Anti-Tyrosinase and Antioxidant Activity of Proanthocyanidins From Cinnamomum Camphora

Haibo Yang  
Henan Agricultural University

Pingluo Xu  
Henan Agricultural University

Wei Song  
Henan University of Urban Construction

Xiaoqiao Zhai (✉ user7117@163.com )  
henan province academy of forestry

Research Article

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Abstract

In this study, the contents of total phenols (TP) and proanthocyanidins (PAs) in the leaves and branches of *Cinnamomum camphora* were investigated, and isolated PAs were determined using reversed-phase HPLC-ESI-MS. The anti-tyrosinase and antioxidant activities were also evaluated. Furthermore, the scanning study and L-DOPA oxidation were performed to further analyzed the inhibition mechanism of PAs on tyrosinase catalytic activity. PAs had strong inhibitory effects on tyrosinase monophenolase activity, with effectively prolonged the delay time and decreased the steady-state of monophenolase activity. For diphenolase activity, the PAs both showed reversible and mixed inhibition. Moreover, the PAs showed strong antioxidant activities in scavenging 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 1,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonicacid) (ABTS) and the ferric reducing antioxidant power (FRAP) assays. The PAs in leaves showed stronger anti-tyrosinase and antioxidant capacity, suggesting that *C. camphora* may be a good resource for tyrosinase inhibitors and antioxidants. This study could provide a scientific basis for the resource utilization of *C. camphora* and the development of new natural tyrosinase inhibitors and antioxidants in medical, cosmetic, food and agricultural industries.

Introduction

Tyrosinase (EC 1.14.18.1), a copper-containing oxidoreductase with a complex structure, is the key enzyme and rate-limiting enzyme in melanin synthesis, and it is closely related to many pigmentation disorders, malignant melanoma, insect fusion and browning of fruits and vegetables (28). Melanin production can be controlled by regulating the activity of tyrosinase; thus, tyrosinase inhibitors have been highly applied into beauty and whitening, health care, storage of fruits and vegetables, biopesticide and so on (30, 28). In addition, due to the long-term use and side effects of synthetic antioxidants and anti-tyrosinase agents, such as kojic acid, hydroquinone and arbutin, in the medicine, food and beauty industries, there are many health problems, including residual toxicity, induced diseases, and carcinogenesis (33). Natural, low toxicity, high efficiency antioxidants and anti-tyrosinase from plants, the natural treasure house of bioactive substances, have the advantages of high selectivity and low residue, which meet the requirements of the human body and the future development direction of antioxidants and anti-tyrosinase agents. Therefore, it is urgent to find natural antioxidants and anti-tyrosinase agents with strong activity and good safety from plants.

*C. camphora* is an evergreen tree of Lauraceae that is usually used in architecture, furniture, sculpture, shipbuilding, beautifying cities, etc. *C. camphora*, as a traditional Chinese medicine in China, contains complex chemical components, including volatile oil, lignans, flavonoids, glycosides and so on (7). Therefore, it has excellent biological activity, with antibacterial, antioxidant, anti-inflammatory, insecticidal, analgesic, anticancer, and other pharmacological effects (35, 12, 15, 11, 10). Recently, quite a few bioactive substances have been extracted from camphora (18, 21). Lee et al. (16) found that *C. camphora* leaves had considerable antioxidant activity. Additionally, flavonoids and polyphenols in plant extracts were discovered to have a significant correlation with their antioxidation and scavenging free radical capabilities (14, 20). However, few reports concern the development of proanthocyanidins (PAs)
from *C. camphora*. Thus, to make full use of *C. camphora*, separating PAs from the leaves and branches of *C. camphora* and examining their biological activities to offer an academic theoretical reference for the exploration of new efficient and safe antioxidants and anti-tyrosinase agents are warranted.

