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Evaluation of the inhibitory activities of aceraceous plants on fatty acid synthase

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Abstract

Fatty acid synthase (FAS) is a very significant lipogenic enzyme participating in energy metabolism in *vivo* and has been reported as a potential new therapeutic target for cancer treatment. The extracts from sixteen *Aceraceae* were prepared to assay their inhibitory activities against duck liver FAS and their correlated antitumor bioactivity. Their inhibition of FAS was composed of a reversible fast-binding inhibition, by which 0.41 μg/mL of the *A. campestre* extract inhibits 50% FAS activity, and an irreversible slow-binding inhibition with inactivation rate constants, k_{obs}, ranging between 1.5×10^{-3} and 10.6×10^{-3} min^{-1}. Three *Aceraceae* extracts were selected from their smaller IC_{50} values to study different type of inhibitions against the three substrates in the FAS overall reaction. As compared with other reported FAS inhibitors including EGCG with regard to inhibition constant and IC_{50} value, the extracts appeared to be more efficient inhibitors, and exhibited a considerable inhibition against the growth of five types of cancer cells (China patent application number 200610088901.6), which may be related to the inhibition of lipogenesis in these cells.

Keywords: *Fatty acid synthase, enzyme inhibition, antitumor, aceraceae, kinetics*

Abbreviations: *FAS, fatty acid synthase; EGCG, (-)-epigallocatechin gallate; DTT, dithiothreitol; EtOAc, ethyl acetate; NDGA, nordihydroguaiaretic acid*

Introduction

The *de novo* synthesis of fatty acids is essential for all organisms. Animal fatty acid synthase (E.C. 2.3. 1.85, FAS) which plays an important role in fatty acid synthesis, one of the most complex cellular multi-enzymes, comprises two identical, 260–270kDa sub-units juxtapositioned head to tail, each containing an acyl carrier protein and seven enzymatic active sites, including acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, β–hydroxyacyl dehydratase, enoyl reductase, and thioesterase [1]. Animal FAS catalyses the *de novo* synthesis of fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH through the reaction which elongates the acetyl group by C2 units derived from malonyl-CoA in a stepwise and sequential manner [2]. The overall architecture of mammalian FAS has been revealed by 4.5 Å resolution x-ray crystallography at intermediate resolution [3].The amino acid sequence of human FAS has 79% and 63% identity with those of the rat and fowl enzymes, respectively [4]. Traditional fowl FAS is a good model for the kinetic study of animal FAS.

It was reported that FAS is selectively highly expressed in certain human cancer cells, including carcinoma of the breast, prostate, colon, ovary, endometrium and lung [5–14]. On the basis of much research, FAS has been suggested as a potential new therapeutic target for the treatment of cancer [15–16] and may lead to new chemotherapeutic methods which

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kill cancer cells but are non-toxic to normal cells [17–18]. Inhibitors of FAS such as cerulenin, synthesized C75 [19], thiolactomycin and its structural analogues [20], and some natural polyphenols [21–24] are known for their effects on some human cancers. However, efficient FAS inhibitors, especially prepared from natural plants are still scarce and novel [25].

The *Acer* genus (*Aceraceae*) consists of approx. 200 species widely distributed in the northern hemisphere with a distribution centre in China [26–27]. They are horticulturally important and widely planted for the brilliant autumn colours of their leaves. Most of them has been used as Chinese folk medicine [28]. *Acer mono* leaves [29], *Acer palmatum* leaves [30], *Acer albopurpurascens* [31] and *Acer truncatum Bunge* show a high antioxidant effect [32]. It was found that compared with extracts from control samples either *Acer rubrum*, *Acer saccharum*, or *Acer saccharinum* extracts increased methaemoglobin formation [33]. *Acer mono* leaves showed significant hepatoprotective activities against H_2O_2-induced toxicity in primary cultures of rat hepatocytes[34]. *Acer nikoense* leaves exhibited inhibitory effects on the release of beta-hexosaminidase in RBL-2H3 cells [35]. Furthermore, *Acer okamotoanum Nakai* leaves was found to potently inhibit HIV-1 integrase [36].

Recently, we have demonstrated that the extract of the leaves of *Acer truncatum Bunge* appeared to be an efficient inhibitor of FAS, and exhibited considerable inhibition of the growth of four types of cancer cells [37]. In this paper, we report the potent inhibitory effects on FAS of the ethyl acetate extracts of sixteen species of *Acer Linn* plants which were collected from Beijing Arboretum, China, and the investigations on their inhibition kinetics as well as antitumor activity experiments.

