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Superoxide anion scavenging activity of alk(en)yl phenol compounds by using PMS-NADH system

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Abstract

Anacardic acid $C_{15:3}$ and cardol $C_{15:3}$ sigmoidally suppressed superoxide anion (O_2-) generation using xanthine oxidase. To study this suppression activity, anacardic acids, cardanols and cardols having different numbers of double bonds in alk(en)yl chains were prepared. The O_2- scavenging activity and H_2O_2 formation from O_2- using PMS-NADH were examined. Anacardic acids and cardols indicated sigmoidal O_2- scavenging activity but cardanols did not. The O_2- scavenging activity of anacardic acid $C_{15:3}$ was weaker than the suppression activity using xanthine oxidase, but the scavenging activity of cardol $C_{15:3}$ was quite similar to the suppression using xanthine oxidase. The H_2O_2 formation from O_2- decreased by the addition of anacardic acids, cardanols and cardols but increased by the addition of gallic and caffeic acids. From these results, we deduced that the O_2- suppression activity of xanthine oxidase reaction with cardols is the O_2- scavenging activity and that anacardic acids and cardols are O_2- scavengers having low prooxidant property.

Keyword: Food science

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1. Introduction

It is well known that superoxide anion (O_2-) is the one-electron reduction products of O_2, and is subsequently reduced to hydrogen peroxide (H_2O_2), and then hydroxyl radical (HO·) is derived from H_2O_2 and O_2-. These reactive oxygen species (ROS) induce oxidative stress containing lipid peroxidation (Frong et al., 1973), and it becomes major problems for health and food manufacturing. Antioxidants are useful for preventing these oxidative damages. To make clear the antioxidant property of alkyl phenols (Fig. 1), flavonoids and the related compounds, we examined these compounds using xanthine oxidase and then O_2-scavenging activity of PMS-NADH system (Masuoka and Kubo, 2004; Masuoka et al., 2006; Masuoka et al., 2012a; Masuoka et al., 2012b; Masuoka et al., 2015; Masuoka and Kubo, 2016). When O_2^- scavenging activity of gallic acid (1), caffeic acid (3) and their esters, gallates (2a, 2b) and caffeates (4a, 4b) having different alkyl chain lengths, was examined by using PMS-NADH system, the scavenging activity changed from hyperbolic activity to sigmoidal one with increase of their alkyl chain lengths (Masuoka et al., 2006; Masuoka and Kubo, 2016). These compounds have conjugated en-diol structures in them. As gallic acid (1) and caffeic acid (3) indicate a strong hyperbolic activity, the hyperbolic scavenging was assigned to conjugated en-diol structures. The hyperbolic scavenging activity became low with increase of alkyl chain lengths. As the sigmoidal scavenging activity came up with increase of their alkyl chain lengths, we deduced that the sigmoidal activity is associated to long alkyl chains. We also

