $14.3 \pm 0.4\%$, and $3.3 \pm 0.1\%$, respectively. Extracts prepared by difference methods were further evaluated their XOD inhibitory activity *in vitro*. As shown in Table 1, the EtOH-Ext and Hot-Ext did not present XOD inhibitory activity, IC_{50} values for both of them were higher than 100 μ g/ml. However, the extract prepared from hydrodistillation, i.e. essential oil (EO), displayed a significant XOD inhibitory activity. When IC_{50} of allopurinol (commercial XOD inhibitor) was 0.6 μ g/ml, the IC_{50} for EO was 16.3 ± 0.2 μ g/ml. Kong et al. (2000) had evaluated the XOD inhibitory activities of 122 traditional Chinese medicinal plants, which were selected according to the clinical efficacy and prescription frequency for the treatment of gout and other hyperuricemia-related disorder. Among 122 medicinal plants, the extract of *C. cassia* twig prepared by methanolic extraction possessed the strongest XOD inhibitory activity ($\text{IC}_{50} = 18$ μ g/ml), followed by the methanolic extract from *Chrysanthemum indicum* flower, the IC_{50} was 22 μ g/ml. Comparing with the extracts of *C. cassia* and *C. indicum*, the EO presented an excellent XOD inhibitory activity, suggesting that it may be a great potential to further investigate its hyperuricemia-reducing and/or even gout treatment effects.

Table 1. Yields and xanthine oxidase (XOD) inhibitory activity of extracts from *C. osmophloeum* leaves

	Yield (%)	IC_{50} of XOD inhibitory activity (μ g/ml)
EtOH-Ext	7.5 ± 0.1	> 100
Hot-Ext	14.3 ± 0.4	> 100
EO	3.3 ± 0.1	16.3 ± 0.2
Allopurinol ^a	–	0.6 ± 0.0

^aAllopurinol was used as a reference compound in this assay.

Table 2. Major components and relative contents of essential oil from *C. osmophloeum* leaves

Peak no.	Compound	RT (min)	Content (%)
1	α -Pinene	9.57	0.23
2	Camphene	10.05	0.15
3	Benzaldehyde	10.36	1.24
4	β -Pinene	11.01	0.10
5	3-Pheayl pionaldehyde	17.87	1.29
6	<i>cis</i> -Cinnamaldehyde	19.98	0.89
7	<i>trans</i> -Cinnamaldehyde	21.86	74.16
8	Isobornyl acetate	22.05	0.52
9	Eugenol	24.67	0.81
10	Cinnamyl acetate	27.43	20.61

RT: retention time.

Chemical composition analysis of essential oil

Since EO revealed a significant XOD inhibitory activity, the composition of EO was analyzed by using the GC–MS technique, the result is shown in Table 2. The main component of EO was cinnamaldehyde (76.16%), followed by cinnamyl acetate (20.61%), and the contents of the other constituents were less than 2%. According to the classification of Hu et al. (1985), the tree used in this study was belonging to cinnamaldehyde type of *C. osmophloeum*. From the chemical composition point of view, *C. osmophloeum* is a unique tree species. The constituents in *C. osmophloeum* leaf essential oil are similar to those of famous *C. cassia*. However, the active compound, e.g. cinnamaldehyde, is richly distributed in the leaves, not in the bark or twig. From forestry conservation point of view, to utilize the leaves of tree without fall it down is the best way for application the natural resource.

XOD inhibitory activity of main components in EO

Based on the results obtained by GC–MS analysis, the compositions of EO were cinnamaldehyde (74.16%), cinnamyl acetate (20.61%), 3-pheayl pinoaldehyde (1.29%), benzaldehyde (1.24%), eugenol (0.81%), isobornyl acetate (0.52%), α -pinene (0.23%), camphene (0.15%), β -pinene (0.10%) (Table 2). The XOD inhibitory activities of major compounds of EO were further evaluated in this study. Fig. 1 demonstrates the IC_{50} values of compounds from *C. osmophloeum* leaves against XOD activity. It is obvious that cinnamaldehyde presented the strongest XOD inhibitory activity ($IC_{50} = 8.4 \mu\text{g/ml}$). The IC_{50} value of allopurinol, being clinically used as a XOD inhibitory drug, was $0.6 \mu\text{g/ml}$. Although benzaldehyde possessed a slight XOD inhibitory activity ($IC_{50} = 27.0 \mu\text{g/ml}$), the amount of benzaldehyde was only 1.24%. Besides, the IC_{50} values for other compounds were higher than $100 \mu\text{g/ml}$. We

