Original Article

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Comparison and combination effects on antioxidant power of curcumin with gallic acid, ascorbic acid, and xanthone

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Summary **Curcumin has been extensively reported as a potential natural antioxidant. However, there was no data on activity comparison as well as the biological interactions of curcumin with other natural antioxidants. The aim of the present study was to investigate the antioxidant power of curcumin in comparison with three important natural antioxidants; gallic acid, ascorbic acid, and xanthone on free radical scavenging action and their combination effects on this activity. The results indicated that the activities of these compounds were dose-dependent. The 50% effective concentration (EC_{50}) of curcumin was found to be 11 µg/mL. Curcumin showed significantly higher antioxidant activity than ascorbic acid and xanthone but less than gallic acid. Interestingly, curcumin revealed synergistic antioxidant effect when combined with gallic acid whereas the antagonistic effect occurred in curcumin combination with ascorbic acid or xanthone. These results suggest that curcumin-gallic acid combination is the potential antioxidant mixture to be used in place of the individual substance whereas using of curcumin in combination with ascorbic acid or xanthone should be avoid.**

Keywords: Antioxidant, curcumin, gallic acid, synergistic effect, antagonistic effect

1. Introduction

There are abundant studies showing the role of antioxidants on reduction of radical oxygen species (ROS) in the maintenance of human health and prevention as well as treatment of various diseases caused by the excessive ROS. Antioxidants from bio-resources have received much attention because of their points of safety and natural aspects. Most plants in the world contain various kinds of phytochemical compounds such as phenolic acids, curcuminoids, carotenoids, and flavonoids which are the major sources of natural antioxidants (*1*).

Curcumin is a well-known natural antioxidant existing in various kinds of plants, especially in *Curcuma longa* L. (turmeric). Curcumin in its crude form has been used for many centuries as spice and dietary supplement as well as component of many traditional Asian medicines. It has been reported that curcumin has a wide range of pharmacological activities against many chronic diseases including type II diabetes, rheumatoid arthritis, multiple sclerosis, Alzheimer's disease and atherosclerosis (*2-6*). Further, curcumin inhibits platelet aggregation, suppresses thrombosis, inhibits human immune deficiency virus (HIV) replication, enhances wound healing and prevents liver injury, cataract formation, pulmonary toxicity and fibrosis (*7-9*). Many reports revealed that curcumin has these pharmacological activities because of its basic beneficial antioxidant, anti-inflammatory, antibacterial, and anticancer activities (*10,11*). Regarding to the antioxidant role, curcumin has been demonstrated to have an effective scavenging property on various harmful free radicals which not only ROS but also nitrogen dioxide radicals, superoxide anions and hydroxyl radicals (*12*). Curcumin also displayed the high binding affinity on metal ions that related to the ROS formation especially on the iron chelating ability (*13*). Moreover, curcumin can inhibit free radicals from mediating lipid peroxidation of membranes or oxidative DNA damage which are the important initiator for carcinogenesis (*14,15*).

Three other natural compounds that are generally classified as potential antioxidants are gallic acid, ascorbic acid, and xanthone. They are the antioxidants found in many kinds of plants. Gallic acid is a phenolic antioxidant commonly found in fruit and flowering

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plants, for example, grapes, berries, lemons, oak barks as well as in wine (*16,17*). It has been reported to have anti-inflammatory, anticancer, and anti-hyperglycemic activities (*18*). Ascorbic acid or vitamin C is an antioxidant exists in many kinds of fruits. It has been effectively used as a standard antioxidant for free radical scavenging evaluation. The previous study demonstrated that ascorbic acid involved in many biological functions such as a cofactor of enzymatic reaction and played a role on cancer and chronic diseases through an antioxidant activity (*19*). Xanthone is one of the important natural antioxidants. It has been studied for recent years and reported that xanthone possesses anticancer, anti-inflammatory, antibacterial, and cardioprotective activities (*20,21*).

Even the antioxidant activity of curcumin has been extensively reported, there was only the action of the individual compound. Taken together of curcumin with gallic acid, ascorbic acid, or xanthone, it is assumed to get the higher beneficial antioxidant effects of free radical scavenging property. However, to our knowledge so far there are no studies on these biological effects of curcumin in combination with these compounds. The present study explores not only the combination effects but also the comparative antioxidant power of curcumin in comparison with gallic acid, ascorbic acid and xanthone individually.

2. Materials and Methods

2.1. *Materials*

Analytical grade curcumin, gallic acid, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Xanthone was extracted from *Garcinia mangostana* fruit peels (*22*). Absolute ethanol was purchased from Merck (Darmstadt, Germany). Other reagents and chemicals were of the highest grade available.