Materials and methods

Preparation of FAS and substrates

Acetyl-CoA and malonyl-CoA were purchased from Sigma, NADPH was from Fluka. All other reagents were local products of analytical grade. The FAS from duck liver was purified with an improved method [38]. The preparation was homogeneous on polyacrylamide gel electrophoresis in the presence and absence of SDS. FAS with specific activity of 150 U/mg was stored in 0.1 M potassium phosphate buffer of pH 7.0 containing 10 mM DTT and 1.0 mM EDTA, and then during all the following experiments, 1.0 mM DTT and 1.0 mM EDTA were added to the reaction solutions to protect the essential thiol groups of FAS and to remove metal ions which could inactivate FAS.

Assay of FAS activity

The assay for FAS activity, described [39] previously, was performed with a Shimadzu UV2550 double wavelength/double beam spectrophotometer at 37°C by following the decrease of NADPH at 340 nm. The reaction system contains 0.1 M potassium phosphate buffer, pH 7.0; 1.0 mM EDTA; 1.0 mM DTT; 3μM acetyl-CoA; 10μM malonyl-CoA; 32μM NADPH and duck liver FAS 10μg in a total volume of 2.0 mL. Fast-binding inhibition was determined by adding the inhibitor (the extracts from each species) to the reaction system before FAS initiated the reaction. After the enzyme solutions were mixed with various concentrations of the extracts, aliquots were taken to measure the remaining activity at the indicated time intervals to follow slow-binding inactivation.

Preparation of the extracts from the leaves of Acer Linn Plants

The leaves of *Acer Linn* Plants were collected from Beijing Arboretum in October 2005 and identified by Professor Chen Yuting of Beijing University of Traditional Chinese Medicine. After appropriate treatment, the cleaned and dried leaves were cut into small pieces and mixed with 70% ethanol in the ratio of 1 to 20 (w/v) followed by magnetic stirring for 24 h at room temperature. A brown concentrated solution was obtained on removal of ethanol under reduced pressure which was then mixed with an equal volume of petroleum ether to remove coloring matter. The extraction was repeated three times to give a residual solution free of lipid which was then extracted thrice with EtOAc of equal volume. The pooled EtOAc extract was evaporated under reduced pressure to yield a brown residue which was further dried in a vacuum at a temperature less than 50°C.

Measurement of inhibition of FAS

IC_{50} values were obtained from the % inhibition-concentration curves for the inhibitors. Inhibition of FAS by different concentrations of inhibitor solution was measured and repeated three times. Reversible inhibition was measured by adding 5μL sample of extracts to the reaction system followed by 10 μg FAS to start the reaction in a total volume of 2 mL. The remaining activity of FAS was assayed as a_i, and the control activity with 5μL solvent instead of extract was assayed as a_o. The inhibition is also termed fast-binding inhibition and the inhibition extent was calculated by the formula: $I_f = (1 - a_i/a_o) \times 100\%$. This inhibition is caused by the non-covalent fast combination of inhibitor with enzyme and is usually reversible.

The time course of inactivation was determined by taking aliquots to measure residual activity at the indicated time intervals after the enzyme solution was mixed with the inhibitors in 0.1 M potassium phosphate buffer, pH 7.0. The time-dependent

inhibition course is often an irreversible process to form a covalent bond between the inhibitor and enzyme known as slow-binding inactivation. The vehicles without inhibitors were used as control in these experiments. FAS activity in the control remained unchanged for 4 h. The apparent first-order inactivation rate constant, k_{obs}, was obtained from the semilogarithmic plot of the inactivation time course. The time course indicates the actual inhibitory effect of the inhibitor, i.e., the more rapid the loss of enzyme activity, the more potent the inhibitior.

The experiments with human cancer cell lines

The inhibitory effects of the extracts on cancer cell growth were investigated using human esophageal cancer cell line (CAES-17), gastric cancer cell line (BGC-823), breast cancer cell line (MCF-7), and the liver cancer cell line (BEL-7402) as well as the human prostate cancer cell line (PC-3M). They were all purchased from the Institute of Medicament Research of Chinese Academy of Medical Sciences. The above various types of cancer cells were seeded in RPMI-1640 containing 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomysin on a 96-well plate with 2×10^5 cells in each well and incubated at 37°C for 24 h in a CO_2 incubator. The plant extracts were then dissolved in dimethylsulfoxide at a preset concentration and added to the cell culture; the control with 200 μL solvent instead of extract was also assayed. The incubation proceeded for an additional 24 h. Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay [40]. The extent of inhibition for each extract concentration is an average value of three independent experiments. The above experiments were carried out with extracts from *Acer tataricum*, *Acer platanoides*, *Acer capillipes, Acer campestre*, and *Acer pseudoplatanus.*

Results

Fast-binding inhibition of FAS by extracts of sixteen species of Acer

The IC_{50} (the inhibitor concentration inhibiting 50% of the activity of FAS) values for extracts from sixteen species *of Acer* indicated their fast binding inhibitory capacities (see Table I).The IC_{50} values were obtained from the % inhibition-concentration curves for the inhibitors (Figure 1). The results showed that *Acer tataricum*, *Acer platanoides*, *Acer capillipes*, *Acer pseudoplatanus, Acer negundo* and *Acer campestr*e possessed highest inhibitory activities, and that *Acer campestr*e was the most potent inhibitor with an IC_{50} value of 0.41 μg/mL, 86-fold less than that of *Acer negundo* '*Vureomarginatum*'.