Fig. 1. IC_{50} values of components of *C. osmophloeum* leaves and allopurinol against xanthine oxidase. The data are representative of three experiments and expressed as mean $\pm$ S.E.

concluded that cinnamaldehyde contributes the most of XOD inhibitory activity in EO. Recently, the XOD inhibitory activity of cassia oil had been reported (Zhao et al., 2006). However, the bioactivity principal contributed the XOD inhibitory activity of cassia oil has not been reported until now. According to the results presented in this study, it was approved that cinnamaldehyde is the principal component for XOD inhibitory activity both in cassia oil and/or EO of *C. osmophloeum*.

Effects of cinnamaldehyde and allopurinol on serum urate levels in hyperuricemic mice induced by potassium oxonate

As the results presented above, cinnamaldehyde exhibited a potent XOD inhibitory activity, the serum uric acid reducing effect by cinnamaldehyde in oxonate-induced mice by cinnamaldehyde was further investigated. The serum level of uric acid in mice was induced by uricase inhibitor potassium oxonate (PO). As shown in Fig. 2, initial serum uric acid level in mice was $1.73 \pm 0.27 \text{ mg/dl}$. After intraperitoneal injection of PO caused a significant increase of serum uric acid level in PO-treated mice, the level of uric acid was reached at $5.25 \pm 0.63 \text{ mg/dl}$ after injection of PO for 3 h later. The hypouricemic effects of cinnamaldehyde and allopurinol on the serum uric acid levels in hyperuricemic mice are shown in Fig. 2. After a single oral administration of cinnamaldehyde at a dosage of 150 mg/kg in hyperuricemic mice, the serum uric acid value was reduced to $2.10 \pm 0.04 \text{ mg/dl}$, the serum uric and levels of mice was lowered down by 84.48% as compared to the hyperuricemic control group. In the same treatment, allopurinol at a dosage of 10 mg/kg , the serum uric acid of mice was

Fig. 2. Effects of cinnamaldehyde and allopurinol on urate levels in serum and liver in the hyperuricemic mice pretreated with potassium oxonate (PO). Normal: mice without treated with PO; PO: mice treated with PO; PO+CA: mice administrated 150 mg/kg cinnamaldehyde after treated with PO; PO+allopurinol: mice administrated 10 mg/kg allopurinol after treated with PO. The data are representative of 10 animals and expressed as mean $\pm$ S.E. ** $p < 0.01$ compared with PO controls.

reduced to 1.84 ± 0.13 mg/dl. Zhao et al. (2006) have investigated hypouricemic effects of cassia oil from *C. cassia* by using the similar animal model, and the cassia oil demonstrated a good inhibitory activity for reducing the XOD activity. Administration of cassia oil significantly reduced the serum uric acid level in hyperuricemic mice at a dosage of 450 mg/kg of cassia oil or above. There are not different from the normal control mice, cassia oil at 600 mg/dl was found to be as potent as allopurinol. In this study, we further proved that cinnamaldehyde is a strong XOD activity inhibitor and presented the strong uric acid reducing effect in PO-induced mice. No matter cassia oil or cinnamaldehyde are common fragrance additives in foods, various food supplements, and cosmetic products, even used in many formulas of traditional herb medicines. In addition to the many biological activities of cinnamaldehyde have been reported previously, it is suggested that cinnamaldehyde may be a potent uric acid lowering agent. Although there are some safety concerns about high intake of cinnamon powders due to the coumarin existed in it, the formulae used in this study are essential oil and cinnamaldehyde. According to the report from US Department of Health and Human Services (NTP TR 514, 2004), oral administration of cinnamaldehyde for long-term period (3 months to 2 years) is safe for rats and mice even at 5475 mg/kg. The highest dosage for cinnamaldehyde used in this study is 150 mg/kg. Thus, it may have a great potential to develop it as an anti-hyperuricemia agent for clinical application.

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