JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

In Vivo Antioxidant Activities of Essential Oils and Their Constituents from Leaves of the Taiwanese *Cinnamomum osmophloeum*

Fu-Lan Hsu,[†] Wen-Hsuan Li,[‡] Chan-Wei Yu,[‡] Yi-Chen Hsieh,[‡] Ying-Fei Yang,[‡] Jui-Tung Liu,[‡] Justin Shih,[‡] Yu-Ju Chu,[‡] Pei-Ling Yen,[§] Shang-Tzen Chang,[§] and Vivian Hsiu-Chuan Liao*[,‡]

[‡]Department of Bioenvironmental Systems Engineering and [§]School of Forestry and Resource Conservation, National Taiwan University, Number 1, Section 4, Roosevelt Road, Taipei 106, Taiwan

[†]Forest Chemistry Division, Taiwan Forestry Research Institute, Number 53, Nanhai Road, Taipei 100, Taiwan

ABSTRACT: *Cinnamomum osmophloeum* Kaneh is an indigenous tree species in Taiwan. In this study, phytochemical characteristics and antioxidant activities of the essential oils and key constituents from the leaves of two *C. osmophloeum* clones were investigated. The two trees possess two chemotypes, which were classified as the cinnamaldehyde type and camphor type. We demonstrated that the essential oils from *C. osmophloeum* leaves exerted in vivo antioxidant activities in *Caenorhabditis elegans*. In addition, *trans*-cinnamaldehyde and D-(+)-camphor, which respectively represent the major compounds in the cinnamaldehyde-type and camphor-type trees, exerted significant in vivo antioxidant activities against juglone-induced oxidative stress in *C. elegans*. Moreover, expressions of antioxidative-related genes, including superoxide dismutase (SOD) and glutathione S-transferase (GST), were significantly induced by *trans*-cinnamaldehyde and D-(+)-camphor from *C. osmophloeum* leaves. Our results showed that the essential oils from *C. osmophloeum* leaves and their major compounds might have good potential for further development as nutraceuticals or antioxidant remedies.

KEYWORDS: *Cinnamomum osmophloeum, antioxidant, Caenorhabditis elegans, superoxide dismutase, glutathione S-transferase*

INTRODUCTION

Plants have potential therapeutic values, and these potentials are still largely unexplored. Plant-derived essential oils have long been used as flavoring agents in food and beverages and as natural agents for food preservation.[1] Cinnamon oil is commonly used in the food industry because of its special aroma. *Cinnamomum osmophloeum* Kaneh (Lauraceae) is an endemic tree that grows in natural hardwood forests of Taiwan at elevations of 400−1500 m. *Cinnamomum osmophloeum* is interesting to study because its chemical constituents are similar to those of *Cinnamomum cassia* bark oil, known as cinnamon oil, commonly used in food and beverages and with great commercial value. Phytochemical and biological activity analyses showed that *C. osmophloeum* leaf essential oils have various biological activities, including antibacterial, antifungal, and anti-inflammatory effects.[2−4] In addition, essential oils from leaves of *C. osmophloeum* also act as a xanthine oxidase inhibitor and reduce serum uric acid levels in oxonate-induced mice, suggesting their potential for development as pharmaceutical antihyperuricemic agents.[5]

Oxidative stress is regarded as a major factor in the pathophysiology of various diseases, including tumor initiation,[6,7] Alzheimer's and Parkinson's diseases,[8] and inflammation associated with atherosclerosis and rheumatoid arthritis.[9,10] Oxidative stress occurs as a result of an imbalance between oxidants, such as reactive oxygen species (ROS), and antioxidants. ROS are considered harmful because they react with proteins, lipids, DNA, and other biomolecules, thus leading to the loss of cellular integrity and functionality.[11] In spite of their deleterious effects, however, recent research has indicated that ROS serve many important and nondamaging roles in both intracellular and extracellular signal transduction that involves diverse functions from vascular health to host defense.[12]