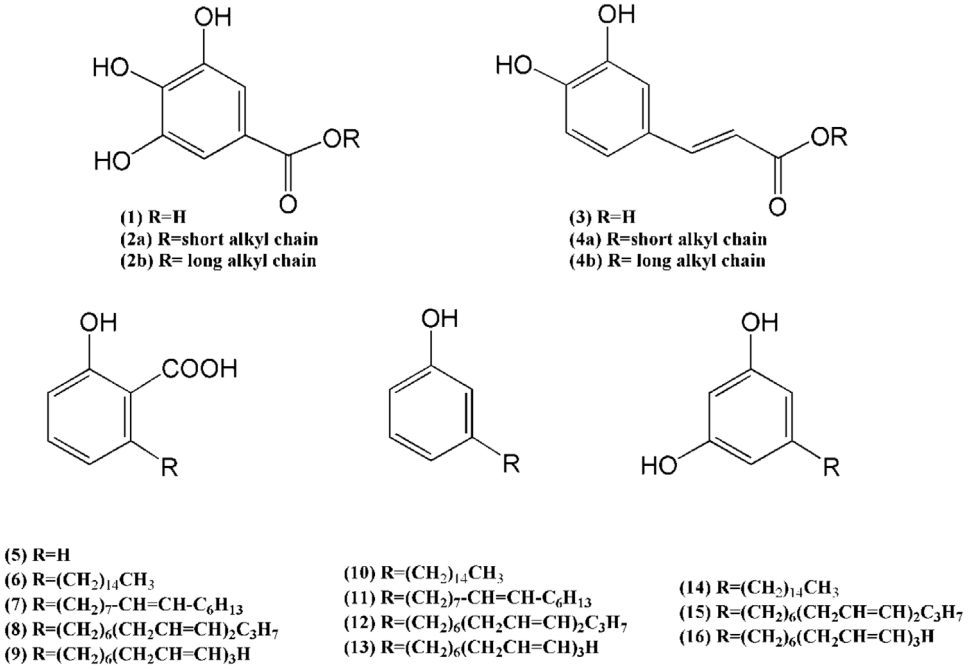

Fig. 1. Phenolic and alk(en)yl phenol compounds.

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indicated that cardols (5-alk[en]ylresorcinols) have no conjugated en-diol structure and have sigmoidal O_2-suppression activity by using xanthine oxidase (Masuoka et al., 2015). Though we considered that the suppression activity is due to a specific enzyme inhibition, it is still unclear about the relation between the suppression activity using xanthine oxidase and O_2- scavenging activity using PMS-NADH. To make them clear, anacardic acids (6-alk[en]ylsalicylic acids) (6–9), cardanols (3-alk[en]ylphenols) (10–13) and cardols (14–16) were prepared. The O_2- scavenging activity was examined using PMS-NADH system and compared with the suppression activity of O_2- generated by xanthine oxidase obtained in previous studies (Masuoka et al., 2015).

2. Methods

2.1. Materials

Salicylic acid (5), DPPH(1,1-diphenyl-2-picrylhydrazyl) radical, EDTA and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemical Co. (Milwaukee, WI) and were of analytical grade. Cashew nut shell liquid (CNSL) was obtained from Udomkij Company (Chonburi, Thailand).

2.1.1. Isolation of anacardic acids $C_{15:0–3}$ (6–9), cardanols $C_{15:1–3}$ (11–13) and cardols $C_{15:2,3}$ (15, 16) from CNSL (Paramashivappa et al., 2001)

CNSL (50 g) was dissolved in 5% aqueous MeOH (300 mL) and $Ca(OH)_2$ (50 g) was added in portions under stirring. After that the reaction mixture was stirred at 50 °C for 5 h. The precipitated calcium anacardate was filtered, washed with MeOH, and dried under vacuum evaporator. Calcium anacardate was suspended in water, acidified with concentrated HCl and stirred for 1 h. The resultant solution was extracted with EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the mixture of anacardic acids (42 g, 84% yield). The filtrate left from the filtration of calcium anacardate was evaporated under reduced pressure and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude product which was purified by silica gel column eluting with hexane/EtOAc to afford the mixture of cardanols (1.1 g, 2.2% yield) and the mixture of cardols (319 mg, 0.6% yield). Each compound in the group was further separated by semi-preparative HPLC.

Anacardic acid $C_{15:0}$ (6-pentadecylsalicylic acid) (6): white solid (9.0 mg, 18% yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.01 (1H, s), 7.36 (1H, t, J = 7.9Hz), 6.87 (1H, dd, J = 8.3, 1.2Hz), 6.78 (1H, dd, J = 7.5, 1.2Hz), 2.98 (2H, t, J = 8.0Hz), 1.59 (2H, m), 1.26 (19H, m), and 0.88 (3H, t, J = 6.8Hz).

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Anacardic acid $C_{15:1}$ (6-[8(Z)-pentadecenyl]salicylic acid) (7): yellow liquid (15.7 mg, 31%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.04 (1H, s), 7.35 (1H, t, J = 7.9Hz), 6.86 (1H, d, J = 8.3Hz), 6.77 (1H, d, J = 7.5Hz), 5.35 (2H, m, J = 4.8Hz), 2.97 (2H, t, J = 7.9Hz), 2.01 (4H, m), 1.60 (2H, m), 1.29 (16H, m), and 0.88 (3H, t, J = 6.5Hz).

