Effect of Baicalein and Acetone Extract of *Scutellaria baicalensis* on Canola Oil Oxidation

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ABSTRACT: There is an increasing interest in natural antioxidants present in traditional Chinese herbal medicines. The present study examined the antioxidant activity of hexane, acetone, and methanol extracts, as well as baicalein purified from the dry roots of *Scutellaria baicalensis* Georgi (common name: Huangqin), in heated canola oil. Oxygen consumption and decreases in linoleic and linolenic acid content were monitored in canola oil held at 90–93°C. Among the three extracts, the acetone extract was most effective against oxidation of canola oil, followed by the methanol extract of the dry roots. The antioxidant activity of these three extracts correlated well with their content of baicalein, which provided strong protection to canola oil from oxidation. The antioxidant activity of Huangqin acetone extract was dose-dependent. The acetone extract at 100 ppm or above was even more effective than butylated hydroxytoluene at 200 ppm in protecting canola oil from oxidation. The present results suggest that the acetone extract of these roots should be further explored as a potential source of natural antioxidants for use in the processed foods.

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KEY WORDS: Antioxidant, baicalein, baicalin, canola oil, huangqin, *Scutellaria baicalensis* Georgi.

The search for and development of antioxidants of natural origin are highly desirable because of general public reluctance to use synthetic antioxidants in foods (1,2). The dry roots of *Scutellaria baicalensis* Georgi (Huangqin) are widely used in traditional Chinese herbal medicine as treatments for discomfort in the chest, nausea, coughing, vomiting, acute dysentery, jaundice, carbuncles, sores, and threatened miscarriage (3). It was previously shown that the flavone extract of *S. baicalensis* exhibits an inhibitory effect on lipid oxidation in rat liver microsomes (4) and in phosphatidylcholine liposome membranes, as induced by ultraviolet light (5). The present study examined the antioxidant activity of hexane, acetone, and methanol extracts of *S. baicalensis* Georgi in canola oil heated at 90–93°C in comparison with that of butylated hydroxytoluene (BHT).

Plant Material. The dry roots of Huangqin were purchased from a local store of traditional Chinese medicine (Hong Kong) and were then cut into small pieces and ground into powder in a coffee grinder. Canola oil without addition of any synthetic antioxidants was obtained from Lam Soon Marketing Service Ltd. (Kowloon, Hong Kong).

Extraction of Huangqin. The ground roots of Huangqin were first extracted with hexane. In brief, 5 g of the sample and 100 mL of hexane were placed in a 500-mL flask attached to a water-cooled condenser. The flask was then placed on a heater, and after refluxing for 2 h, the flask was cooled to room temperature followed by filtration through filter paper. The residue was refluxed once more with an additional 100 mL of hexane for the same period. The hexane extracts were then pooled and saved. Similarly, the acetone extract was obtained by refluxing the residue remaining after two hexane extractions with 100 mL of acetone followed by filtration. Finally, the methanol extract was obtained by extracting the residue after two acetone extractions with 100 mL of methanol in a similar way. The solvents in the three extracts were then evaporated using a vacuum evaporator. The resulting three extracts were then weighed and stored at –20°C prior to the oxygen consumption test. The dry weight of each extract is shown in Table 1.

Purification of baicalein. Acetone extract **(**2.5 g) was dissolved in 150 mL methanol and then placed overnight at –20°C in a freezer. A yellow precipitate formed, which was then filtered and redissolved in methanol followed by recrystallization overnight at –20°C. The crystallization process was repeated three more times. The final yellow crystals obtained (210 mg) had a melting point of 268–271°C. Thin-layer chromatographic analysis of this material was performed using two developing solvent systems (chloroform/methanol/acetic acid, 20:2:1; vol/vol/vol; butanol/water/acetic acid, 4:1:1; vol/vol/vol) and only one single spot was visualized under ultraviolet (UV) light, indicating that it was

TABLE 1
Dry Weight of Hexane, Acetone, and Methanol Extracts and Their Content of Baicalein and Baicalin[a]

	Extracts (mg/g dry roots)	Baicalein (% extract)	Baicalin (% extract)
Hexane	12.1 ± 1.5	0	0
Acetone	70.8 ± 7.2	17.3 ± 1.4	7.9 ± 0.9
Methanol	187.6 ± 13.5	2.4 ± 0.3	34.4 ± 3.8

[a]Data are expressed as mean ± SD of the three extracts.