In contrast to cell-free studies and cell-culture systems, *Caenorhabditis elegans* allows examination within the context of a whole organism with many different organs and tissues.[13] Many key findings with relevance for mammals were discovered in the well-characterized *C. elegans*. This was possible because of the strong conservation of biological principles between *C. elegans* and mammals, and 60∼80% of human gene homologues have been identified in *C. elegans*.[13] Moreover, *C. elegans* has become a popular model for studying stress resistance and longevity, because of its relatively short life span and rapid generation time, and the well-defined genetic and environmental factors that affect its life span.[14−16] *Caenorhabditis elegans* is increasingly used to study the effects of pharmacologically active compounds of herbal origin on biological processes and identify new targets for pharmacological interventions.[13,17,18]

Herein, we used *C. elegans* as an in vivo model to examine the protective potential and the mode of action of essential oils and their constituents from leaves of *C. osmophloeum*. Due to the chemical polymorphism of leaf essential oils from different provenances of *C. osmophloeum*,[19] it is of interest to study differences in the bioactivities of varieties of indigenous cinnamon leaf oils. Therefore, in this study we investigated the chemical composition of leaf essential oils from two *C. osmophloeum* provenances using gas chromatography (GC)−

Received: November 7, 2011
Revised: January 15, 2012
Accepted: March 1, 2012
Published: March 1, 2012

mass spectrometry (MS) and GC-flame ionization detector (FID) analyses, followed by examination of their antioxidant properties. We attempted to determine whether essential oils of *C. osmophloeum* and their specific constituents could (1) enhance oxidative stress resistance in a whole organism, and if so, (2) what potential mechanism is involved. The effects of essential oils of *C. osmophloeum* and their specific constituents on the expressions of the antioxidant enzyme superoxide dismutase (SOD)-3 (mitochondrial manganese superoxide dismutase) and the phase II enzyme glutathione S-transferase (GST)-4 were also examined.

MATERIALS AND METHODS

Plant Material and Essential Oil Preparation. Mature healthy leaves of *C. osmophloeum* were collected in October 2005 from the Taiwan Sugar Company Research Center located in Nantou County, central Taiwan. The species was identified by the Taiwan Forestry Research Institute, and voucher specimens were deposited at the Laboratory of Wood Chemistry, School of Forestry and Resource Conservation, National Taiwan University. Fresh leaves (in triplicate 200-g batches) were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h, followed by determination of the oil contents. The yellow essential oil with a characteristic odor was obtained and stored in an airtight sample vial prior to analysis by GC-MS and bioactivity evaluation.

GC-MS and GC-FID Analyses of Essential Oils. Leaf essential oils were analyzed by a Trace GC Ultra (Thermo, Austin, TX) gas chromatograph coupled with a Polaris Q (Thermo) ion-trap mass spectrometer, equipped with a 30 m × 0.25 mm × 0.25 μm DB-5 ms column (Agilent J&W Scientific, Santa Clara, CA). The mass spectrometer was operated in the electron-impact mode, with an ionization energy of 70 eV. The oven temperature was held at 60 °C for 1 min, then programmed to increase from 60 to 220 at 4 °C/min, hold for 2 min, rose to 250 at 20 °C/min, and hold for 3 min. The injector temperature was 250 °C, and the flow rate of the carrier gas, helium, was 1.0 mL/min in a 1:10 split ratio. Diluted samples (1.0 μL, $1/10^4$ v/v, in ethyl acetate) were manually injected in the split mode. Kovats indices were calculated for all volatile constituents by use of a homologous series of C9∼C19 *n*-alkanes on the DB-5 ms column. Quantification was performed by percentage peak area calculations via GC-FID, and the major constituents were identified by coinjection with standards (wherever possible) and confirmed by the Kovats indices by use of the Wiley (v 7.0) and National Institute of Standards and Technology (NIST) v 2.0 GC-MS libraries. The relative concentration of each compound was quantified by integrating the peak area of the chromatograms.

