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Evaluation and analysis of phytochemical antioxidant capacity.

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Abstract

According to previous studies, phytochemicals have many pharmacological activities. Chinese herbal remedies that have potential use in many diseases. Caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid are bioactive compounds from plants. In the present study, the antioxidant properties of the caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid were evaluated by using different *in vitro* antioxidant assays such as 1, 1-diphenyl-2-picryl-hydrazyl free radical (DPPH•) scavenging, metal chelating activities and cytochrome c reduced method. The result of this study showed that caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid all have apparent antioxidant activity by these assays. Among these compounds, oleanolic acid was capable of inhibiting xanthine oxidase and showed a significant antioxidant effect. Thus, these compounds have potential to be used as natural antioxidants against oxidative deprivation for preventing carcinogenesis and other diseases.

Keywords: DPPH radical activity, Antioxidant activity.

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Introduction

It has been indicated that oxygen molecules and most of the organisms present in our environment produce Reactive Oxygen Species (ROS) through the metabolism of oxygen to produce energy, and that these ROS are pathologic mediators in many diseases. Thus, many scientists have conducted extensive studies on the prevention or improvement of tissue damage resulting from active oxygen [1-6]. The main active oxygen species stored in cells are superoxide and peroxide, and the generation of excessive reactive oxygen species causes depletion of intracellular GSH (Glutathione), lipid peroxidation, changes in enzyme activity and DNA damage [7]. Therefore, if cells succumb to excessive exposure to reactive oxygen species, this exposure would induce large amounts of ROS damage and excessive lipid peroxidation in the organism body and finally result in cell apoptosis or necrosis [8,9]. While the mitochondria within a cell are the main sites for the generation of ROS, they mostly come from the mitochondrial electron transport chain. Some studies have further pointed out that the mitochondria play a key role in regulating the process of cell death and many mitochondrial-related proteins dominate the apoptosis process.

Hence, any kind of substance with antioxidant effects, could remove ROS or prevent lipid peroxidation [10,11]. Such a substance might even affect the biochemical conversion of the mitochondria and cause potential changes in the mitochondrial membranes, which regulate cell death messages. This possibility has led many scientists to conduct extensive research toward finding ways to prevent, improve, and reduce tissue damage caused by ROS in order to achieve anti-aging effects and disease prevention. Antioxidant effects elicited by plant species have a full range of prospective applications in human health care. Therefore, attention has shifted to non-nutritive phytochemicals present in a plant-based diet. It is estimated that more than 1000 different phytochemicals possess chemopreventive properties [12,13]. A lot of antioxidants minimize actions of ROS production. In this study was aimed at evaluating their antioxidative activities of caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid by DPPH radical activity, chelating activity and cytochrome c reduced method.

Materials and Methods

Chemicals

DMSO, DPPH, Vitamin C, EDTA, caffeic acid, p-coumaric acid, ursolic acid and oleanolic acid were purchased from Sigma chemical Co, USA.

DPPH radical activity

Because fats produce free radicals in the oxidation process and result in fat rancidity, common antioxidants provide hydrogen (hydrogen donor) to remove peroxyl radicals and thus inhibit the oxidation chain reaction. The present study used DPPH to assess the hydrogen donor ability of a given antioxidant. Since DPPH has a strong absorption at 517 nm in methanolic solution, as antioxidants restore it, the absorbance value is decreased and the spectrophotometric method could be used to measure the absorbance values at 517 nm. If the absorbance value decreases, then this means that the antioxidant has stronger hydrogen donor ability [14]. Radical scavenging activity (%)=(Control OD 517 nm-sample OD 562 nm))/ Control OD 562 nm × 100%.