The extracts of the above six species of aceraceous plants were further used for measurement of their IC_{50} values for the FAS ketoacyl and enoyl reduction reactions (Figure 1). As shown in Figure 1, compared to the considerable inhibition against the FAS overall reaction, the inhibition of the enoyl reduction reaction was not obvious for both *Acer tataricum* and *platanoides*, but for all *Acer* extracts the FAS ketoacyl reduction reaction appeared to be inhibited with further increasing concentrations of extracts. It is worthy to note that with increasing concentration of extracts of *Acer capillipes and Acer pseudoplatanus* the inhibition of the enoyl- and the ketoacyl reduction reactions were enhanced almost to the same extent (Figure 1,C, D). Moreover, the inhibition by *Acer negundo* of the ketoacyl reduction reaction became actually not as much as that of the enoyl reduction reaction. The IC_{50} values of these six

Table I. The reversible inhibition activity of extracts of Aceraceous plants.

Acer Linn	Raw material (g)	Yield of extract (mg)	IC_{50} for the overall reaction (μg/mL)
1. *A. platanoides*	1.00	87.9	0.60
2. *A. tataricum*	1.00	47.8	1.81
3. *A. truncatum*	1.00	55.6	1.30
4. *A. mono*	1.00	87.7	10.0
5. *A. sacharinum* '*Laciniatum wieri*'	1.00	105	3.25
6. *A. triflorum*	1.00	84.2	2.05
7. *A. capillipes*	1.00	57.8	1.50
8. *A. dasyphylla*	1.00	36.7	3.75
9. *A. dervidii*	1.00	39.8	10.0
10. *A.pseudoplatanus*	1.00	59.9	1.76
11. *A. negundo* '*Vureomarginatum*'	1.00	29.8	35.4
12. *A. stenolobum*	1.00	37.1	6.88
13. *A. negundo*	1.00	67.2	1.35
14. *A. griseum*	1.00	114	18.8
15. *A. palmatum*	1.00	134	2.95
16. *A. campestre*	1.00	68.0	0.41