***Caenorhabditis elegans* Strains and Handling Procedures.** Strains used in this study were Bristol N2 (wild-type); CL2166, dvIs19[pAF15(*gst-4*::GFP::NLS)]; and CF1553, muIs84[pAD76(*sod-3*::GFP)]. All *C. elegans* strains as well as the *Escherichia coli* OP50 strain were obtained from the Caenorhabditis Genetics Center (CGC; University of Minnesota). Worms were maintained and assayed (unless otherwise stated) at 20 °C on nematode growth medium (NGM) agar plates carrying a lawn of *E. coli* OP50. Synchronization of worm cultures was achieved by hypochlorite treatment of gravid hermaphrodites.[20]

Oxidative Stress Resistance Assay. Synchronized wild-type L1 larvae were incubated in liquid S-basal medium containing *E. coli* OP50 bacteria at 10^9 cells/mL and essential oil, or 0.1% dimethyl sulfoxide (DMSO) (Wako, Saitama, Japan) as the solvent control for 72 h. Subsequently, adult worms were subjected to an oxidative stress assay. For the oxidative stress assay, juglone (5-hydroxy-1,4-naphthoquinone) (Sigma, St. Louis, MO), an ROS-generating compound, was used to induce oxidative stress in worms. Essential oil-treated and control adult worms were transferred to S-basal medium containing 250 μM juglone for 3 h and then scored for viability. The survival of worms was determined by touch-provoked movement.[21] Worms were scored as dead when they failed to respond to repeated touching with a platinum wire pick. The test was performed at least three times.

Induction of a Stress-Response Reporter. Synchronized L1 larvae containing an inducible green fluorescent protein (GFP) reporter for *gst-4* and *sod-3* were incubated in liquid S-basal medium containing *E. coli* OP50 bacteria at 10^9 cells/mL and essential oil, or the 0.1% DMSO solvent control, for 72 h. Transgenic worms were incubated for 1 h in liquid medium containing 150 μM juglone to generate oxidative stress.[22] The expression of SOD-3 and GST-4 was directly measured by observing the fluorescence of the GFP reporter.

Randomly selected worms from each set of experiments were mounted onto microscope slides coated with 3% agarose, anesthetized with 2% sodium azide, and capped with coverslips. Epifluorescence images were captured with a Leica epifluorescence microscope (Leica, Wetzlar, Germany) by use of a filter set (excitation at 480 ± 20 nm; emission at 510 ± 20 nm) with a cooled charge-coupled device (CCD) camera. Adult worms were examined, and total GFP fluorescence of each whole worm was quantified by Image-Pro Plus software (Media Cybernetics, Bethesda, MD).

Data Analysis. Statistical analyses were performed by SAS 9.2 software (SAS Institute, Cary, NC). Results are presented as the mean ± standard error. The statistical significance of differences between populations was demonstrated by a one-way analysis of variance (ANOVA) and least significant difference (LSD) post hoc test. Differences were considered significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$ (see figures).

RESULTS

Yield and Constituents of Essential Oils. Yields of leaf oils from the two *C. osmophloeum* trees (Y3 and Y6) respectively constituted 1.28% and 0.36% of the leaves. Table 1 shows the main components and their relative contents of leaf essential oils distilled from *C. osmophloeum*. Relative amounts of each component were determined by the GC-FID analysis. *trans*-Cinnamaldehyde (73.99%) was the major compound in the essential oils of the indigenous Y3 tree, followed by cinnamyl acetate (12.61%) and 3-phenylpropionaldehyde (3.69%). In contrast, in the Y6 tree, D-(+)-camphor was the major compound (58.04%), followed by L-bornyl acetate (27.90%) (Table 1).

Table 1. Major Components and Their Relative Contents in Essential Oils from *C. osmophloeum* Leaves

	compd[a]	RT[b] (min)	KI[c]	content (%)
	Tree Y3			
1	*trans*-cinnamaldehyde	16.67	1273	73.99
2	*trans*-cinnamyl acetate	22.26	1444	12.61
3	3-phenylpropionaldehyde	12.93	1164	3.69
4	L-bornyl acetate	17.09	1285	1.19
5	4-allylanisole	14.17	1198	0.81
6	*trans*-β-caryophyllene	21.52	1240	0.49
	Tree Y6			
1	D-(+)-camphor	12.49	1151	58.04
2	L-bornyl acetate	17.10	1285	27.90
3	α-terpineol	14.08	1195	1.08
4	T-cadinol	28.22	1641	1.00
5	caryophyllene oxide	26.48	1581	0.92
6	coumarin	21.91	1433	0.85

[a]Compounds presented here are the top six main components (by percentage) in the essential oils from *C. osmophloeum* leaves according to a GC-MS analysis. [b]Retention time. [c]Kovats index relative to *n*-alkanes (C9∼C25) on a DB-5 ms column.