2.2. *Samples preparation*

Stock solution of curcumin, gallic acid, ascorbic acid, and xanthone were prepared by dissolving in ethanol and diluted to get the concentrations of 1000, 500, 250, 125, and 62.5 µg/mL. Stock standard Trolox solution was prepared and diluted to have the concentration series of 125, 100, 75, 50, 25, and 12.5 µg/mL.

2.3. *Effect of reaction time on antioxidant activity*

The DPPH free radical-scavenging method performed by Okonogi *et al*. (*23*) with some modification was used to measure the free radical scavenging activity of the samples. Briefly, 20 µL of the ethanolic solution of curcumin, gallic acid, ascorbic acid, or xanthone at a concentration of 50 µg/mL was pipetted into a microtiter plate. Then 180 µL of 10^{-4} M DPPH in ethanol or 180 µL of absolute ethanol as a negative control was added and mixed rapidly. The absorbance of the samples was measured at 540 nm by using microtiter plate reader (Biorad 680, Hercules, CA, USA) every 10 min over 60 min time period.

2.4. *Determination of antioxidant activity of a single compound and a combination*

In this study, the determination of antioxidant activity of the test samples was performed as described in section 2.3, however the absorbance was recorded at 30 min of reaction and the concentration of the test substances was varied as followings. For the antioxidant comparative test of the individual compound, 20 µL of the ethanolic solution of curcumin, gallic acid, ascorbic acid, or xanthone at the concentration ranging from 6.25 µg/mL to 100 µg/mL was added into a microtiter plate. Trolox was used as a positive control. For the combination test samples, 10 µL of curcumin were firstly mixed with 10 µL of 0.14 µg/mL gallic acid or 25 µg/mL of ascorbic acid or 25 µg/mL of xanthone. Then 180 µL of 10^{-4} M DPPH in ethanol was added. For a negative control, 180 µL of absolute ethanol was added instead of 10^{-4} M DPPH ethanolic solution. The mixtures were kept for 30 min then the absorbance of each sample was measured at 540 nm by using microtiter plate reader. The antioxidant activity was expressed as the 50% effective concentration (EC_{50}) which 50% free radicals was scavenged by this concentration and as Trolox equivalent antioxidant capacity (TEAC) which obtained from 1 mg of the test sample. The lower the EC_{50} value or the higher the TEAC value, the higher the antioxidant power of the compound.

2.5. *Calculation of antioxidant activity and combination index*

The antioxidant activity of the single compound and mixture was calculated by following equation; % Antioxidant activity = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$, where $Abs_{control}$ is the absorbance value of DPPH solution and Abs_{sample} is the absorbance value of the test sample plus DPPH solution.

To evaluate the interaction biological activity of the mixture, the experimental data were transformed into the combination index (CI) parameter (*24*). This parameter was calculated with the following equation; $CI = (MC_a/SC_a) + MC_b/SC_b)$, where MC_a and MC_b are the concentration of compound A and compound B in the mixture to achieve 50% of antioxidant activity. SC_a and SC_b are the EC_{50} of the single compound A and the single compound B, respectively. The CI value

indicates a synergistic, additive, or antagonistic effect if it is < 1, = 1, or > 1, respectively.

2.6. *Statistical analysis*

The experiments were done in triplicate and the results are expressed as mean ± SD. Statistical analysis was done by using ANOVA and *p* value at a level of 95% confidence limit.

3. Results and Discussion

3.1. *Effect of reaction time on antioxidant activity*

The detection of antioxidant activity based on free radical scavenging mechanism investigated by DPPH method was according to the color changes of the DPPH free radicals. The purple radical DPPH solution was converted to the yellow non-radical DPPH by the antioxidant having electron donating activity. The rate of this reaction was found to be depended on the different chemical structure of a substance (Figure 1). At the fixed concentration of 50 µg/mL, curcumin and gallic acid reached the maximum action within 10 min, whereas ascorbic acid achieved the maximum activity within 20 min (Figure 2). Xanthone showed significant lowest rate of scavenging activity ($p < 0.05$) by using 30 min for reaching the maximum activity. After that, the antioxidant activity of all compounds did not increase until the end of the test period. Therefore, the scavenging reaction time of 30 min was selected for the further study.