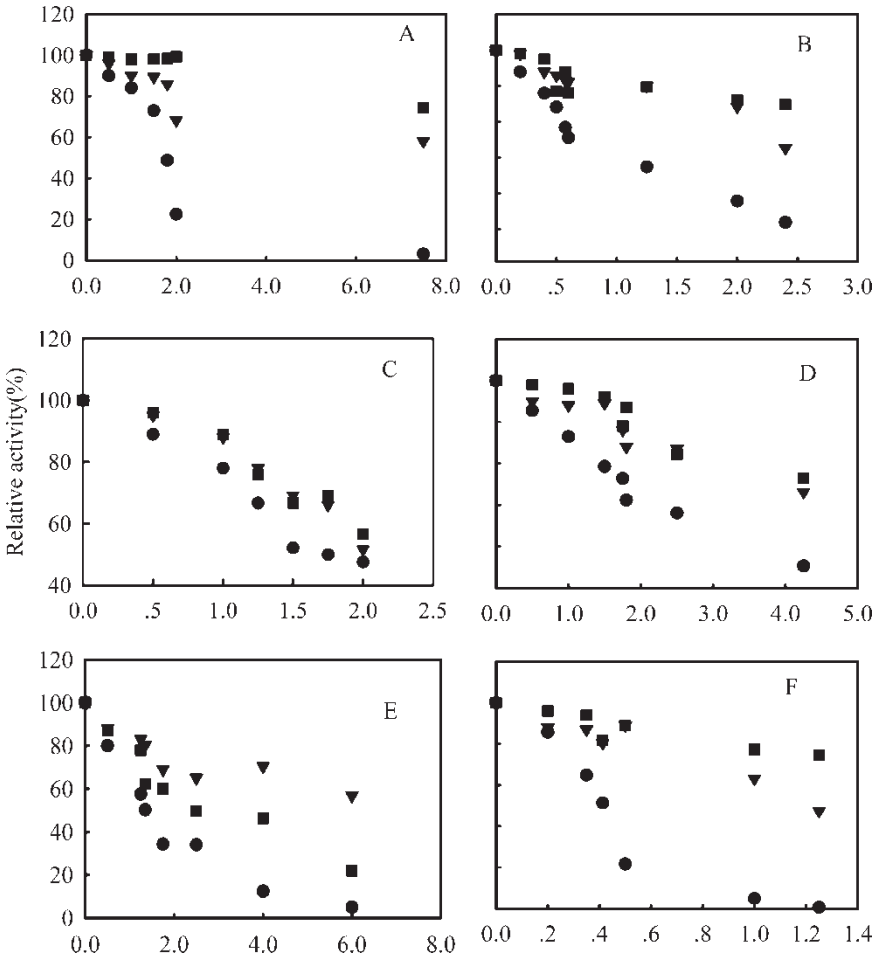

Figure 1. Fast-binding inhibition of FAS by the extracts of above six species of Aceraceous plants:(A) *Acer tataricum,* (B) *Acer platanoides,*(C) *Acer capillipes,*(D) *Acer pseudoplatanus,* (E) *Acer negundo,* (F) *Acer campestre.* (●) the overall reaction, (◆) ketoacyl reduction reaction, and (▲) enoyl reduction reaction. The reaction system contains 0.1 M potassium phosphate buffer, pH 7.0; 1.0 mM EDTA; 1.0 mM DTT; 3μM acetyl-CoA; 10μM malonyl-CoA; 32μM NADPH and FAS 20 μg in a total volume of 2.0 mL, 37°C, by following the decrease of NADPH at 340 nm within 1.5 min.

species of *Acer* for the FAS overall reaction and ketoacyl reduction reaction are listed in Table II.

The inhibition time courses of FAS by Acer extracts

The time courses of inhibition of the FAS overall reaction are shown in Figure 2. They are biphasic. In the first three minutes FAS activity rapidly decreased by 20–40%, which is attributed to fast and reversible binding to the enzyme before the time-dependent irreversible step which further decreased FAS activity. The semilogarithmic plot of relative activity (ln R.A.) versus time (insert of Figure 2) exhibited two phases and the line, initially appeared concave, but later became linear which gave the inactivation rate constant, k_{obs}, equal to the fitted slope of the plots. The k_{obs} values are listed in Table II. Although the IC_{50} value for the FAS overall reaction of *A. tataricum* approached those of *A. capillipes* and *A. pseudoplatanus*, the smaller k_{obs} value demonstrated its weak slow-binding irreversible inhibition of FAS.

The inhibition pattern studies

Considering the smaller IC_{50} value representing a higher fast binding inhibitory capacity, *A. Platanoids and A. Campestre* were selected for studying the inhibition patterns against the FAS overall reaction in the presence of three fixed extract concentrations and NADPH, Malonyl-CoA, and Acetyl-CoA as the variable substrates, (Figures 3–8) and for estimating the respective kinetic parameters which were calculated by secondary plots on the basis of Figures 3–8 and listed in Table III. It can bee seen from Figure 3 that the extract of *Acer platanoides* was a competitive inhibitor of acetyl-CoA, but for malonyl-CoA nearly noncompetitive while for NADPH the extract appeared to be a mixed type of inhibitor (Figures 4–5). The extract of *Acer campestre* is nearly a noncompetitive inhibitor of acetyl-CoA (Figure 6), whereas it was a mixed type inhibitor against malonyl-CoA and NADPH (Figures 7–8).

Table II. IC_{50} values of extracts of six species of Aceraceous plants for the FAS overall reaction and ketoacyl reduction reaction, and k_{obs} (min^{-1}) values.

Acer linn	IC_{50} for the overall reaction (μg/mL)	IC_{50} for the β-ketoacyl reduction reaction(μg/mL)	k_{obs} (min^{-1})
A. platanoides	0.60	2.35	10.6×10^{-3}
A. tataricum	1.81	7.56	1.5×10^{-3}
A. capillipes	1.50	2.07	8.5×10^{-3}
A. pseudoplatanus	1.76	4.16	7.6×10^{-3}
A. negundo	1.35	6.54	4.8×10^{-3}
A. campestre	0.41	1.21	7.2×10^{-3}

A 5 μL sample of the EtoAc extract was added to the assay mixture, followed by 20 μg FAS starting the reaction in a total volume of 2 mL. The remaining activity of FAS was assayed as A_i, and the control activity with 5 μl solvent instead of extract was assayed as A_o. The reversible inhibition was calculated by the formula: $I = (1 - A_i/A_o) \times 100\%$.

Effects of five species of Acer extracts on growth of various cancer cell lines

Effects of extracts of five species of *Acer* on cancer cells growth were investigated using CAES-17, BGC-823, MCF-7, BEL-7402, and PC-3M cells. Treatment with serial dilutions of *Acer* extracts at 5–80 μg/mL exhibited significant growth inhibition on all these cells, and the extent of inhibition exceeded 50% when the extract concentration reached 60 μg/mL (Table IV) In addition, the IC_{50} of *Acer tataricum* and *Acer capillipes* for MCF-7 and PC-3M cells was only 9 and 11 μg/mL, respectively.