Essential Oils from the Leaves of *C. osmophloeum* Enhance the Oxidative Stress Resistance of Wild-Type *C.*

***elegans*.** To investigate whether leaf essential oils from the two *C. osmophloeum* trees have protective effects against oxidative stress in *C. elegans*, wild-type N2 worms were pretreated with essential oils from the leaves of *C. osmophloeum*, followed by exposure to juglone-induced oxidative stresses. Wild-type N2 synchronized L1 larvae were pretreated with 0, 10, and 20 μg/mL essential oils and 0.1% DMSO as the solvent control for 72 h before being exposed to juglone (250 μM), a redox cycler that generates intracellular oxidative stress,[23] and then they were incubated for 3 h.

During pretreatment with the essential oils, no adverse effects on the worms, including survival, growth rate, progeny production, body length, or morphological changes, were observed. The results showed that pretreatment with 10 and 20 μg/mL essential oils from the Y3 tree significantly increased the survival of worms exposed to juglone-induced oxidative stress (Figure 1). Similarly, pretreatment with 10 and 20 μg/mL

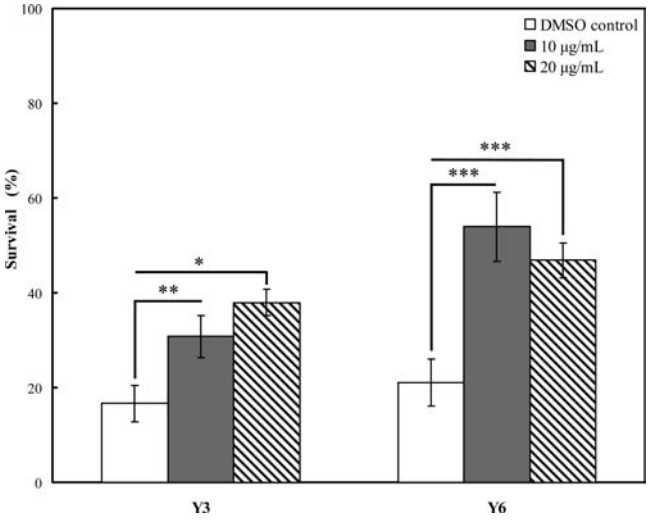

Figure 1. Essential oils from leaves of *C. osmophloeum* enhanced the oxidative stress resistance of wild-type *C. elegans*. Synchronized wild-type L1 larvae were pretreated with essential oils or 0.1% DMSO as the solvent control for 72 h. Subsequently, adult worms were subjected to oxidative stress assays. Essential oil-treated (n = 120) and control (0.1% DMSO, n = 120) adult worms were exposed to 250 μM juglone for 3 h and then scored for viability. The test was performed three times. Error bars represent the standard error, and differences compared to the control (0 μg/mL, 0.1% DMSO) were considered significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ by one-way ANOVA and the LSD post hoc test.

essential oils from the Y6 tree caused an increase in the survival of juglone-treated worms (Figure 1). The results demonstrated that essential oils from the two *C. osmophloeum* trees (Y3 and Y6) enhanced the stress resistance of *C. elegans* under juglone-induced oxidative stress.