Proanthocyanidins (PAs) are secondary metabolites synthesized during plant development. They are a class of polyphenols based on flavan-3-ol and linked by carbon bonds. Monoploids are the structural units of PAs and consist of two benzene rings, A and B, and a heterocycle, C (4). There are two kinds of connections, including A-type PAs linked by C2-O-C7 ether bonds and C4→C8 (C6) bonds, and B-type PAs linked together by a C4→C8 (C6) bond, existing in most plants in nature (22). In addition, the hydroxyl part of PAs is the donor of hydrogen atoms, and the hydroxyl part has many electrons, which can react with double bonds. Moreover, PAs are soluble in organic solvents and form stable macromolecular complexes with proteins and polysaccharides through certain kinds of chemical bonds. Therefore, PAs have the biochemical properties of convergence, solubility, UV absorption and stability, which result in many special biological functions. For example, due to its strong free radical scavenging ability and inhibit oxidative damage, PA has become an internationally recognized natural antioxidant; it can also prevent hypertension, protect cardiovascular, and possess anti-aging, antitumor, skin care, and beauty effects. In addition, the anti-tyrosinase activity of PAs was also reported (27, 5).

In this study, PAs from the leaves and branches of *C. camphora* were isolated and purified, and the antioxidant activity and anti-tyrosinase activity were also investigated. To the best of our knowledge, there is currently no report on the structure and composition of PAs from *C. camphora*, or the antioxidant and anti-tyrosinase activities of the PAs. Therefore, this study could provide a theoretical foundation for the comprehensive development of high value-added products of *C. camphora* and provide a greater possibility for potent antioxidant and anti-tyrosinase agents.

**Materials And Methods**

**Preparation of *C. camphora* PAs**

Mature leaves (fully expanded leaves without aging symptoms) and newly-grown branches of *C. camphora* were randomly selected from Xiangyun Park, Pingdingshan City. After being rinsed with deionized water several times, samples were grinded into powder after freeze-drying and stored at -20 °C. 20 g of freeze-dried ground powder was added to 250 mL 70% (V/V) acetone aqueous solution and ultrasonically extracted for 30 min, followed by vacuum filtration to obtain the filtrate. The residue was extracted three times, and the filtrate was combined. The acetone was eliminated using vacuum-rotary evaporation and then extracted by petroleum ether and ethyl acetate successively. The aqueous phase kept was performed concentration and freeze-drying to obtain the crude extract of PAs from *C. camphora*. The crude extracts were dissolved in 50% (V/V) methanol solution, followed by supernatant added into a Sephadex LH-20 column (Pharmacia Biotech, Uppsala, Sweden). Used 50% methanol to remove the impurities and the purified PAs was collected after elution with 70% acetone. Acetone was removed by rotary decompression at 30 °C, and purified PAs were stored at -20 °C after freeze-drying.
Determination of total polyphenols by the Folin phenol method

The method for the determination of total phenols with FC reagent in reference (19) was slightly modified. 1 mL of sample solution was transferred, 5 mL 10% Folin phenol reagent and 4 mL 7.5% Na₂CO₃ solution was added within 5 min after mixing, and then water was added to fix the volume. The standard solution and blank sample were placed at 20°C for 1 h, and then the absorption value was measured by Beckman DU-800 spectrophotometer at 765 nm.

Determination of PAs

The procedure for the determination of PAs referred to reference (19, 25). 1 mL of sample solution was added to 6 mL N-butanol-HCl solution and then boiled for 75 min. After cooling to room temperature, colorimetry was performed at 550 nm and took distilled water and N-butanol-HCl as a control.

RP-HPLC-ESI-MS analysis

The procedure carried out in RP-HPLC-ESI-MS analysis followed references (8). The sample was taken for analysis by RP-HPLC-ESI-MS on an Agilent 1100 system (Agilent, Santa Clara, CA, USA) with a diode array detector (25); further analysis was performed by LC/MS (QTRAP 3200, USA) equipped with a Hypersil ODS column (4.6 × 250 mm) (Elite, Dalian, China) (25). Catechin and epicatechin were used as standards.