Discussion

In the present work we have shown that extracts from sixteen species of *Acer* leaves inhibit the overall reaction and ketoacyl reduction of FAS effectively and significantly inhibited growth of cancer cells. These enzyme inhibition involved both reversible and irreversible inhibition. Especially for the fast-binding inhibition, the IC_{50} values of the extracts of six species (Table II) for both the overall reaction and ketoacyl reduction reaction of FAS clearly demonstrate that the inhibitory effects of the extract remarkably exceed those of polyphenols like NDGA [41].

It can be seen that the IC_{50} of *A. capillipes* for ketoacyl reduction reaction at 2.07 μg/mL is close to that for the FAS overall reaction (1.5 μg/mL) implying that inhibition of the FAS overall reaction is mainly ascribed to the inhibition of the ketoacyl reduction reaction, which is also supported by Figure 1C. However for other *Acer species* the several fold discrepancy between the IC_{50} values for the ketoacyl reduction reaction and for the FAS overall reaction implied that inhibition of ketoacyl reduction is not the predominant reason for FAS inactivation and other intermediate step-reactions of FAS are also somewhat inhibited. As summarized in Table III for the overall reaction of FAS, there are different types of inhibition between the extracts using the three substrates (Table III). Extracts show inhibitiory effect on many steps of the FAS overall reaction, including acetyl transacylation and malonyl transacylation and β-ketoacyl reduction.

The synthesized C75 and cerulenin are the main well-known inhibitors of FAS. C75 showed only

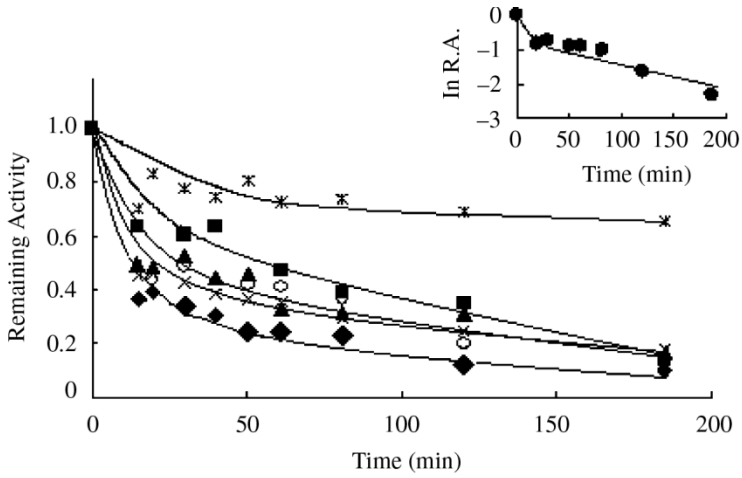

Figure 2. Kinetic time courses of inhibition of the overall reaction in the presence of the extracts of six species of Acer:(○) *Acer platanoides*, (▲) *Acer pseudoplatanus*, (⋆) *Acer tataricum*,(×) *Acer campestre*, (◆) *Acer truncatum*, and (■) *Acer capillipes*. The insert is a plot of ln R.A.(relative activity) versus time calculated from Figure1 data (*Acer platanoides*). The FAS solution (0.6μM) was mixed with the extract (0.5 μg/mL) and the aliquots were assayed for remaining activity at the indicated time intervals.

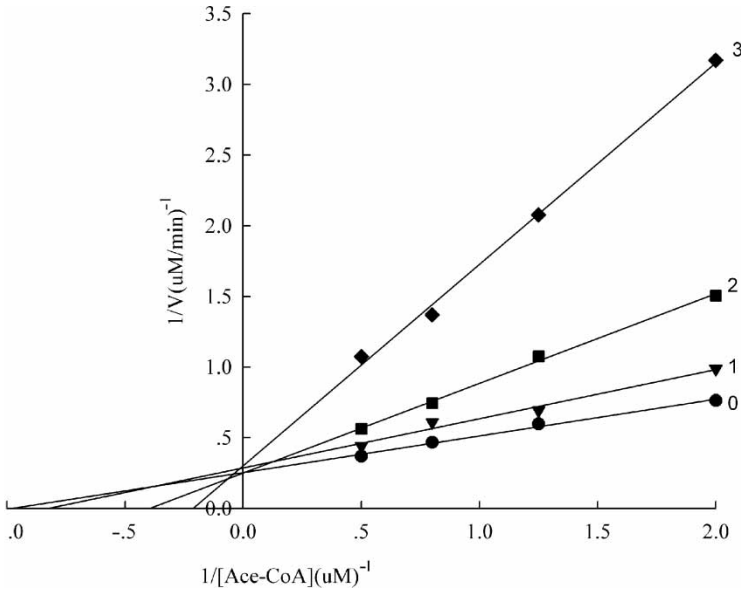

Figure 3. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by *A. platanoides* extracts. The concentration of extract in the reaction system was 0 (0), 0. 25 μg/mL (1), 0.5 μg/mL (2), and 0.75 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of NADPH and Malonyl-CoA were 32 and 10μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