3.2. *Determination of antioxidant activity of a single compound*

The free radical scavenging activity of various concentrations of individual curcumin, gallic acid, ascorbic acid, and xanthone at a reaction time of 30 min was compared. It was found that all substances showed a concentration dependent activity (Figure 3). At the highest concentration of 100 µg/mL, all substances exhibited the highest antioxidant activity with more than 90% free radical inhibition. The results in Table 1 confirmed that the antioxidant power of the compounds were significantly different. The EC_{50} of curcumin (10.6 ± 0.4 µg/mL) was significantly lower than that of ascorbic acid (24.7 ± 1.4 µg/mL) and xanthone (32.9 ± 0.9 µg/mL) indicating that curcumin has more powerful in antioxidant activity than ascorbic acid and xanthone. This was confirmed by the TEAC value of these compounds that the value of curcumin was significantly higher than these two compounds. However, in comparison with gallic acid, it was found that curcumin has lower antioxidant power than this compound. The EC_{50} of gallic acid (1.2 ± 0.1 µg/mL) obtained from the present study was in the agreement of that from the previous study (0.011 mM or 1.8 µg/

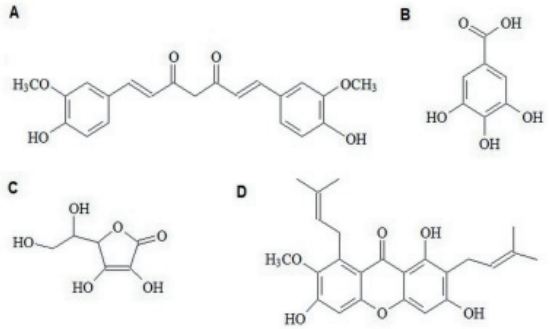

Figure 1. Chemical structure of curcumin (A), gallic acid (B), ascorbic acid (C), and xanthone (D).

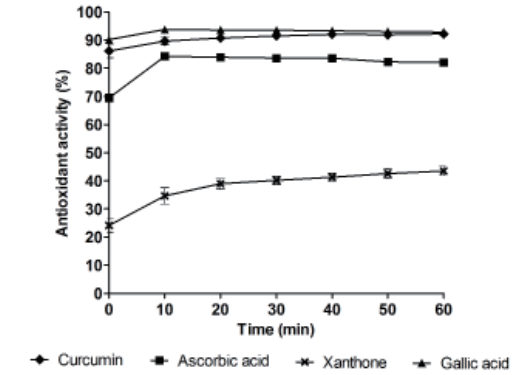

Figure 2. Antioxidant activity of single compound at different time points (*n* = 3).

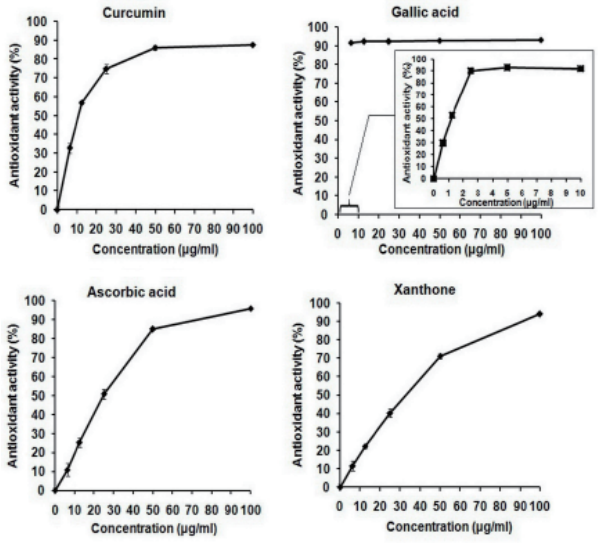

Figure 3. Antioxidant activity of single compound at different concentrations (*n* = 3).

Table 1. The antioxidant activity of a single compound expressed as 50% effective concentration (EC_{50}) and Trolox equivalent antioxidant capacity (TEAC)

Compound	EC_{50} (µg/mL)	TEAC (µM/mg)
Curcumin	10.6 ± 0.4	13.1 ± 0.6
Gallic acid	1.2 ± 0.1	26.4 ± 0.1
Ascorbic acid	24.7 ± 1.4	5.8 ± 0.7
Xanthone	32.9 ± 0.9	5.1 ± 0.4

mL) (*25*). The EC_{50} values of curcumin, ascorbic acid, and xanthone in this study were comparable to the previous studies which reported that of 29 µM or 11 µg/mL (*26*), 138 µM or 24 µg/mL (*27*), and 33 µg/mL (*28*), respectively. From these results, it was considered that the power of antioxidant potency of these four compounds was gallic acid > curcumin > ascorbic acid > xanthone. The different in antioxidant power reflected the number of reduced DPPH radicals involved in the free radical scavenging reaction. It was reported that gallic acid, curcumin, and ascorbic acid reduced 6.9, 3.7, and 2.9 molecules of the radical DPPH, respectively (*29*), whereas the ability of xanthone on this action was still unclear. It was reported that α-mangostin, the major compound existing > 90% in xanthone played the most important role on the antioxidant activity of xanthone. The antioxidant potency of α-mangostin was noted by the hydroxyl group that related to free radical scavenging activity (*30*). It was considered that the hydroxyl groups of xanthone might be hindered by the steric effect that made xanthone hardly accessed to the radical site of DPPH.