Effect of C. camphora PAs on the activity of monophenolase

The 3 mL reaction system including 0.1 mL PAs solution, 2.8 mL phosphate buffer containing substrate (final concentration of L-Tyr was 0.05 mol/L, pH 6.8) and 0.1 mL 0.2 mg/mL mushroom tyrosinase solution was controlled at 30 °C using a constant temperature water bath and determined at 475 nm. The optical density value was read once every 5 s, with a total determination time of 300 s by a Beckman DU-800 spectrophotometer.

Effect of C. camphora PAs on the activity of diphenolase

The 3 mL reaction system containing 0.1 mL PAs, 2.8 mL of phosphate buffer containing substrate L-DOPA (dihydroxyphenylalanine) (final concentration of 0.05 mol/L, pH 6.8), and 0.1 mL 0.2 mg/mL mushroom tyrosinase solution was detected at 475 nm. The detection was carried out every 5 s, with a total time of 300 s by a Beckman DU-800 spectrophotometer.

Inhibition mechanism of C. camphora PAs on diphenolase

According to the method in reference (26, 27), 0.5 mmol/L L-DOPA was used as the substrate, and the concentration of the enzyme was changed in a system containing PAs at different concentrations (1.33, 3.33, 5.33, 7.33 and 9.33 μg/mL). The relationship between enzymatic reaction rate and tyrosinase concentration was determined, and the inhibition mechanism was judged according to the correlation.

Study on inhibition type and inhibition constant of C. camphora PAs on diphenolase
In a 3 mL reaction system, the effect of different concentrations of PAs on the catalytic activity of L-DOPA catalyzed by tyrosinase was determined, with fixed enzyme concentration and varied L-DOPA amount. The inhibition type of the inhibitor was determined by a Lineweaver-Burk double reciprocal plot. The inhibition constant $K_I$ and $K_{IS}$ of the PAs can be obtained by plotting the concentration of the PAs with the slope of the straight line and the intercept of the Y axis, respectively.

**Scanning study**

Took 0.5 mmol/L L-Tyr as substrate, 125 μL 2 mg/mL PAs (leaves) and 2.3 mg/mL PAs (branches) were added in 50 mmol/L Na$_2$HPO$_4$-NaH$_2$PO$_4$ buffer (pH 6.8) at 25 °C. The final concentration of tyrosinase in 3 mL reaction system was 16.67 μg/mL and the spectrophotometer (Beckman DU-8000) was used to record continuously at scanning wavelength of 240-800 nm for 10 minutes.

**Effect of PAs on the oxidation spectra of L-DOPA by sodium periodate**

The 3 mL reaction system containing 0.3 mL 1 mg/mL L-DOPA and 0.5 mL phosphate buffer saline (50 mmol/L, pH 6.8) was tested in the absence and present of 1 mg/mL NaIO$_4$. The spectral changes of oxidation products of L-DOPA were detected using Du-650 spectrophotometer at 230 - 800 nm.

**Determination of antioxidant activity**

**Evaluation of DPPH radical scavenging ability**

3 mL DPPH solution (25 μg/mL methanol dissolution) was added to 0.1 mL sample solutions of different concentrations, and methanol solution was used as a blank instead of sample solution(25). Followed by reacted for 30 min and the tested absorbance was 517 nm. The determination was carried out twice, and three parallel tests were carried out according to the above operation steps. The clearance formula of DPPH was $SA_{DPPH} (%) = [(A_1 - A_2)/A_1] \times 100 \%$. $A_1$ is the blank absorbance; $A_2$ is the absorbance after adding the sample solution to be tested. The IC$_{50}$ value is the sample concentration when the absorbance value is reduced by 50 %.

**Determination of ABTS free radical scavenging ability**

Referring to ReRobert et al. (24), 0.1 mL sample solution and 3.9 mL ABTS free radical working solution were fully mixed. After reacting for 6 min, the absorbance $A_2$ was measured at 734 nm. Took mixture of 80% ethanol solution and ABTS as a control, and the absorbance was $A_1$. The formula for the ABTS radical scavenging rate (%) = $[(A_1 - A_2)/A_1] \times 100\%$.