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a pure compound. It also had the same R_f value as pure baicalein (Sigma Chemical Co., St. Louis, MO). The UV spectrum further demonstrated that this compound had λ_{max} at 325, 275, and 251 nm, which were similar to those of baicalein reported by Liu *et al.* (6). The ^{1}H nuclear magnetic resonance (NMR) spectrum in $DOCD_3$ had the following characteristic (δ): 7.79 (*d*, 2H, C2′ and C6′-H), 7.40–7.37 (*m*, 3H, C3′, C4′, and C5′-H), 6.54 (*s*, 1H, C3-H), and 6.43 (*s*, 1H, C8-H), proving that this compound is baicalein (Fig. 1).

Purification of baicalin. The ground root powder of Huangqin was first extracted twice using hexane and acetone as described above. To the remaining residue, 400 mL of distilled water was added followed by boiling for 2 h. After filtration, the water extract was adjusted to pH 1.2 using 1 N HCl and then kept at 40°C for 1 h. After centrifugation, the yellow precipitate was separated and dissolved in 50 mL of distilled water. After adjusting to pH 6.5 using 40% NaOH and then adding an equal volume of ethanol, the water extract was centrifuged; the supernatant was separated and immediately adjusted to pH 8.0 and then centrifuged again. After centrifugation, the gel-like substance was separated, adjusted to pH 1.5 and kept at 40°C for 1 h. After centrifugation, the yellow precipitate was separated and redissolved in methanol and recrystallized. The crystallization was performed four more times in methanol at –4°C.

The yellow crystals obtained were found to be a pure compound, namely baicalin (Fig. 2), using the thin-layer chromatographic technique already described. It had a melting point of 222–224°C and λ_{max} at 279 and 314 nm in methanol, which were similar to those reported by Liu *et al.* (6). ^{1}H NMR spectrum in $DOCD_3$ had the following characteristic (δ): 7.84 (*d*, 2H, C2′ and C6′-H), 7.40 (*m*, 2H, C3′ and C5′-H), 7.41 (*m*, 1H, C4′-H), 6.84 (*s*, 1H, C3-H), 6.62 (*s*, 1H, C8-H), 5.04 (*d*, 1H, C1′′-H), 4.00 (*d*, 1H, C5′′-H), 3.37–3.54 (*m*, 3H, C2′′, C3′′, and C4′′-H), indicating that this compound is baicalin (Fig. 2).

Determination of baicalein and baicalin in hexane, acetone, and methanol extracts. Two milligrams of each extract was dissolved in 10 mL of methanol using an ultrasonicator. An aliquot (20 μL) was applied on a silica gel 60 F_{254} thin-layer plate (4 × 6 cm; Merck, Darmstadt, Germany) and developed in a solvent system of chloroform/methanol/acetic acid (20:2:1,vol/vol/vol). The band containing baicalein was scraped off the plate and eluted with 2 mL of 2% boric acid in methanol. The concentration of baicalein in the extracts was determined by measuring the absorbance at 275 nm against the standard curve of pure baicalein.

The baicalin in each extract was similarly determined. In brief, 100 mg of extract was dissolved in 10 mL of methanol.