Identification of Key Compounds That Contributed to the Oxidative Stress Resistance in *C. elegans*. We next determined which constituents from the two trees (Y3 and Y6) contributed to the observed oxidative stress resistance. We selected the major compounds, *trans*-cinnamaldehyde (73.99%), cinnamyl acetate (12.61%), and 3-phenylpropionaldehyde (3.69%) from the Y3 tree and D-(+)-camphor (58.04%) and L-bornyl acetate (27.90%) from the Y6 tree, for further oxidative stress resistance assays. Figure 2A shows that, in the Y3 tree, 1 μM (0.13 μg/mL) *trans*-cinnamaldehyde pretreat-

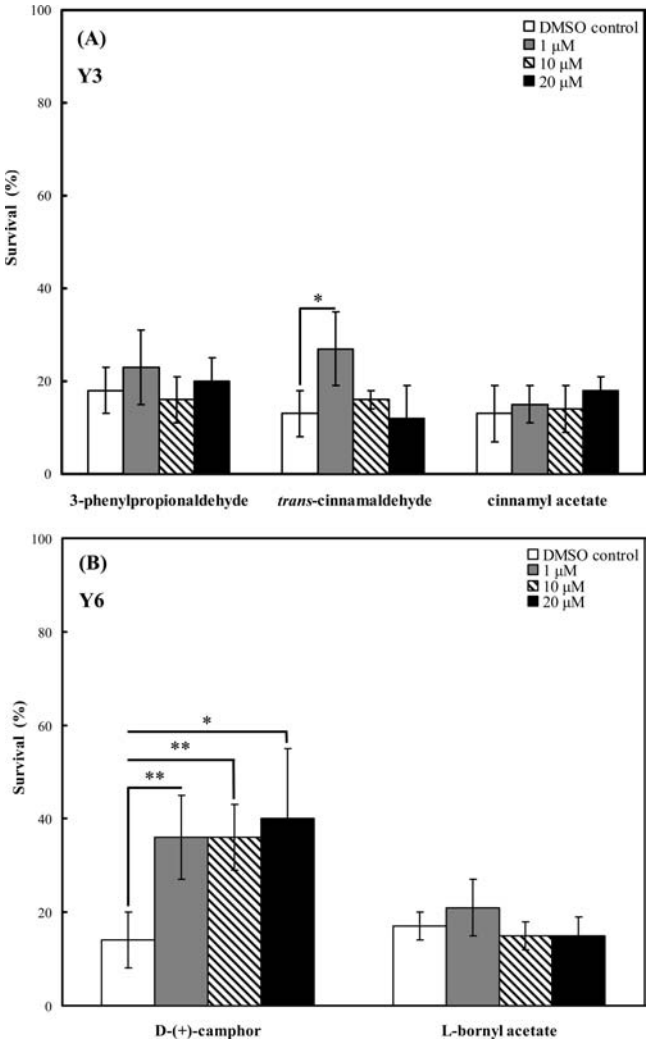

Figure 2. Key compounds contributed to oxidative stress resistance of *C. elegans*. Synchronized wild-type L1 larvae were pretreated with 1, 10, and 20 μM *trans*-cinnamaldehyde (0.13, 1.30, and 2.60 μg/mL, respectively), cinnamyl acetate (0.18, 1.80, and 3.60 μg/mL, respectively), 3-phenylpropionaldehyde (0.13, 1.30, and 2.60 μg/mL, respectively), D-(+)-camphor (0.15, 1.50, and 3.00 μg/mL, respectively), L-bornyl acetate (0.20, 2.00, and 4.00 μg/mL, respectively), or 0.1% DMSO as the solvent control for 72 h. Subsequently, adult worms were subjected to oxidative stress assays. Compound-treated (n = 120) and control (0.1% DMSO, n = 120) adult worms were exposed to 250 μM juglone for 3 h and then scored for viability. The test was performed three times. (A) Major compounds of the Y3 cinnamon tree were *trans*-cinnamaldehyde (73.99%) cinnamyl acetate (12.61%), and 3-phenylpropionaldehyde (3.69%). (B) Major compounds of the Y6 cinnamon tree were D-(+)-camphor (58.04%) and L-bornyl acetate (27.90%). Error bars represent the standard error, and differences compared to the control (0 μg/mL, 0.1% DMSO) were considered significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ by one-way ANOVA and the LSD post hoc test.

ment enhanced the survival of worms, whereas neither cinnamyl acetate nor 3-phenylpropionaldehyde produced a significant increase in the survival of worms exposed to juglone-induced oxidative stress. This suggests that *trans*-cinnamaldehyde might have played a key role in the observed oxidative stress reduction in Figure 1. In the Y6 tree, 1, 10, and 20 μM (0.15, 1.52, and 3.04 μg/mL) D-(+)-camphor, but not L-bornyl acetate, significantly enhanced the survival of worms (Figure

2B), suggesting that the oxidative stress resistance observed in Figure 1 could be attributed to D-(+)-camphor.