**Determination of the total oxidation capacity by the FRAP method**

A 0.1 mL sample solution and 3 mL FRAP working solution were added to the centrifuge tube, and after incubation for 5 min at 25 °C, the absorbance value ($A$ value) was tested at 593 nm. Deionized water was added into the FRAP working solution as a blank. According to the $A$ value after the reaction, the
corresponding concentration was obtained on the FeSO$_4$ standard curve, which was defined as the FRAP value.

**Results And Discussion**

**Determination of total phenol (TP) and PAs**

Due to the strong correlation between the biological activity and the content of polyphenols, the determination of polyphenol content by Folin phenol colorimetry can explain the mechanism of biological activity of the samples to be tested. Thus, first, we used the FC method to determine TP content and the HCl-N-butanol method to determine PAs content. The contents of TP and PAs in the leaves of *C. camphora* were 220.6 ± 18.3 mg/g and 152 ± 9.4 mg/g, respectively, and in the branches of *C. camphora*, they were 118.04 ± 11.9 mg/g and 89.6 ± 7.5 mg/g, respectively. The results showed that the content of TP and PAs in the leaves of *C. camphora* was 22% and 15%, respectively, and that in the branches of *C. camphora* was 12% and 9%, respectively. The content of PAs in leaves was more abundant than that in branches, suggesting that leaves may be a good raw material for PAs supply.

**Reversed-phase HPLC-ESI-MS**

The basic structural units and polymer chain lengths of PAs were predicted by measuring the conjugates of acid-catalyzed PAs with benzylmercaptan. The HPLC-ESI-MS results of PAs from *C. camphora* leaves and branches are shown in Fig. 1. It can be seen that the PAs from leaves and branches have obvious difference in the peak position and intensity, indicating that there are difference in the composition of two parts in *C. camphora*, in which catechin benzylthioether (C-thio) and afzelechin/epiafzelechin (AF/EAF) were detected in leaves, while no significant AF/EAF was detected in branches. But for both, the terminal units were catechin (C) and the extension units were mainly catechin/epicatechin benzylthioether (C/EC-thio). The difference of active phenolic hydroxyl groups and dihydroxy phthalic groups may be the main reason for the better anti-tyrosinase and antioxidant activity of leaves PAs.

**Effect of *C. camphora* PAs on the activity of tyrosinase monophenolase and diphenolase**

Tyrosinase has monophenolase activity of hydroxylation of monophenol to diphenol and diphenolase activity of oxidation of bisphenol to quinine (26, 3). Therefore, we first measured the monophenolase activity using L-tyrosine (L-Tyr) as the substrate. Fig. 2 a-ı shows the catalytic reaction of the inhibition of monophenolase activity by the PAs and plots the concentration of PAs with steady state activity and lag time (Fig. 2 a-ı and Fig. 2 a-ıı). The results showed that the PAs from the leaves and branches of *C. camphora* could both effectively prolong the delay time of monophenolase activity and decrease its steady-state activity, and the inhibitory effect showed an obvious dose-dependent manner. It was found that when the concentration of the PAs from leaves and branches reached 200 μg/mL and 306.67 μg/mL, the lag time increased from 13.14 s to 120 s and 3.6 s to 33 s, respectively, while the steady-state activity decreased from 100% to 43.23% and 42.86%, respectively. The IC$_{50}$ of PAs was 166.65 ± 18.1 μg/mL and
268.38 ± 23.9 μg/mL, respectively (Table 1), indicating that PAs extracted from leaves possess better inhibitory effect on tyrosinase monophenolase activity than that from branches.