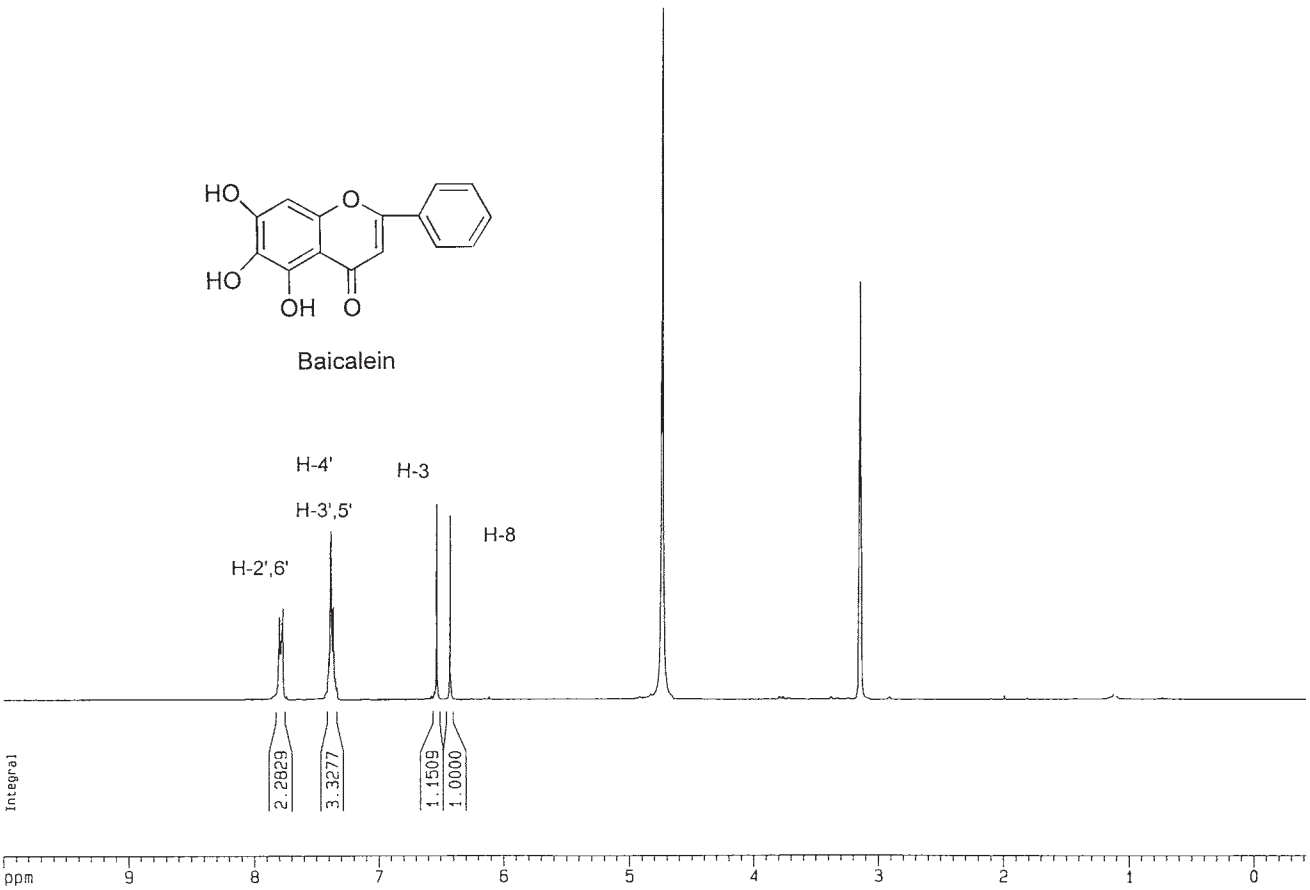

FIG. 1. Chemical structure and ^{1}H nuclear magnetic resonance (NMR) spectrum of baicalein in $DOCD_3$ isolated from Huangqin.