Anacardic acid $C_{15:2}$ (6-[8(Z), 11(Z)-pentadecadienyl]salicylic acid) (8): yellow liquid (12.8 mg, 26%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.07 (1H, s), 7.35 (1H, t, J = 7.9Hz), 6.86 (1H, d, J = 8.3Hz), 6.77 (1H, d, J = 7.5Hz), 5.37 (4H, m), 2.97 (2H, t, J = 7.9Hz), 2.77 (2H, t, J = 6.3Hz), 2.01 (4H, m), 1.33 (12H, m), and 0.90 (3H, t, J = 7.4Hz).

Anacardic acid $C_{15:3}$ (6-[8(Z), 11(Z), 14-pentadecatrienyl]salicylic acid) (9): yellow liquid (12.3 mg, 25%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 11.09 (1H, s), 7.35 (1H, t, J = 7.9Hz), 6.86 (1H, d, J = 8.3Hz), 6.76 (1H, d, J = 7.5Hz), 5.81 (1H, m), 5.39 (4H, m), 5.01 (2H, m), 2.97 (3H, t, J = 7.9Hz), 2.79 (4H, dd, J = 13.7, 7.5Hz), 2.05 (2H, m), 1.59 (2H, m), and 1.34 (8H, m).

Cardanol $C_{15:1}$ (3-[8(Z)-pentadecenyl]phenol) (11): yellow liquid (15.4 mg, 31% yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (1H, t, J = 7.7Hz), 6.75 (1H, d, J = 7.6Hz), 6.65 (2H, d, J = 8.4Hz), 5.35 (2H, m), 2.55 (2H, t, J = 7.8Hz), 2.01 (4H, m), 1.59 (2H, m), 1.29 (16H, m), and 0.89 (3H, t, J = 6.5Hz).

Cardanol $C_{15:2}$ (3-[8(Z), 11(Z)-pentadecadienyl]phenol) (12): yellow liquid (22.8 mg, 46%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (1H, t, J = 7.7Hz), 6.75 (1H, d, J = 7.5Hz), 6.65 (2H, d, J = 8.1Hz), 5.36 (4H, m), 2.78 (2H, t, J = 6.3Hz), 2.55 (2H, t, J = 7.8Hz), 2.04 (4H, m), 1.58 (12H, m), and 0.91 (3H, t, J = 7.4Hz).

Cardanol $C_{15:3}$ (3-[8(Z), 11(Z), 14-pentadecatrienyl]phenol) (13): yellow liquid (11.4 mg, 23%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.14 (1H, t, J = 7.6Hz), 6.76 (1H, d, J = 7.6Hz), 6.65 (2H, d, J = 7.9Hz), 5.83 (1H, m), 5.40 (4H, m), 5.03 (2H, m), 2.82 (4H, dt, J = 16.1, 6.1Hz), 2.56 (2H, t, J = 7.7Hz), 2.04 (2H, m), 1.60 (2H, m), and 1.29 (8H, m).

Cardol $C_{15:2}$ (5-[8(Z), 11(Z)-pentadecadienyl]resorcinol) (15): brown liquid (36.5 mg, 73%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.24 (2H, s), 6.17 (1H, s), 5.36 (4H, m, J = 8.1, 4.8Hz), 2.78 (2H, t, J = 6.3Hz), 2.48 (2H, t, J = 7.8Hz), 2.04 (4H, m), 1.56 (2H, m), 1.33 (8H, m), and 0.91 (3H, t, J = 7.4Hz).

Cardol $C_{15:3}$ (5-[8(Z), 11(Z), 14-pentadecatrienyl]resorcinol) (16): brown liquid (13.4 mg, 27%yield) ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.25 (2H, s), 6.17 (1H, s), 5.82 (1H, m), 5.39 (4H, m), 5.02 (2H, m), 2.80 (4H, dt, J = 14.1, 7.9Hz), 2.46 (2H, t, J = 7.7Hz), 2.04 (2H, m), 1.55 (2H, m), and 1.28 (8H, m).

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2.1.2. Preparation of saturated anacardic acid $C_{15:0}$, cardanol $C_{15:0}$ and cardol $C_{15:0}$

These compounds were prepared by using hydrogen in the presence of 10% Pd/C, and these products were purified by silica gel column and crystallization. Anacardic acid $C_{15:0}$ (6) (16.7 g, 84% yield) was obtained from the mixture of anacardic acids (20 g). Cardanol $C_{15:0}$ *(3-pentadecylphenol)* (10) (14.9 g, 75% yield) was obtained from the mixture of cardanols (20 g), and cardol $C_{15:0}$ *(5-pentadecylresorcinol)* (14) (16.1 mg, 83% yield) was obtained from the mixture of cardols (1 mmol).