Chelating activity of Fe^{2+} ions

The present study used the chelating activity of Fe^{2+} ions to determine the pro-oxidant effect of metal ions, which is often the main factor that causes lipid oxidation. Through redox cycle reactions, only a small amount of metal ions could effectively produce free radicals and accelerate lipid oxidation. From a variety of metal ions, Fe^{2+} is often the most influential pro-oxidant, which would promote lipid oxidation. Using the absorbance value of Fe^{2+} and ferrozine complexes at 562 nm, the chelating ability of test samples with Fe^{2+} ions could be measured. If the absorbance value decreases, this represents the chelating ability of test samples with Fe^{2+} ions. If this value is low, the test sample has a stronger chelating ability with Fe^{2+} ions. When the chelating activity of Fe^{2+} ions is expressed in percentages, the higher the chelating ability, then the stronger the antioxidant effects [15]. Chelating effects (%)=(1-(sample OD 562 nm-Control OD 562 nm))/Control OD 562 nm × 100%.

Cytochrome c reduced method

The cytochrome c reduction method was used to conduct an antioxidant study. Xanthine oxidase catalyses xanthine to produce uric acid and the maximum absorption occurs at a wavelength of 295 nm through uric acid. Then the spectrophotometric method could be used to determine the absorbance change of uric acid and could be used as an indicator for xanthine oxidase activity. At the same time, when xanthine oxidase catalyses xanthine to produce uric acid, it would be accompanied by superoxide ($\cdot O^{2-}$) generation. If cytochrome c (the most important electron carrier in the mitochondrial electron transport chain) is used as a detection agent, the redox reaction between cytochrome c and $\cdot O^{2-}$ could result in a substantial increase in the amounts of reduced state cytochrome c (Ferro-cytochrome c). Due to the fact that reduced state cytochrome c has a specific absorption at 550 nm [16,17], the spectrophotometer could be used to detect absorbance change at 550 nm and the amount of reduced state cytochrome c could be determined and could be used indirectly to assess the ability of drugs removing superoxide [18]. Cytochrome c reduction (%)=(Control OD 550 nm-sample OD 550 nm))/Control OD 550 nm × 100%.

Statistical analysis

All data were analysed using the Statistical Package for the Social Sciences Ver 22 software (SPSS Inc., Chicago, IL).

Results

The antioxidation activity of isolated compounds

DPPH radical activity was used to evaluate an antioxidant's capacity for hydrogen donation, the determination of chelating activity of Fe^{2+} ions and cytochrome c reduction methods in antioxidant studies. The results showed that DPPH radical activity analysed antioxidant capacity based on free radical removal capability and the activity order was caffeic acid>p-coumaric acid>ursolic acid> oleanolic acid (Table 1) with IC_{50} values of 0.97, 6.65, 6.88, and 26.47 μM, respectively. The IC_{50} value of caffeic acid was less than the IC_{50} value of Vitamin C (1.94 μM) (Table 1). Moreover, when using chelating activity of Fe^{2+} ions to measure antioxidant ability, the order of free radical removal ability was caffeic acid>p-coumaric acid> oleanolic acid >ursolic acid (Table 2), but their IC_{50} values were 655.3, 1234.1, 1860.6 and 9310 μM, respectively (Table 2). The order of cytochrome c inhibition ability of cytochrome c reduction method was oleanolic acid>ursolic acid>p-coumaric acid>caffeic acid (Table 3) and the IC_{50} values were 10.3, 15.3, 51.5 and 88.4 μM, respectively. The IC_{50} value of oleanolic acid is less than the IC_{50} value of Vitamin C (14.09 μM) (Table 3). From the above experimental results, we discovered that while oleanolic acid, ursolic acid, p-coumaric acid and caffeic acid all have antioxidant effects and their main mechanisms of action were different. The antioxidant effect of caffeic acid was mainly used for the removal of peroxyl radicals by being a hydrogen donor for the inhibition of the oxidation chain reaction or for the chelating of Fe^{2+} ions.

Table 1. *The IC_{50} value of DPPH radical activity of caffeic acid, p-coumaric acid, ursolic acid, and oleanolic acid.*

Compound	Inhibition (%)			IC_{50} (μM)
	1 μM	2.5 μM	5 μM	
Vitamin C	33.54 ± 4.1	67.57 ± 7.3	73.37 ± 10.0	1.94
caffeic acid	46.12 ± 6.2	67.20 ± 5.2	75.83 ± 8.9	0.97
p-coumaric acid	30.09 ± 3.0	37.61 ± 6.1	43.90 ± 9.2	6.65
ursolic acid	22.32 ± 3.1	29.22 ± 2.0	41.18 ± 4.0	6.88

oleanolic acid	22.19 ± 2.0	22.07 ± 5.1	26.39 ± 3.2	26.47

Not determined. Values represent mean ± SD of three independent experiments. Vitamin C was used as a positive control in DPPH radical activity.