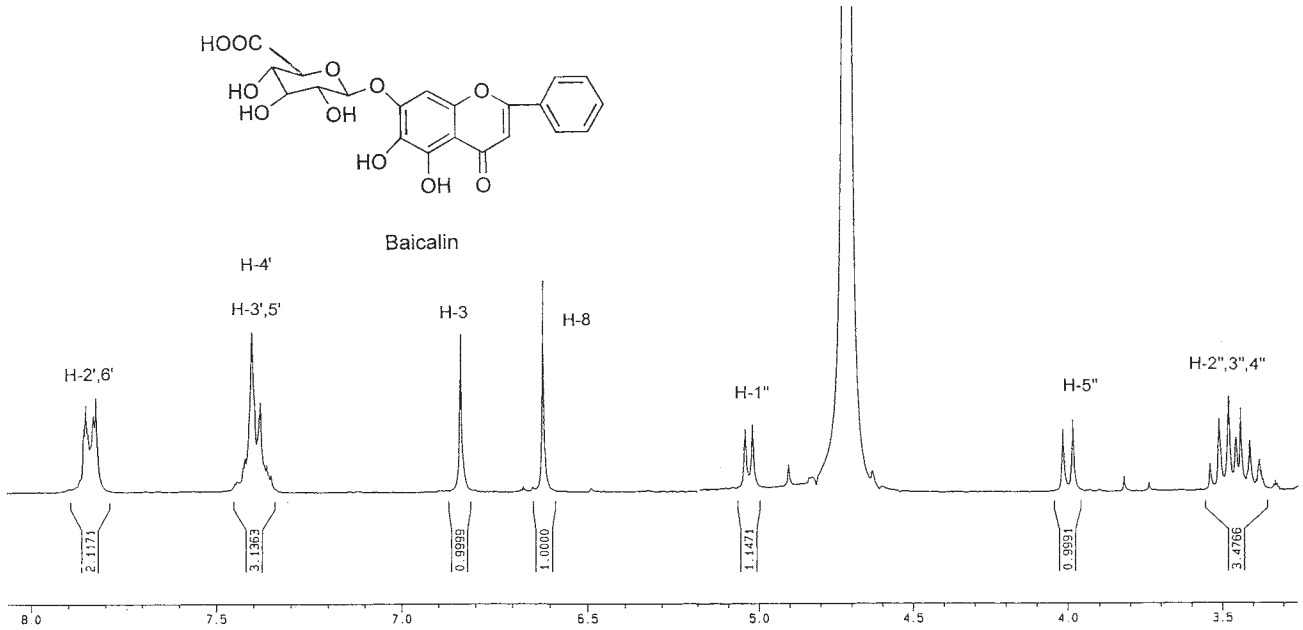

FIG. 2. Chemical structure and ^{1}H NMR spectrum of baicalin in $DOCD_3$ isolated from Huangqin. For abbreviation see Figure 1.

An aliquot (10 μL) was applied on a silica gel 60 F_{254} plate and developed in butanol/ethyl acetate/water (4:1:2, vol/vol/vol). The band containing baicalin was visualized under UV light and scraped off and eluted with 2 mL of 2% boric acid in methanol. The amount of baicalin in the extracts was determined by measuring the absorbance at 270 nm against the standard curve of pure baicalin.

Oxygen consumption test. The oxygen uptake by canola oil was monitored as previously described (7,8). In brief, 1 mL of hexane containing 200 mg of canola oil was placed in a glass tube (150 × 16 mm, o.d.) followed by adding 1 mL of acetone containing varying amounts of the acetone extract or purified baicalein and baicalin. Similarly, the hexane and methanol extracts were added to the reaction tubes in 1 mL of hexane and methanol, respectively. The components were then mixed thoroughly. The solvents were removed under a gentle stream of nitrogen at 45°C. The final concentrations of different extracts were set at 50, 100, 200, 300, and 400 ppm in canola oil. The reaction tube was then flushed with air and sealed tightly with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm o.d.; Becton-Dickinson, Rutherford, NJ), which usually maintains a vacuum for 2–3 yr. The sealed reaction tube was leak-free and was verified by filling the tube with nitrogen gas and monitoring by gas chromatography (GC) if headspace oxygen concentration decreased. Oxidation was conducted at 90–93°C. The headspace oxygen was sampled periodically with a gas-tight syringe and analyzed in a Hewlett-Packard 5890 series II gas–solid chromatograph (Palo Alto, CA) fitted with a 1/8″ × 6′ stainless-steel column packed with Molecular Sieve 5A (60/80 mesh) and a thermal conductivity detector. The percent oxygen in the headspace was calculated from the ratio of oxygen to nitrogen. After the headspace oxygen analysis, the canola oil was dissolved in 10 mL chloroform, and an aliquot containing 20 mg canola oil was then taken for fatty acid analysis.