Cardanol $C_{15:0}$ (10): ^{1}H NMR ($CDCl_3$) δ_H (ppm): 7.15 (1H, t, J = 7.7Hz), 6.77 (1H, d, J = 7.6Hz), 6.68 (2H, d, J = 8.4Hz), 5.91 (1H, s), 2.56 (2H, t, J = 7.9Hz), 1.60 (2H, t, J = 7.6Hz), 1.30 (24H, m), 0.91 (3H, t, J = 6.6Hz).

Cardol $C_{15:0}$ (14): ^{1}H NMR ($CDCl_3$) δ_H (ppm): 6.24 (2H, s), 6.18 (1H, s), 2.47 (2H, t, J = 7.8Hz), 1.55 (2H, m), 1.25 (24H, m), 0.87 (3H, t, J = 6.6Hz).

2.2. Preparation of sample solution

Phenol and alkyl phenol compounds were dissolved with dimethyl sulfoxide (DMSO). Each a 10 mM solution was prepared and examined.

2.3. Scavenging activity on DPPH radical (Blois, 1958)

One mL of 100 mM acetate buffer (pH 5.5), 1.87 mL of ethanol and 0.1 mL of ethanolic solution of 3 mM DPPH were put into a test tube. Then, 0.03 mL of the sample solution was added to the tube and incubated at 25 °C for 20 min. The absorbance at 517 nm (DPPH, $\varepsilon = 8.32 \times 10^3$) was recorded. As control, 0.03 mL of DMSO was added to the tube. From decrease of the absorbance, scavenging activity was calculated and expressed as scavenged DPPH molecules per each sample molecule.

2.4. Scavenging activity for the O2- generated by the PMS-NADH system

Superoxide anion was generated nonenzymatically with a PMS-NADH system. The reaction mixture (final volume was 3.0 mL) consisted of 2.82 ml of 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0), 0.03 mL of 0.5% bovine serum albumin, 0.03 mL of 2.5 mM nitroblue tetrazolium, 0.06 mL of sample solution and 0.03 mL of 7.8 mM NADH. The mixture was kept at 25 or 37 °C and reaction was carried out. The reaction start by the addition of 0.03 mL of 155 μM PMS, and the absorbance at 560 nm was recorded for 60 sec (Nishikimi et al., 1972). As the control, 0.06 mL of DMSO was used. The reaction rate was

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calculated from the proportional increase of absorbance, and scavenging activity of sample was expressed as the inhibition percentage.

2.5. Assay of hydrogen peroxide generated by PMS-NADH system

The reaction mixture consisted of 5.70 ml of 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0), 0.12 mL of sample solution, 0.06 mL of 5% albumin and 0.06 mL of 7.8 mM NADH was prepared. To the reaction mixture at 25 °C, 0.06 mL of 155 μM PMS was added. After 0, 60 and 120 s, 1.5 mL of the reaction mixture was taken out and was immediately put into the test tube containing 1.5 mL of a reagent solution (64.5 μM *meso*-tetrakis(4-methyl-pyridyl)-porphinatoiron(III) pentachloride, 13.3 mM *N*,*N*-dimethylaniline, 2.76 mM 3-methyl-2-benzothiazolinone hydrazone, and 1.0 mM EDTA in 0.13 M hydrochloric acid) for stopping the reaction and determination of hydrogen peroxide (Masuoka et al., 1996). The mixture was incubated at 25 °C for 1 h, and then the absorbance at 590 nm was recorded. As the control, 0.03 mL of DMSO was used.

2.6. Assay of hydrogen peroxide scavenging activity

To 5.88 ml of 70 μM hydrogen peroxide in 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0), 0.12 mL of 10 mM sample solution was added. After 0, 2 and 4 min, a 1.5 ml of the reaction mixture was added to 1.5 mL of the reagent solution for determination of hydrogen peroxide. The mixture was incubated at 25 °C for 1 h, and then the absorbance at 590 nm was recorded. As the control, 0.12 mL of DMSO was used.