irreversible inhibition against FAS without obvious reversible inhibition [11]. The reported IC_{50} value of cerulenin for FAS was 20 μg/mL [42] which is larger than that for any of the 15 species of *Acer* listed in Table 1, except for *A. negundo* '*Vureomarginatum*'.

In recent years gallated catechins such as EGCG, ECG have become known for their inhibitory effects on FAS and have been extensively studied [43–45]. The IC_{50} of EGCG and ECG for the FAS overall reaction are 24 and 18 μg/mL while for the ketoacyl reduction reaction their values are 46 and 30 μg/mL [46], respectively, obviously larger than those for the six species of *Acer* shown in Table II. The extracts as compared with EGCG in the values of their inhibition constants (k_i) (Table III) were demonstrated to be much more potent on FAS. The k_i values of *A. platanoides* for acetyl-CoA, malonyl-CoA, and for NADPH are several hundredfold smaller than those of EGCG, showing *A. platanoides's* much more potent fast-binding inhibitory capacity. Overall, for the FAS

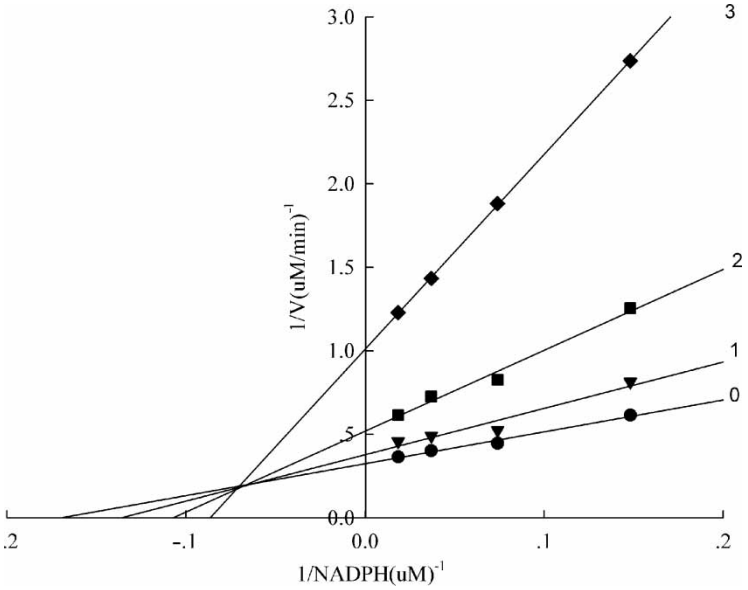

Figure 4. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by *A. platanoides* extract. The concentration of extract in the reaction system was 0 (0), 0. 25 μg/mL (1), 0.5 μg/mL (2), and 0.75 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of Malonyl-CoA and Acetyl-CoA were 10 and 2.5μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

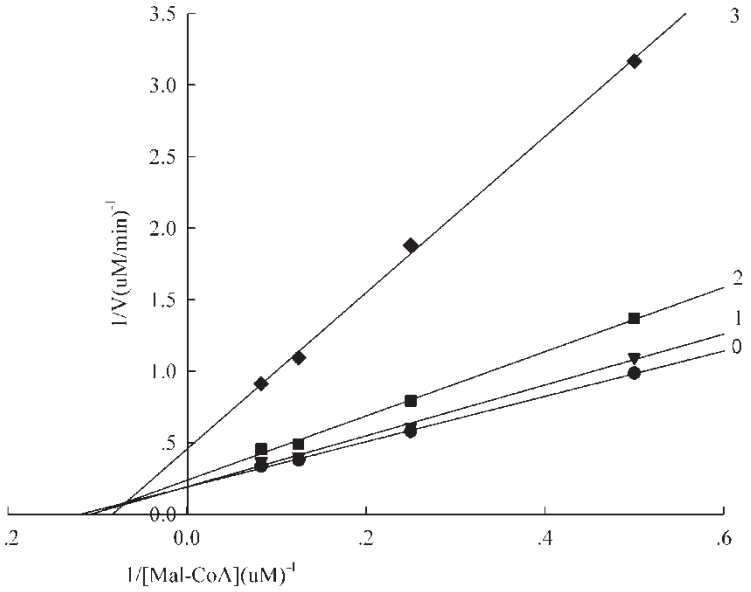

Figure 5. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by *A. platanoides* extract. The concentration of extract in the reaction system was 0 (0), 0. 25 μg/mL (1), 0.5 μg/mL (2), and 0.75 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of NADPH and Acetyl-CoA were 32 and 2.5μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

overall reaction the aceraceous plants possess more inhibitory capacity than these catechins.