Fatty acid analysis. Fatty acids of heated canola oil sample with or without addition of Huangqin extracts were converted to the corresponding methyl esters with a mixture of 14% BF_3 in methanol (Sigma Chemical Co.) and toluene (1:1, vol/vol) under nitrogen at 90°C for 45 min (3). Fatty acid methyl esters were analyzed on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm; i.d.; Supelco, Inc., Bellefonte, PA) in a Hewlett-Packard 5980 series II gas–liquid chromatograph equipped with a flame-ionization detector. Column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 10 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Hydrogen was used as the carrier gas at a head pressure of 15 psi.

Statistics. All experiments were repeated two or three times. Data were pooled from each experiment in which four to five replicates were conducted. Data for the headspace oxygen consumption and fatty acid analysis were subjected to analysis of variance, and the means were compared between treatments by using Duncan's multiple range test (9). This was done by running data on the PC ANOVA software (PC ANOVA for the IBM personal computer, Version 1.1, 1985, IBM, Armonk, New York).

RESULTS AND DISCUSSION

The antioxidant activity of Huangqin was first compared among three extracts, namely, hexane, acetone and methanol extracts. As shown in Figure 3, the acetone extract was the most effective in slowing the oxygen consumption of canola

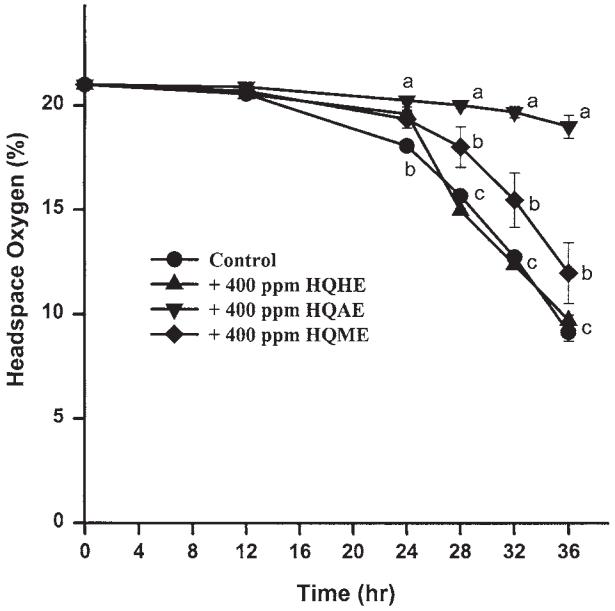

FIG. 3. Effect of Huangqin (*Scutellaria baicalensis* Georgi) hexane extract (HQHE), acetone extract (HQAE), and methanol extract (HQME) on oxidation of canola oil heated at 91 ± 2°C. Data are expressed as mean ± SD of n = 4–5 samples. Means at the same time point with different superscript letters (a–c) differ significantly ($P < 0.05$).

oil, followed by methanol extract. To simplify the presentation, only data for the acetone extract are shown hereafter. The protective effect of Huangqin acetone extract on oxidation of canola oil was dose-dependent (Fig. 4). Compared with BHT at 200 ppm, the acetone extract at 100 ppm or above was more effective against lipid oxidation of canola oil heated at 93°C.