2.7. Assay and data analysis

Each assay was performed more than three times in separate experiments, and the analysis was performed with Sigma plot 2001 (SPSS Inc., Chicago, IL). The inhibition mode and kinetic parameters were analyzed with Enzyme Kinetics Module 1.1(SPSS Inc.) equipped with Sigma Plot 2001. When the sigmoidal inhibition was observed with the concrete compounds, the inhibited rate (iv_i) was calculated as a difference between the rate ($v_{i=0}$) in the absence of the compound and the rate (v_i) in the presence of the compound and analyzed using a Hill equation: $iv_i = v_{i=0}\,[I]^n/(K_i + [I]^n)$; [I] indicates concentration of the compound; n is a slope factor of the sigmoidal curve; K_i is a constant. When K_i is equal to $[I]^n$, K_i indicates IC_{50}. Data were analyzed using Student's t-test, and the difference ($p < 0.05$) was considered significant.

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3. Results

3.1. DPPH scavenging activity of phenol and alkyl phenol compounds

The DPPH scavenging activity of anacardic acids (6–9), cardanols (10–13) and cardols (14–16) was examined, and the results indicated in Table 1. Anacardic acids and cardanols showed no scavenging activity, and cardols indicated weak scavenging activity (less than 0.53 scavenged DPPH molecules/a molecule).

3.2. O_2- scavenging activity of alkyl phenolic compounds

Anacardic acids (6–9) and cardols (14–16) sigmoidally scavenged superoxide anion at 25 °C, but salicylic acid and cardanol (10–13) did not. In the scavenging activity of anacardic acids (Fig. 2A), IC_{50}s of anacardic acid $C_{15:0}$, $C_{15:1}$, $C_{15:2}$, and $C_{15:3}$ were 41 ± 3 (n = 9.4 ± 1.5), 72 ± 5 (n = 10.5 ± 2.2), 95 ± 3 (n = 5.0 ± 1.8) and 98 ± 2 μM (n = 6.7 ± 0.9), respectively. (Numbers in parentheses indicated slope factors.) The activity of anacardic acid $C_{15:0}$ is highest and then follows $C_{15:1}$, $C_{15:2}$ and $C_{15:3}$. However, the scavenging activity of cardols (Fig. 2B) was not affected by numbers of the double bonds in alk(en)yl chains. IC_{50}s of cardol $C_{15:0}$, $C_{15:2}$, and $C_{15:3}$ were 117 ± 4 (n = 6.4 ± 1.5), 116 ± 8 (n = 8.1 ± 1.3) and 116 ± 10 μM (n = 8.7 ± 1.8), respectively. The strong activity of anacardic acids

Table 1. DPPH Scavenging activity of alkyl phenol compounds.

Compound	Activity (DPPH molecules/a compound molecule)
Salicylic acid (5)	0.01 ± 0.00
Anacardic acid $C_{15:0}$ (6)	0.00 ± 0.00
Anacardic acid $C_{15:1}$ (7)	0.01 ± 0.01
Anacardic acid $C_{15:2}$ (8)	0.00 ± 0.00
Anacardic acid $C_{15:3}$ (9)	0.01 ± 0.01
Cardanol $C_{15:0}$ (10)	0.01 ± 0.01
Cardanol $C_{15:1}$ (11)	0.02 ± 0.01
Cardanol $C_{15:2}$ (12)	0.02 ± 0.01
Cardanol $C_{15:3}$ (13)	0.02 ± 0.01
Cardol $C_{15:0}$ (14)	0.53 ± 0.00
Cardol $C_{15:2}$ (15)	0.49 ± 0.01
Cardol $C_{15:3}$(16)	0.50 ± 0.01
Gallic acid (1)	5.94 ± 0.01
Ethyl gallate (2a)	6.18 ± 0.01
Caffeic acid (3)	5.05 ± 0.20

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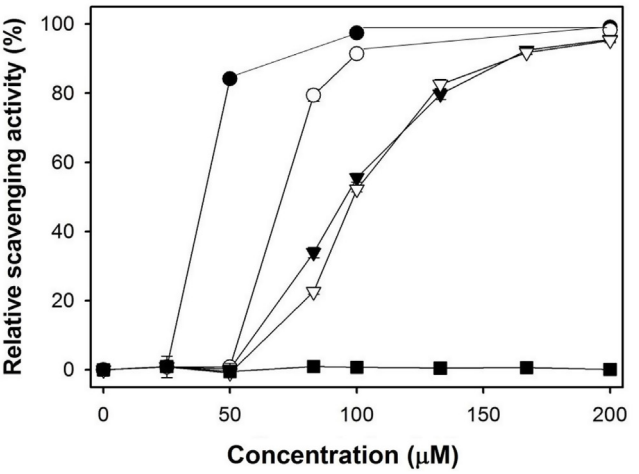
(A) Anacardic acids.