L-DOPA was used as a substrate to determine the activity of tyrosinase diphenolase, and the reaction curve obtained is shown in Fig. 2 b. The enzyme activity decreased with increasing PAs concentration, and the IC\textsubscript{50} values were 70.31 ± 6.62 μg/mL and 90.93 ± 8.15 μg/mL, respectively (Table 1). Both of them had good inhibitory activity on tyrosinase diphenolase, especially the extract of PAs from leaves of C. camphora. Qu et al. (23) reported the effects of puerarin on the monophenolase activity with an IC\textsubscript{50} value of 0.537 mg/mL. Cui et al. (9) reported that T. grandis "Xiangyafei" seed oil (XYSO) exhibited the highest activities in the tested seed oils against tyrosinase monophenolase (IC\textsubscript{50} = 817.5 μg/mL). XYSO in T. grandis was tested against diphenolase with IC\textsubscript{50} values of 227.01 ± 2.68 μg/mL (9). A. aucheri oil exhibited anti-tyrosinase activity at a 50% concentration (IC\textsubscript{50}) of 6.43 mg/mL (29). Obviously, PAs extracted from the leaves and branches of C. camphora both showed better inhibitory effect on monophenolase and diphenolase, suggesting that C. camphora may be a good resource for tyrosinase inhibitors. Thus, it is feasible to seek potent tyrosinase inhibitor PAs from C. camphora.

**Inhibition mechanism of PAs from the leaves and branches of C. camphora on tyrosinase**

The inhibition mechanism of PAs on tyrosinase is shown in Fig. 2 c. The slope of straight lines decreased with increasing PAs concentration, and two sets of straight lines passing through the origin were obtained by mapping the enzyme activity to the PAs concentration. These suggested that instead of reducing the amount of effective enzyme, PAs reduced the catalytic efficiency of enzyme to achieve the inhibitory activity, which can be concluded that the inhibition of tyrosinase by PAs is a reversible process. Reduced catalytic efficiency reversible has been a common inhibition mode of PAs from plants on tyrosinase diphenolase (2, 6) (26). As shown in HPLC-ESI-MS, the basic structural units of C/EC and AF/EAF in PAs, which have very active phenolic hydroxy groups and dihydroxy phthalic groups, may be the structural reason for its reversible inhibition of tyrosinase activity.

**Determination of the inhibition type and inhibition constant of PAs from C. camphora on tyrosinase**

In the diphenolase detection system, by measuring the effect of L-DOPA on tyrosinase activity, a group of straight lines with different slope intersected in the second quadrant were obtained, as shown in Fig. 3 a and Fig. 3 b, in which the Km increased and Vm decreased with the increase of substrate concentration, showed mixed competitive inhibition. The inhibition constant $K_I$ and $K_{IS}$ of leaves PAs can be calculated as 33.36 μg/mL and 344.44 μg/mL, respectively (Table 1). For PAs from branches of C. camphora, the inhibition constants $K_I$ and $K_{IS}$ were 17.97 μg/mL and 349.89 μg/mL, respectively. For PAs from the leaves and branches of C. camphora, $K_{IS}$ was 10 and 19 times that of $K_I$, respectively. $K_{IS}$ were much larger than $K_I$ further indicated that the binding ability of PAs extracted from the two parts of C. camphora to free enzyme were much stronger than that to the enzyme substrate complex. In conclusion, the results demonstrated for the first time that the leaves and branches of C. camphora might be good sources for
further development of tyrosinase inhibitors, which indicated the possible application of these compounds, especially PAs from leaves, in food, agricultural, cosmetic and medical industries.

**Scanning study**

To further analyzed the inhibition mechanism of PAs on tyrosinase catalytic activity, UV-Vis spectroscopy was used to assess the L-Tyr oxidation and L-DOPA oxidation of PAs. Fig. 4 showed the time-dependent UV spectra of PAs acting on tyrosinase catalyzed oxidation of tyrosine. The absorption value in 475 nm increased with the increase of catalytic time, suggesting that 475 nm is the characteristic peak of the oxidation of L-Tyr by tyrosinase. Compared with Fig. 4a, the absorption values of absorption peaks at 475 nm caused by PAs decreased with time (Fig. 4 b-c), indicating that PAs could effectively inhibit the oxidation of L-Tyr by tyrosinase.