Table 2. *The IC_{50} value of chelating activity of Fe^{2+} ions of caffeic acid, p-coumaric acid, ursolic acid, and oleanolic acid.*

Compound	Inhibition (%)			IC_{50} (μM)
	25 μM	50 μM	100 μM	
EDTA	40.48 ± 6.2	77.34 ± 14.0	96.34 ± 17.0	27.5
caffeic acid	-	2.19 ± 3.6	5.90 ± 2.1	655.3
p-coumaric acid	1.11 ± 0.2	0.43 ± 0.1	3.85 ± 1.0	1234.1
ursolic acid	-	-	-	9310
oleanolic acid	0.79 ± 0.2	3.33 ± 0.7	3.14 ± 1.1	1860.6

Not determined. Values represent mean ± SD of three independent experiments. EDTA was used as a positive control in chelating activity of Fe^{2+} ions.

Table 3. *The IC_{50} value of the cytochrome c reduction of caffeic acid, p-coumaric acid, ursolic acid, and oleanolic acid.*

Compound	Inhibition (%)			IC_{50} (μM)
	25 μM	50 μM	100 μM	
Vitamin C	49.7 ± 14.2	71.5 ± 8.2	82.1 ± 15.0	14.1
Caffeic acid	-	26.1 ± 7.3	56.9 ± 5.2	88.4
p-coumaric acid	45.6 ± 7.1	50.3 ± 13.0	55.7 ± 7.0	51.5
Ursolic acid	50.3 ± 7.2	61.6 ± 6.0	69.8 ± 10.2	15.3
Oleanolic acid	51.6 ± 8.0	57.9 ± 10.2	64.6 ± 17.4	10.3

Not determined. Values represent mean ± SD of three independent experiments. Vitamin C was used as a positive control.

Discussion

Free radicals have potential of reacting with biological macromolecules and inducing tissue damage such as oxidative damage in lipids, proteins and DNA. Medical plants play an important role in maintaining the health and cure disease over the thousand years in Asia. Many phytochemicals including flavonoids, terpenoids, phenolic compounds, saponins, plant sterols, and curcumins have been identified and evaluated the pharmacological activities. Some of these phytochemicals have been found to be potent antioxidants and free radical scavengers with anti-cancer activities [19-21]. In order to further examine the antioxidant mechanism of compounds, different experiments were performed. This experiment includes the radical scavenger, the metal chelating mechanisms and reduction of cytochrome c. The scavenger mechanism (DPPH) is based on the reaction of the antioxidant directly with the hydroxyl radical. In metal chelation assay, these compounds may chelate the ferrous ions with hydroxyl groups. Oleanolic acid and ursolic acid are extracted from many plants [22]. Oleanolic acid and ursolic acid are triterpenoid compounds and widely used in food and folk medicine. Oleanolic acid and ursolic acid have antiinflammatory and antihyperlipidemic properties *in vitro* and *in vivo* study [22]. The hepatoprotection of two compounds may result from the inhibition of toxicant activation. The current antioxidant effect of oleanolic acid was present in the inhibition of xanthine oxidase. The two compounds displayed anti-cancer activities could result from antioxidant effect. The detail mechanism of pharmacological activities can be examined in the future. Caffeic acid (3, 4-dihydroxycinnamic acid) is phenolic compounds with hydroxycinnamic acids chemical structure. Based on recent researches, caffeic acid and p-coumaric inhibited LDL oxidation and quenche radicals [23,24]. Based on these results, we found that caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid have antioxidation activity by different mechanism.

Conclusion

According to data obtained from the present study, caffeic acid, p-coumaric acid, oleanolic acid and ursolic acid was found to be an effective antioxidant in different *in vitro* assays. However, further studies are required to determine the exact pharmacological mechanisms involved in preventing carcinogenesis, diseases or food products.

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