(B) Cardols and cardanols.

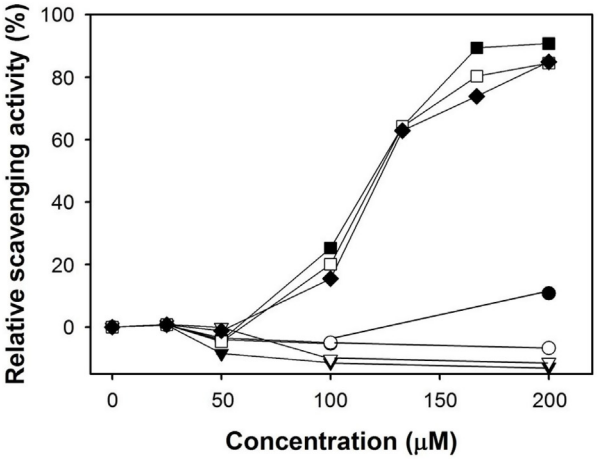


Fig. 2. Superoxide anion scavenging activity of phenol and alkyl phenols. (A) Anacardic acids. ●Anacardic acid $C_{15:0}$, ○Anacardic acid $C_{15:1}$, ▼Anacardic acid $C_{15:2}$, ▽Anacardic acid $C_{15:3}$, ■salicylic acid at 25 °C. (B) Cardols and cardanols. ●Cardanol $C_{15:0}$, ○Cardanol $C_{15:1}$, ▼Cardanol $C_{15:2}$, ▽Cardanol $C_{15:3}$, ■Cardol $C_{15:0}$, □Cardol $C_{15:2}$, ♦Cardol $C_{15:3}$.

may be explained with hydrogen bond formation in the head portions (Tsujimoto et al., 2008). When O_2- scavenging activity was measured at 37 °C, IC_{50} values of anacardic acids and cardols were not essentially affected (data not shown).

3.3. Hydrogen peroxide generated by PMS-NADH and H_2O_2 scavenging activity

Hydrogen peroxide generation from O_2- using PMS-NADH system became low by the addition of anacardic acids, cardanols and cardols. It may be explained that

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O_2- were scavenged with these alk(en)yl phenols and turned to non radical compounds (Rodrigues et al., 2006). However, H_2O_2 formation is strongly increased by the addition of 200 μM gallic and caffeic acids (Table 2). Even in the presence of 20 μM gallic acid, caffeic acid and ethyl gallate, H_2O_2 generations from PMS-NADH were significantly increased to 120 ± 8, 133 ± 4 and 118 ± 2%, respectively. These results suggested that these compounds were further oxidized with O_2- and generated H_2O_2 as they were oxidized with DPPH radical (Kawabata et al., 2002).

In the addition of H_2O_2, anacardic acids cardanols and cardols hardly indicated the scavenging activity. Gallic acid and caffeic acid increased the H_2O_2 concentration. It suggested that alk(en)yl phenol compounds did not react with H_2O_2 but phenol compounds slightly did.

4. Discussion

Anacardic acids, cardanols and cardols are natural products found in CNSL and cereal gains (Kamal-Eldin et al., 2000; Masuoka and Kubo, 2004). Anacardic acids

Table 2. Hydrogen peroxide generation by PMS-NADH and H_2O_2 scavenging reactions in the presence of 200 μM phenol and alk(en)yl phenol compounds.