**L-DOPA oxidation**

The product of L-DOPA catalyzed by tyrosinase can also be obtained from the non enzymatic catalysis such as oxidized by NaIO$_4$. Using spectral analysis to assess the effect of PAs on the oxidation of L-DOPA, and Fig. 5 showed that the characteristic peaks of L-DOPA oxidized by NaIO$_4$ are located at 475 nm after adding PAs, and the absorbance value obviously decreased. It is suggested that PAs could decline the L-DOPA oxidation products. Therefore, PAs in *C. camphora* could inhibit the oxidation of L-Tyr by tyrosinase, and the effect of PAs on the spectra of oxidation products of L-DOPA indicated that PAs could depress the oxidation products of L-DOPA and prevent the formation of L-DOPA pigment, which leads to the reduction of characteristic absorption peak of the product.

**Determination of antioxidant capacity**

Due to the rich hydroxyl structure, PAs can prevent the chain reaction of free radicals by releasing H$^+$ in the structure to compete with free radicals, eliminate free radicals and protect lipids from oxidation (34, 31, 1, 32, 13). Thus, DPPH, ABTS and FRAP methods were used to investigate the antioxidant capacity of PAs in leaves and branches of *C. camphora*. The relationship between the scavenging capacity of PAs from two plant parts on DPPH free radicals were shown in Fig. 6 a. In the range of experimental concentrations, with increasing concentration, the scavenging effect of each part on DPPH free radicals also showed an increasing trend. The antioxidant activities of different extracts were significantly different, and the DPPH scavenging ability of PAs extracted from these two parts was relatively weak compared to that of VC. The IC$_{50}$ of DPPH radical scavenging activity of leaves and branches were 77.51 ± 12.6 µg/mL and 273.53 ± 28.3 µg/mL, respectively (Table 2). The DPPH scavenging ability of leaves was higher than that of branches. Fig. 6 b shows that the ABTS radical scavenging rate of PAs and VC were positively correlated with the concentration, and the change trend was similar to the scavenging rate of DPPH radicals. The IC$_{50}$ values of leaves and branches in Table 2 are greater than those of VC. The results of the FRAP assay were expressed in the form of VC equivalent, that is, the ability of a 1 g sample to scavenge free radicals is equivalent to the capacity in mmol of VC. It can be seen from the results in
Table 2 that leaves PAs (4.74 ± 0.46 mmol AAE/g) > branches PAs (3.58 ± 0.35 mmol AAE/g). Wang et al. reported that the Fuwan 8, Dongliang and FD97 varieties of Dimocarpus longan Lour. had the strongest DPPH scavenging activity, with an IC$_{50}$ of 1.03 g/mL (31). The anthocyanins isolated from Lycium ruthenicum Murr possessed ABTS radical scavenging capacity, with IC$_{50}$ of 0.5023 ± 0.011 mg/mL (17). Because the total phenol content (TPC) is the greatest antioxidant contributor in the DPPH and FRAP assays, the radical scavenging ability of PAs in leaves was better than that of PAs in branches, which may be due to the relatively high TPC. In short, the results demonstrated for the first time that PAs from the leaves and branches of C. camphora might be good sources for the further development of antioxidants.

**Conclusions**

In this study, the separation, purification and determination of the content of total phenols and PAs in leaves and branches of C. camphora were carried out first. Then, through the analysis of their structure and evaluation of their activity, including anti-tyrosinase and antioxidant activity, the resource availability of PAs from the leaves and branches of C. camphora was clarified and the relationship between the two was identified based on structure and activity. Therefore, this study could lay a theoretical foundation for better research, development and utilization of the biological activity of PAs and provide a possibility for the development of new efficient, natural tyrosinase inhibitors and antioxidants.

**Declarations**

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**Compliance with Ethical Standards**

**Ethical Approval** Not applicable

**Consent to Participate** Not applicable

**Consent to Publish** All authors consented to the publication of this work. Authors all confirm the permission of publication for this study.