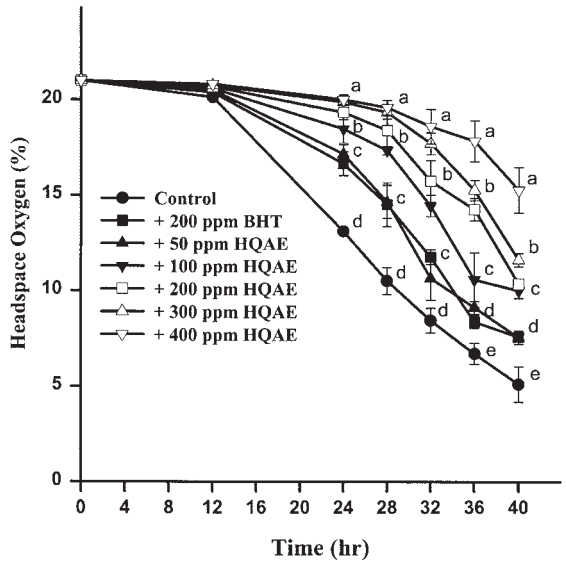

FIG. 4. Dose-dependent effect of HQAE and of butylated hydroxytoluene (BHT) on oxidation of canola oil heated at 91 ± 2°C. Data are expressed as mean ± SD of n = 4–5 samples. Means at the same time point with different superscript letters (a–e) differ significantly ($P < 0.05$). For abbreviation see Figure 3.

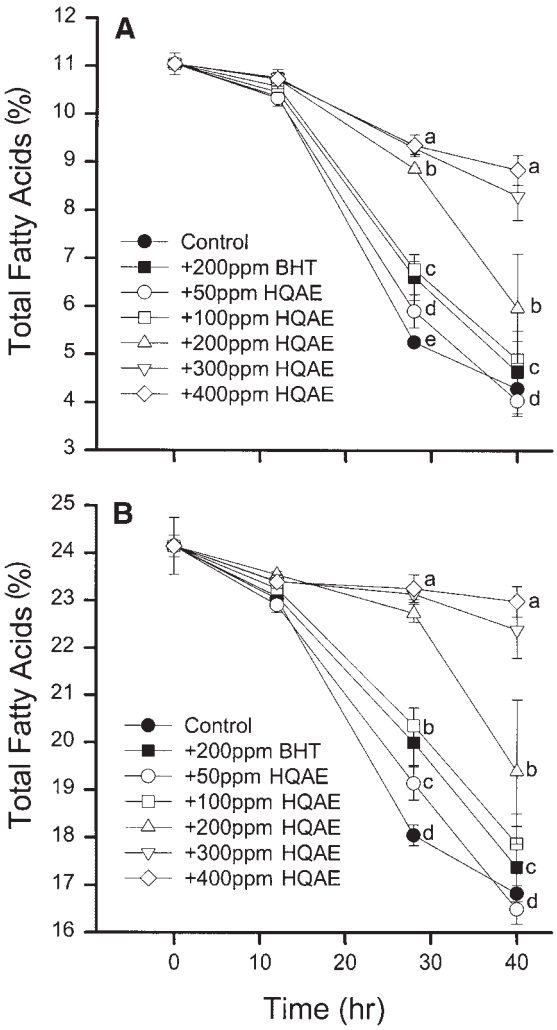

FIG. 5. Dose-dependent effect of HQAE on change in (A) linolenic acid and (B) linoleic acid of canola oil at 91 ± 2°C. Data are expressed as mean ± SD of n = 5 samples. Means at the same time point with different superscript letters (a–e) differ significantly ($P < 0.05$). For abbreviations see Figure 4.

Fatty acid data from fatty acid methyl esters were consistent with the oxygen consumption test (Fig. 5). The oxygen consumption by the sample was positively correlated with the oxidation of linoleic and linolenic acids in canola oil. Addition of the Huangqin acetone extract significantly prevented oxidative loss of these two polyunsaturated fatty acids in canola oil heated at 93°C (Fig. 5). The protective effect of Huangqin acetone extract on linoleic and linolenic acids also appeared to be dose-dependent. The present results suggest that the crude Huangqin acetone extract contains an antioxidant or a mixture of antioxidants that is more effective than BHT under the present experimental conditions.

To explain the antioxidant activity of the acetone extract, the two compounds were isolated and tested for their relative ability to protect canola oil against oxidation. They turned out to be baicalein and baicalin, which have previously been described by Liu *et al.* (6). Of these two compounds, only