Compounds (200 μM)	H_2O_2 formation (%) from O_2-*	H_2O_2 scavenging activity (%) **
Salicylic acid (5)	107 ± 1	0 ± 0
Anacardic acid $C_{15:0}$ (6)	18 ± 0	–2 ± 1
Anacardic acid $C_{15:1}$ (7)	48 ± 2	1 ± 0
Anacardic acid $C_{15:2}$ (8)	83 ± 2	–1 ± 0
Anacardic acid $C_{15:3}$ (9)	89 ± 1	1 ± 3
Cardanol $C_{15:0}$ (10)	14 ± 4	0 ± 1
Cardanol $C_{15:1}$ (11)	29 ± 9	3 ± 2
Cardanol $C_{15:2}$ (12)	39 ± 1	1 ± 2
Cardanol $C_{15:3}$ (13)	28 ± 0	2 ± 0
Cardol $C_{15:0}$ (14)	22 ± 3	–6 ± 0
Cardol $C_{15:2}$ (15)	14 ± 2	–8 ± 1
Cardol $C_{15:3}$(16)	42 ± 2	–7 ± 2
Gallic acid (1)	319 ±14	–35 ± 1
Caffeic acid (3)	308 ± 2	–66 ± 2

* Values of H_2O_2 generation from O_2- in the presence of the compound were indicated as the H_2O_2 generation in the presence of DMSO is 100%.

**Values were indicated the decrease (%) of H_2O_2 concentration from 70 μM H_2O_2 (100%) after 4 min from the addition of the compound.

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(6–9), cardanols (10–13) and cardols (14–16) were prepared from CNSL. These compounds showed low DPPH scavenging activity (Table 1). O_2- Scavenging activity of anacardic acids (6–9) and cardols (14–16) using PMS-NADH indicated a sigmoidal activity (Fig. 2) like as alkyl gallates and caffeates having long alkyl chains and that a conjugated en-diol structure is unnecessary for the sigmoidal O_2- scavenging activity. Cardanol $C_{15:3}$ (10) and the hydrogenated derivatives (11–13) did not indicate this activity though these cardanols showed considerable antioxidant property for thermaloxidation (Rodrigues et al., 2006). As the sigmoidal activity is similar to that of anacardic acid $C_{13:3}$ and cardol $C_{13:3}$ using xanthine oxidase (Masuoka and Kubo, 2004; Masuoka et al., 2015), the activity strength is compared. The O_2- scavenging activity of anacardic acid $C_{15:3}$ (IC_{50} value is 98 ± 2 μM) was lower than the O_2- suppression activity (51 ± 2 μM) using xanthine oxidase since anacardic acid $C_{15:3}$ sigmoidally inhibited xanthine oxidase reaction (Masuoka, & Kubo, 2004). As cardol $C_{15:3}$ did not inhibit xanthine oxidase, IC_{50} value (116 ± 3 μM) of O_2- scavenging by cardol $C_{15:3}$ using PMS-NADH system was compared to the value (115 ± 10 μM) of the O_2- suppression using xanthine oxidase. There is no difference between them ($p = 0.87$), and we deduced that the O_2- suppression activity of xanthine oxidase reaction with cardol $C_{15:3}$ is the O_2- scavenging activity and that the O_2- scavenging activity of antioxidants is determined using xanthine oxidase when antioxidants do not inhibit the enzyme and have no conjugated en-diol structure.

The sigmoidal scavenging activity may be explained as follows. Anacardic acids and cardols are amphiphilic molecules and make micelle formation over a critical micelle concentration (Stasiuk and Kozubek, 2008). It is suggested that the concentrations of hydroxyl group or/and carboxyl group of head portion in the aqueous medium are sigmoidally increased. As hydrophilic groups in anacardic acids and cardols serve as hydrogen donation and scavenge O_2- (Hladyszowski et al., 1998; Kamal-Eldin et al., 2000), it is deduced that the scavenging activity becomes sigmoidal.

To examine other antioxidant property of these alk(en)yl phenols, H_2O_2 generation from O_2- generated with PMS-NADH and H_2O_2 scavenging were examined. Anacardic acids, cardanols and cardols indicated low H_2O_2 generation, and low H_2O_2 scavenging activity (Table 2). It suggests that these alk(en)yl phenols suppress ROS generation from O_2- but phenols do not. As it was also known that anacardic acids (Kubo et al., 2008) and cardols (unpublished data) worked as lipoxygenase inhibitors, it is suggested that the addition of anacardic acids or cardols is useful for preventing oxidative stress. Further study is currently progressing.

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Declarations

Author contribution statement

Noriyoshi Masuoka, Kulwadee Tamsampaoloet, Warinthorn Chavasiri, Isao Kubo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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