***trans*-Cinnamaldehyde and D-(+)-Camphor Enhance Expression of GST-4 and SOD-3 in *C. elegans*.** To elucidate whether the above-described increase in oxidative stress resistance could be due to regulation of a specific stress-response gene by *trans*-cinnamaldehyde or D-(+)-camphor, we examined the responsiveness of the antioxidant enzymes SOD and GST to *trans*-cinnamaldehyde and D-(+)-camphor treatments. Superoxide dismutase (SOD) is a major enzyme that protects against oxidative stress by catalyzing the removal of O_2^-.[24] The *C. elegans* manganese SOD, SOD-3, is an antioxidant enzyme that is induced in response to stress.[25] Enzymes of the glutathione S-transferase (GST) family are involved in the phase II detoxification process, and *C. elegans* GST-4 is involved in the oxidative-stress response.[22]

We treated transgenic *C. elegans* (CF1553 and CL2166) expressing GFP as a reporter transgene for inducible *sod-3* and *gst-4* expression with *trans*-cinnamaldehyde and D-(+)-camphor, respectively. Given that the oxidative stress resistance of wild-type nematodes was achieved at 1 μM levels of both *trans*-cinnamaldehyde and D-(+)-camphor (Figure 2), changes in gene expressions of SOD-3 and GST-4 were examined after treatment with 1 μM (0.13 μg/mL) *trans*-cinnamaldehyde and 1 μM (0.15 μg/mL) D-(+)-camphor.

Changes in gene expression of SOD-3 and GST-4 were studied under non-stressed conditions and in cultures exposed to juglone. SOD-3 and GST-4 expression was significantly induced by *trans*-cinnamaldehyde and D-(+)-camphor in transgenic worms grown in non-stressed normal culture conditions (Figure 3). In addition, the CF1553 and CL2166 transgenic strains were challenged with juglone, and the effects were more profound than that in the non-stressed condition (Figure 3).