It was reported that some Chinese medicinal herbs such as Loranthus Parasiticus and green tea have inhibitory effects on FAS [46–47]. The IC_{50} values of their extracts were 0.48 and 12.2 μg/mL, respectively. In comparison with reported medicinal herbs, the average value of IC_{50} for the aceraceous plants in Table II which are comparable in fast-binding inhibitory capacity, 1.20 μg/mL, is remarkably less than that for the above herbal inhibitors.

Wang and Tian reported that EGCG acts on the binding site of NADPH in β-ketoacyl reductase, and is a specific inhibitor of ketoacyl reductase [48].

The IC_{50} values of extracts for the ketoacyl reduction reaction are within the range 1.21–7.56 μg/mL; but for the FAS overall reaction the IC_{50} values range from 0.60–1.81 μg/mL (Table II). This remarkable difference in IC_{50} values indicates that, except for *A. capillipes*, in contrast with EGCG, the inhibition of ketoacyl reduction is not the main reason for FAS inactivation. Inactivation of FAS could

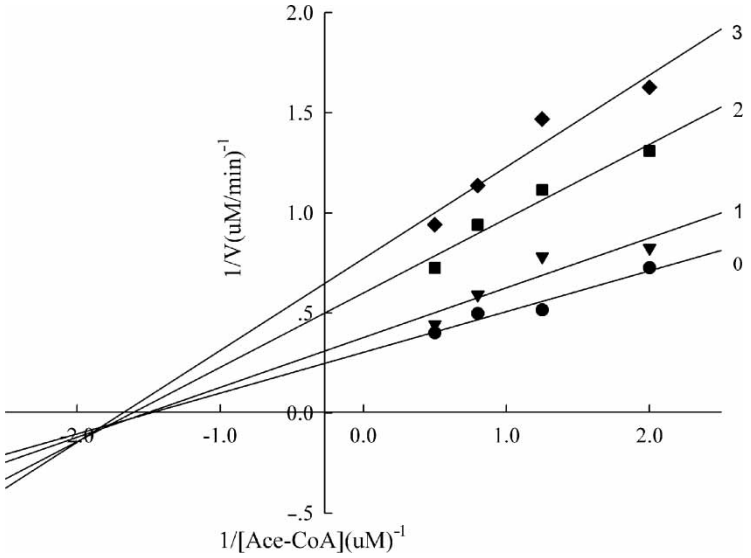

Figure 6. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by *A. campestre* extract. The concentration of extract in the reaction system was 0 (0), 0. 2 μg/mL (1), 0.4 μg/mL (2), and 0.5 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of NADPH and Malonyl-CoA were 32 and 10μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

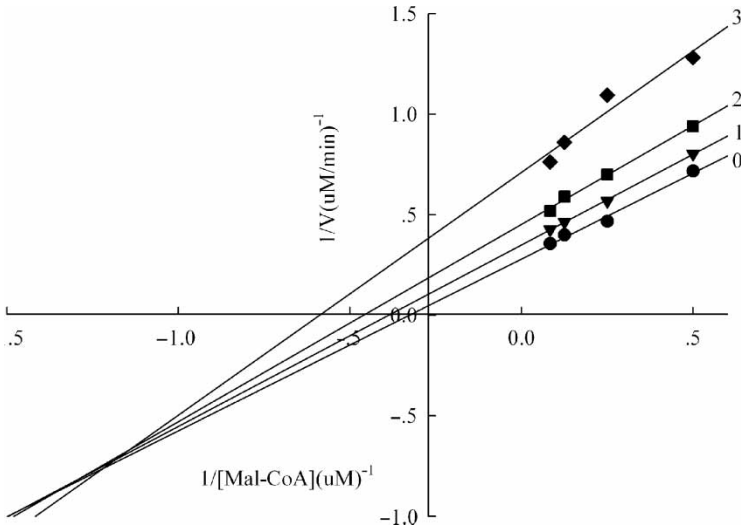

Figure 7. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by *A. campestre* extracts.The concentration of extracts in the reaction system was 0 (0), 0. 2 μg/mL (1), 0.4 μg/mL (2), and 0.5 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of NADPH and Acetyl-CoA were 32 and 2.5μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

rather be attributed to the cooperative effect of the components of extracts on the intermediate step-reactions of FAS. Overall, *Aceraceae* extracts are more efficient inhibitors of FAS.