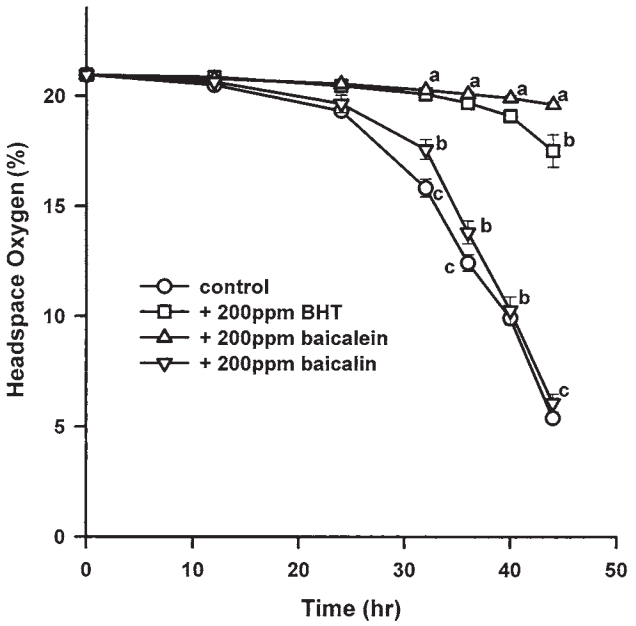

FIG. 6. Effect of baicalein on the oxidation of canola oil heated at 90 ± 2°C compared with that of baicalin. Data are expressed as mean of n = 4 samples. Means at the same time point with different superscript letters (a–c) differ significantly ($P < 0.05$). For abbreviation see Figure 4.

baicalein was found to possess stronger activity than BHT (Figs. 6, 7). It was interesting to note that its derivative, baicalin, exhibited a weaker activity (Fig. 6). This indicates either that the blockage of C7-OH of baicalein almost completely abolishes its antioxidant activity or instead, that the glucuronate group may possess some prooxidant activity, which may counteract the antioxidant activity of baicalein. This speculation deserves further investigation.

The antioxidant activity of the three extracts corresponded well to their content of baicalein. As shown in Table 1, the content of baicalein was highest in the acetone extract followed by the methanol extract, as was the antioxidant activity. In contrast, the hexane extract contained no baicalein and thus possessed no antioxidant activity (Fig. 3). The present data were in agreement with those reported by Guo *et al.* (10*)* who showed that baicalein inhibited lipid oxidation in rat liver microsomes and by Gabrielska *et al.* (5), who demonstrated that baicalein was protective to phosphatidylcholine liposome membranes against lipid oxidation.

In addition to baicalein and baicalin, several other structure-related flavonoids including wogonin, oroxylin, and neobaicalein have been isolated and characterized from Huangqin (10–14). However, they are quantitatively minor compared with baicalein and baicalin. The present study was the first to explore the possibility of using Huangqin extract as a food antioxidant. We are currently using column chromatography and high-performance liquid chromatography to isolate the other possible antioxidants present in Huangqin acetone extract and assess their individual contribution to antioxidant activity.

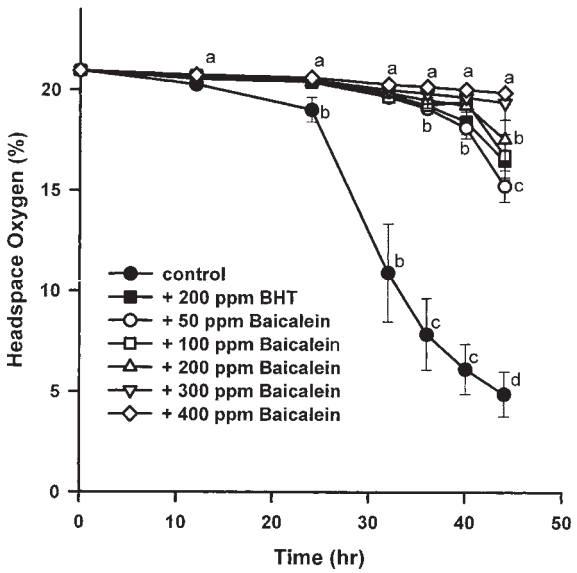

FIG. 7. Dose-dependent effect of baicalein on oxidation of canola oil heated at 90 ± 2°C. Data are expressed as mean ± SD of n = 4–5 samples. Means at the same time point with different superscript letters (a–d) differ significantly ($P < 0.05$). For abbreviation see Figure 4.

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