**Competing Interests** The authors declare that they have no competing interests.

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Tables

**Table 1.** Inhibition activity and constants of proanthocyanidins (PAs) extracted from leaves and branches of *Cinnamomum camphora*

| Samples   | IC$_{50}$ (μg/mL) | Inhibition | Inhibition constants (μg/mL) |
|-----------|-------------------|------------|------------------------------|
|           | monophenolase     | diphenolase| mechanism  | type    | $K_I$   | $K_{IS}$ |
| Leaves    | 166.65 ± 18.1     | 70.31 ± 6.62 | Reversible | mixed   | 33.36   | 344.44   |
| Branches  | 268.38 ± 23.9     | 90.93 ± 8.15 | Reversible | mixed   | 17.97   | 349.89   |
Table 2. Comparison of antioxidant capacity between leaves and branches proanthocyanidins (PAs) from *Cinnamomum camphora*. and VC

| Samples | DPPH ($IC_{50}$ μg/ml) | ABTS ($IC_{50}$ μg/ml) | FRAP (mmol AAE/g) |
|---------|-------------------------|-------------------------|------------------|
| Leaves  | 77.51 ± 12.6            | 117.16 ± 15.9           | 4.74 ± 0.46      |
| Branches| 273.53 ± 28.3           | 229.59 ± 30.1           | 3.58 ± 0.35      |
| Vc      | 72.87 ± 0.47            | 74.31 ± 9.34            | —                |

Figures
Figure 1

Reversed-phase HPLC-ESI-MS of proanthocyanidins (PAs) extracted from leaves (a) and branches (b) in Cinnamomum camphora. C: catechin; C-thio: catechin benzylthioether; C/EC-thio: catechin/epicatechin benzylthioether; AF/EAF: afzelechin/epiafzelechin; BM: benzyl mercaptan.
Figure 2

Inhibition effects of proanthocyanidins (PAs) extracted from leaves and branches in Cinnamomum camphora on monophenolase (a), diphenolase (b) activity and inhibitory mechanism (c) of mushroom tyrosinase. (i) Progress curve for the oxidation of L-Tyr by the enzyme. In leaves, the concentrations of proanthocyanidins (PAs) for curve 0-4 was 0, 33.33, 66.67, 133.33 and 200 μg/mL, respectively. In branches, curves 0-4 represent the reaction curve of the system when the concentration of proanthocyanidins (PAs) was 0, 76.67, 153.33, 230 and 306.67 μg/mL, respectively. (ii) Effects of proanthocyanidins (PAs) on the steady-state rate of monophenolase. (iii) Effects of proanthocyanidins...
(PAs) on the lag time of mushroom tyrosinase. In leaves, lines 0-4 represent the reaction curve of the system when the concentration of proanthocyanidins (PAs) was 0, 13.33, 40, 66.67, 93.33 μg/mL. In branches, lines 0-4 represent the reaction curve of the system when the concentration of proanthocyanidins (PAs) was 0, 30.67, 46, 76.67, 92 μg/mL.

Figure 3

Determination of the inhibitory type and constants of proanthocyanidins (PAs) extracted from leaves (a) and branches (b) of Cinnamomum camphora. In leaves, lines 0-4 represent the reaction curve of the system when the concentration of proanthocyanidins (PAs) was 0, 13.33, 40, 66.67, 93.33 μg/mL with L-DOPA as substrate. In branches, lines 0-4 represent the reaction curve of the system when the concentration of proanthocyanidins (PAs) was 0, 30.67, 46, 76.67, 92 μg/mL. (ii) The plot of intercept versus proanthocyanidins (PAs) concentration for determining the inhibition constants KIS. (iii) The plot of slope versus PAs concentration for determining the inhibition constants KI.

Figure 4
The UV-Vis spectra of the oxidation of L-Tyr by tyrosinase. (a): L-Tyr; (b): L-Tyr + leaves proanthocyanidins (PAs); (c): L-Tyr + branches proanthocyanidins (PAs). Lines 0-11 represent 0-11 minutes after enzyme addition.

Figure 5

The UV-Vis spectra of the oxidation of L-DOPA by NaIO4 in the absence and presence of proanthocyanidins (PAs) from leaves (a) and branches (b) of Cinnamomum camphora.
Figure 6

The antioxidant activity of the leaves and branch proanthocyanidins (PAs) from Cinnamomum camphora is determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (a), 1,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonicacid) (ABTS) assay (b) and ferric reducing antioxidant power (FRAP) assay (c).