For the overall reaction of FAS there are different inhibition patterns (Table III) between the extracts of *A. Platanoids* and *A. campestre* using the three substrates. The extracts of *Acer campestre* and *Acer platanoides* show inhibitory effect on the intermediate steps of the FAS overall reaction, including acetyl transacylation, malonyl transacylation, β-ketoacyl reduction.

Up-regulation of FAS occurs early in tumor development and is further enhanced in more advanced tumors including prostate and breast carcinomas, ovarian and endometrium cancers [49]. In addition, high FAS expression levels often predict a poor outcome for cancer patients. Recently, FAS hyperactivity was also found in squamous cell carcinoma of the lung and intestinal metaplasia of the stomach [50]. Pharmacological inhibition of tumour-associated FAS hyperactivity is under investigation as a chemotherapeutic target. The above five types of cancer cells other than breast cancer and prostate

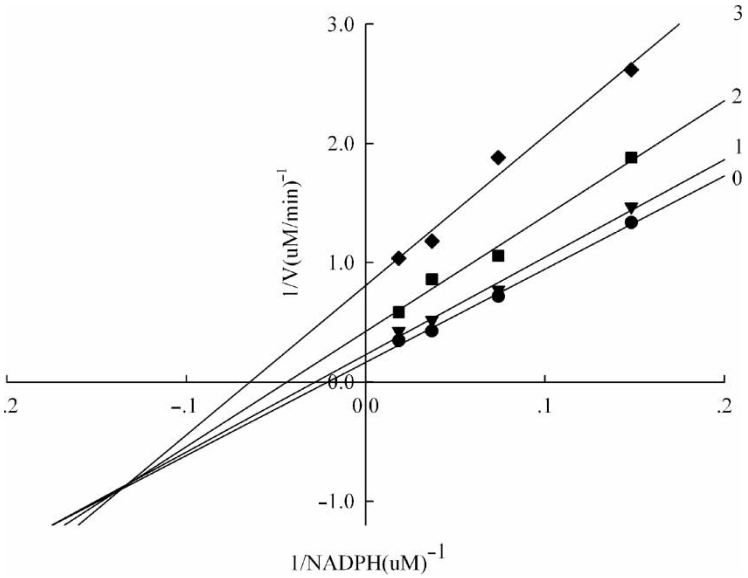

Figure 8. Lineweaver-Burk plot of the inhibition of the overall reaction of FAS by extracts.The concentration of extracts in the reaction system was 0 (0), 0. 2 μg/mL (1), 0.4 μg/mL (2), and 0.5 μg/mL (3). The FAS concentration was 0.012μM, and the fixed concentrations of Malonyl-CoA and Acetyl-CoA were 10 and 2.5μM, respectively. The reaction rate was the concentration of NADPH per min consumed in the overall reaction.

Table III. The inhibition types for the extracts from Aceraceous plants against the FAS overall reaction.

	A. platanoides		*A. campestre*		*A. truncatum*		EGCG	
Substrate	type	K_i(μg/ml)	type	K_i(μg/ml)	type	K_i(μg/ml)	type	K_i(μg/ml)
Acetyl-CoA	C	0.066	N	0.36	C + N	2.61	C + N	16.9
Malonyl-CoA	C + N	0.20	C + N	1.31	C	1.87	N	21.1
NADPH	C + N	0.048	C + N	0.84	U	2.43	C + N	9.16

C, competitive inhibition;N, noncompetitive inhibition;and U, uncompetitive inhibition. The K_i values for EGCG and *A. truncatum* are cited from (Zhang et al., 2006), and (Zhao et al.,2006), respectively.

Table IV. The IC_{50} of the extracts of five species of *Acer* against various cancer cells.

Species	CAES-17	BGC-823	MCF-7	BEL-7402	PC-3M
			(IC_{50}) (μg/mL)		
Acer tataricum	43	22	9	24	25
Acer platanoides	45	36	17	23	24
Acer capillipes	42	18	21	13	11
*Acer campestr*e	62	39	46	24	62
Acer pseudoplatanus	57	46	36	56	61

cancer are non sex-steroid-related, but were similarly inhibited by the extracts (Table IV), which may be related to the inhibition of lipogenesis in these cells. Brusselmans [51] et al investigated the EGCG inhibition of FAS in cultured prostate cancer cells and how this inhibition affected endogenous lipid synthesis, cell proliferation and cell viability. Their findings established EGCG as a potent natural inhibitor of FAS in intact cells and strengthen the molecular basis for the use of EGCG as a chemopreventive and therapeutic antineoplastic agent.

Historically, traditional herbal medicine has gained much practical experience for the control of tumors and understanding their physiological and biochemical mechanism is important for the application of the natural product in the cancer preventive field. We believe that these natural products represent a promising source of new therapies for tumors.

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