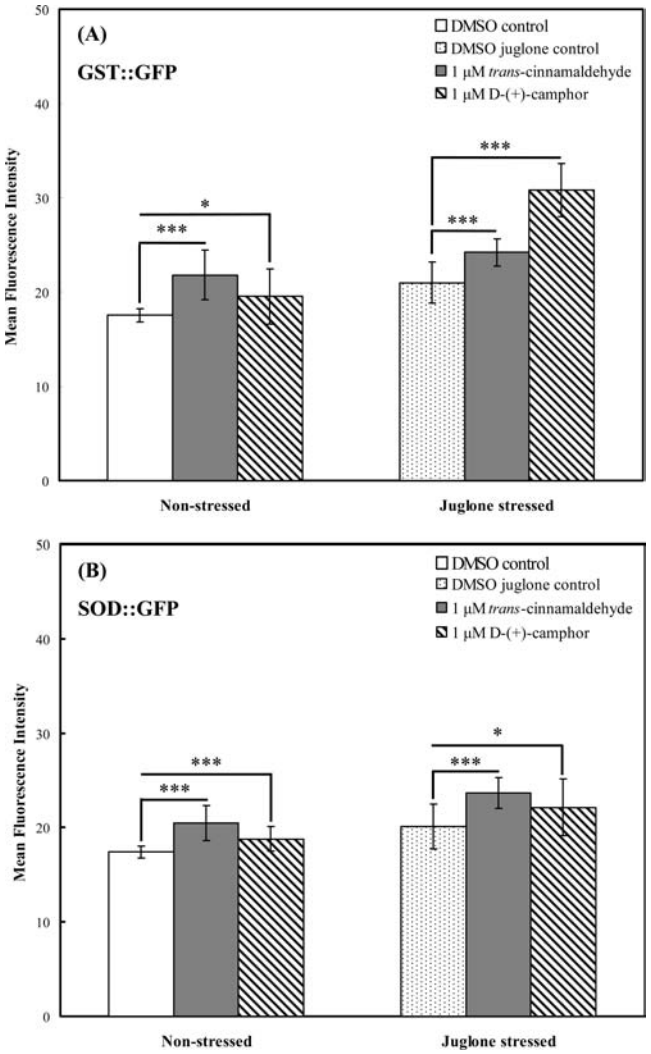

Figure 3. *trans*-Cinnamaldehyde and D-(+)-camphor enhance expression of GST-4 and SOD-3 in *C. elegans*. Immediately after hatching, age-synchronized, transgenic worms of the CF1553 strain (SOD-3::GFP) and CL2166 strain (GST-4::GFP) were incubated with 1 μM (0.13 μg/mL) *trans*-cinnamaldehyde, 1 μM (0.15 μg/mL) D-(+)-camphor, or 0.1% DMSO as the solvent control in liquid S-basal medium for 72 h. In addition, the CF1553 and CL2166 strains were also challenged with 150 μM juglone for 1 h. Total GFP fluorescence of each whole worm prior to and after application of juglone stress was quantified by Image-Pro Plus software. Data shown are the average number of pixels in the transgenic *C. elegans* (n = 60) at each indicated treatment. The test was performed three times. Error bars represent the standard error, and differences compared to the control (0 μg/mL, 0.1% DMSO) were considered significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ by one-way ANOVA and the LSD post hoc test.

DISCUSSION

Cinnamomum osmophloeum has been used as a medicinal plant in Taiwan for decades. To explore the potential usefulness of the leaf essential oils of *C. osmophloeum*, it is important to know the chemical constituents. Although the chemical constituents and biological activities of leaf essential oils of various *C. osmophloeum* clones were studied,[2−4,26] the potential antioxidant activities of the leaf essential oils and their constituents have not yet been evaluated. In addition, most biological activities of leaf essential oils from *C. osmophloeum* were examined in cell cultures or with in vitro systems but not with in vivo studies. In this study, the essential oils from leaves of two *C. osmophloeum* clones (Y3 and Y6) were distilled, and their chemical constituents were analyzed. By use of the model organism *C. elegans*, the antioxidant activities of the essential oils were first evaluated, and subsequently the efficacy of the chemical constituents against oxidative stress was further investigated. Finally, potential mechanisms associated with oxidative stress resistance by essential oils in the nematode *C. elegans* were investigated.

The constituents and their relative contents of essential oils from the same plant species may vary due to ecological and plant growth factors.[27] In this study, the major chemical constituents of indigenous cinnamon Y3 and Y6 leaf oils and their relative contents were determined by GC-MS and GC-FID analyses and are presented in Table 1. Indigenous cinnamon Y3 and Y6 leaf oils are composed of different compounds with various relative contents (Table 1). There are nine different types of *C. osmophloeum* as classified by the chemical constituents: cassia, cinnamaldehyde, coumarin, linalool, eugenol, camphor, terpineol-4-ol, linalool−terpineol, and mixed types.[28] On the basis of this classification, the Y3 indigenous cinnamon tree, which was analyzed in this study, belongs to the cinnamaldehyde type, while the indigenous Y6 cinnamon tree belongs to the camphor type.

We showed that essential oils from both the Y3 and Y6 cinnamon trees significantly increased the survival of worms exposed to juglone-induced oxidative stress (Figure 1). Juglone is an ROS-generating compound; this thus suggests that

extracts from the two *C. osmophloeum* trees might produce their effects by scavenging free radicals, as the toxicity of oxidative stress associated with damage caused by an accumulation of ROS was described.[11,29]

To identify the relationship of the constituents in indigenous cinnamon leaf oils and the antioxidant activities, the major constituents of indigenous cinnamon leaf oils from Y3 and Y6 were further examined for antioxidant activity against juglone-induced oxidative stress. The results showed that, among the three constituents (*trans*-cinnamaldehyde, cinnamyl acetate, and 3-phenylpropionaldehyde), only *trans*-cinnamaldehyde, the major compound in the Y3 cinnamon leaf oil, possessed antioxidative activity in *C. elegans* (Figure 2A). Similarly, only the major compound D-(+)-camphor in the Y6 cinnamon leaf oil exerted antioxidative activity against juglone-induced oxidative stress (Figure 2B).