3.3. *Antioxidant activity of curcumin mixture*

The mixtures of curcumin with other antioxidant compounds were studied to examine the combination effects. The CI value was calculated based on the obtained data from the single compounds and the mixtures. Figure 4 shows the antioxidant activities of each mixture compared with that of each single compound. Interestingly, it was found that the mixture of curcumin at various concentrations with gallic acid (the EC_{50} of the mixture = 4.7 ± 1.8 µg/mL) revealed the extremely higher antioxidant activity than that of curcumin or gallic acid alone or in addition. This was confirmed by the calculated CI value presented in Table 2 that the value of this mixture is less than 1. This result indicated that the mixture of curcumin and gallic acid possessed the synergistic antioxidant effect. On the contrary, the mixtures of curcumin and ascorbic acid or xanthone did not display the synergistic effect. Moreover, the calculated CI values of both mixtures demonstrated the value of more than 1 indicating the antagonistic effect. The synergism might be hypothesized by the regeneration mechanism or sacrificial oxidation or exertion of different mechanism of action or combination of different mechanisms (*31*). The interaction of curcumin and gallic acid might be regenerated from their oxidized form by the phenolic hydroxyl group. It was also possible for the sacrificial oxidation that curcumin or gallic acid protected the other one by radical scavenging resulting in the increase antioxidant effect from the non-oxidized form. It was still unclear for the explanation of the antagonism of curcumin and ascorbic acid or xanthone. One possible reason was considered to be according

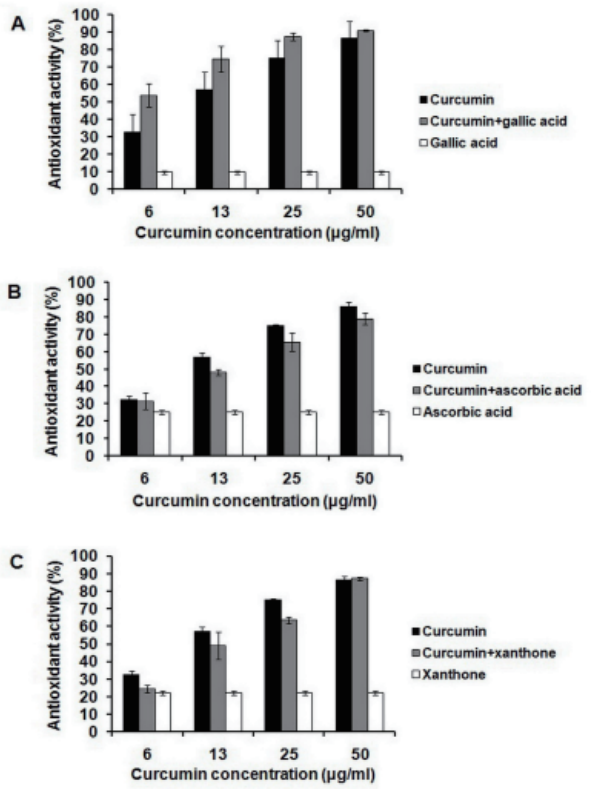

Figure 4. Antioxidant activity of curcumin in combination with gallic acid (A), ascorbic acid (B), and xanthone (C) in comparison with the anitioxidant activity of the respective individual compound.

Table 2. The antioxidant activity of curcumin mixed with other antioxidant compounds expressed as 50% effective concentration (EC_{50}) and combination index (CI)

Mixtures	EC_{50} (µg/mL)	CI
Curcumin + Gallic acid	4.7 ± 1.8	0.5
Curcumin + Ascorbic acid	13.8 ± 2.2	1.9
Curcumin + Xanthone	13.2 ± 0.9	2.0

to the interference of each other that could provide the reduction of their individual activity (*32*). Also, for xanthone, the hydroxyl group was protected from the steric effect that might obstruct the scavenging ability and decrease the activity when combined with curcumin.

4. Conclusion

The present study explores the comparative antioxidant power of curcumin with the other three natural antioxidants; gallic acid, ascorbic acid, and xanthone. It was found that the antioxidant activity of all compounds was dose and time dependent free radical scavenging. The antioxidant power of curcumin was higher than that of ascorbic acid and xanthone, respectively but lower than gallic acid. Interestingly, curcumin and a low dose of gallic acid showed the synergism on antioxidant activity while curcumin with ascorbic acid or xanthone did not act synergistically but agonistically. Therefore,

the combination of curcumin and gallic acid is a promising mixture for the highest antioxidant activity.

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