In Figure 2A, 1 μM (0.13 μg/mL) *trans*-cinnamaldehyde induced stress resistance in worms, whereas 10 (1.30 μg/mL) and 20 μM (2.60 μg/mL) *trans*-cinnamaldehyde did not enhance the stress resistance of worms. However, much higher doses of *trans*-cinnamaldehyde (7.40 and 14.80 μg/mL) were present in the Y3 impure oil that produced increased stress resistance in nematodes (Figure 1). Although *trans*-cinnamaldehyde is the major component (73.99%) in the Y3 impure oil, it is possible that the effect of *trans*-cinnamaldehyde might not completely account for the effect of the Y3 impure oil. It is believed that herbal combinations act synergistically to harmonize and neutralize beneficial effects or minimize toxic or adverse effects of individual constituents.[30] Therefore, it is possible that the combination of components in the Y3 impure oil acts synergistically to account for its effects (Figure 1). Additionally, some unidentified components in the Y3 impure oil might also contribute to its effects (Figure 1). Moreover, results also suggest that a minor component in the oil may have great activity in inhibiting juglone-induced toxicity in *C. elegans*.

To further understand the essential oil's mechanism of action in vivo, we examined the responsiveness of the antioxidant enzymes SOD and GST to 1 μM *trans*-cinnamaldehyde and D-(+)-camphor treatments. Results showed that 1 μM *trans*-cinnamaldehyde or D-(+)-camphor treatment caused increases in the expressions of SOD-3 and GST-4 in transgenic *C. elegans* expressing GFP as a reporter transgene (Figure 3), which might explain why the essential oils could significantly increase the survival of *C. elegans* under oxidative stress.

It was noted that results from the oxidative stress assay showed that the most effective concentration (1 μM *trans*-cinnamaldehyde) resulted in increased survival; the effectiveness, however, declined to the control level at higher doses (10 and 20 μM *trans*-cinnamaldehyde). This suggests that *trans*-cinnamaldehyde at high doses might induce toxicity to *C. elegans*. Similar findings were described for tannic acid, catechin, plant adaptogens, quercetin, and rosmarinic acid.[31−34] With this in mind, it is proposed that low doses of stressful stimuli activate an adaptive response resulting in increased resistance of cells or organisms.[35] The effect of a low concentration of *trans*-cinnamaldehyde or D-(+)-camphor (1 μM) appears to be in agreement with such observations. We found that both *trans*-cinnamaldehyde and D-(+)-camphor at a low concentration (1 μM) significantly increased expression of antioxidant-related genes including SOD-3 and GST-4 (Figure 3). It is possible that by acting as a mild stress stimulus, essential oils activate an adaptive response leading to an increase in the resistance of *C. elegans* to subsequent stress and at the same time increasing maintenance, repair, and resistance processes.

To the best of our knowledge, this is the first report demonstrating that the essential oils from *C. osmophloeum* leaves exert antioxidant activity in vivo. The antioxidant constituents were also isolated and identified from *C. osmophloeum* leaves. Moreover, the potential mechanisms associated with oxidative stress resistance in vivo were studied, and results showed that expression of antioxidative-related genes, including SOD-3 and GST-4, was significantly induced by essential oils from *C. osmophloeum* leaves. Our results showed that essential oils from *C. osmophloeum* leaves may have good potential for further development as nutraceuticals or antioxidant remedies.

AUTHOR INFORMATION

Corresponding Author

*Telephone +886-2-33665239; fax +886-2-33663462; e-mail vivianliao@ntu.edu.tw.

Notes

The authors declare that no competing interests exist.

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Professor Tzu-Ming Pan, Department of Biochemical Science and Technology, National Taiwan University, for reviewing the manuscript.

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