Review Article

Pharmaceutical Values of Calycosin: One Type of Flavonoid Isolated from Astragalus

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Astragalus membranaceus, Huangqi in Chinese, as a classic traditional herbal medicine, is commonly used in a variety of traditional Chinese medicine prescriptions [5, 6]. The major pharmaceutical functions of this Materia Medica are boosting immune and hematopoietic systems [7, 8]. Previous studies have reported that a plethora of flavonoids have been identified and isolated from Astragalus. Flavonoids are classified as polyphenolic compounds and they are ubiquitously enriched in the plant kingdom [9, 10]. It is estimated that over 4000 flavonoids have been reported and identified, and they could be clustered into 8 subclasses, that is, flavanole, flavanonole, chalkone, anthocyanidine, aurone, flavone, flavanone, and isoflavone [9, 10]. Calycosin is the most enriched isoflavone found abundantly in Astragalus. This molecule has gained attention for its myriad medical functions both in vitro and in vivo [2, 5, 6, 11]. Hence, this review emphasizes the effects of calycosin on anticancer, antioxidative, immune-modulatory, and estrogenic-like properties.

1. Introduction

The development of traditional Chinese medicine (TCM) has a history of thousands of years, and it has accumulated myriad medical experience and summarized pharmacological effects of Materia Medica playing a pivotal role in modernization of TCM [1, 2]. With the development of modern medicine, the pharmaceutical properties of raw herbal extract can no longer satisfy today’s sophisticated biomedical research. The bioactive molecules selected and isolated from plant are more suitable as potential medicine [3, 4]. In fact, plant-derived chemicals are associated with drug development, such as Taxol isolated from Taxus chinensis and camptothecin identified and enriched from Camptotheca acuminata.

Astragalus membranaceus, Huangqi in Chinese, as a classic traditional herbal medicine, is commonly used in a variety of traditional Chinese medicine prescriptions [5, 6]. The major pharmaceutical functions of this Materia Medica are boosting immune and hematopoietic systems [7, 8].

2. Pharmacological Activities of Calycosin

2.1. Anticancer Functions. Breast cancer is one of the most common cancers threatening women globally and it accounts for approximately 15% of female cancer-related
deaths in the United States [12, 13]. Human breast cancer is classified into estrogen receptor-positive (ER+) and estrogen receptor-negative (ER−) subtypes. Tian et al. have reported that calycosin was able to inhibit both ER− and ER+ breast cancer cell proliferation in a dose-dependent manner and the inhibitory effects were associated with noncoding RNA WDR7-7 expression level by inducing G-protein coupled estrogen receptor 30 (GPR30) and RAD1 via Erk1/2 and Akt transduction pathway [14, 15]. The apoptosis-related protein, cleaved caspase 3/9, and Bax were significantly stimulated under the treatment of calycosin in ER+ cancer cell type MCF-7 [14]. Li group published similar data and confirmed that calycosin at 150 μM was capable of blocking MCF-7 and T47D cells migration and invasion by wound healing and Transwell assays [16]. Interestingly, calycosin at 2 μmol/L already triggered MCF-7 cell apoptosis by flow cytometry analysis [17]. Additionally, treatment of calycosin could downregulate forkhead box P3, vascular endothelial growth factor (VEGF), and matrix metalloproteinase 9 (MMP9) in MCF-7 and T47D [17]. Furthermore, Chen group (2014) confirmed that calycosin induced ER+ MCF-7 cell apoptosis via the blocking insulin-like growth factor 1 receptor (IGF-1R) pathway after 48-hour treatment [18]. On the other hand, Wu et al. (2019) found that the application of calycosin decreased invasive and migratory effects in ER-brazen MDA-MB231 cells by suppressing Rab27B, β-catenin, and VEGF levels. More importantly, the inhibitory activities under the challenge of calycosin were recovered by the overexpression of Rab27B [19].

Colorectal cancer has a high mortality rate, which is also named as bowel cancer, colon cancer, or rectal cancer, claiming at least 500 thousand lives every year globally [20, 21]. Colorectal cancer is the third highest incidence of all cancers worldwide. The early symptoms of colorectal cancer are hard to detect, and the terminal stage of colorectal cancer is barely treated due to lack of effective biomarkers for clinical screening [22]. The study found that the potential targets of calycosin on colorectal cancer were ERα, ERβ, ATP-binding cassette subfamily G member 2, breast cancer type 1 susceptibility protein, CYP19A1, and epidermal growth factor receptor (EGFR) [22]. Therefore, these targets could be used as monitor for colorectal cancer treatment. Besides, the in vitro and in vivo against colorectal properties of calycosin have been widely documented [23–25]. Zhao et al. have published that calycosin suppressed colorectal cancer cell line SW480 dose-dependently by Hoechst 33258 assay [25]. Furthermore, the xenograft tumor size in nude mice was decreased by the calycosin treatment [25]. Impressively, calycosin significantly enhanced autophagy specific protein expressions, that is, Beclin-1 and LC-3II, after 48-hour incubation in cultured HT-29 cells [26]. However, cotreatment of HT-29 with IGF-1 could recover calycosin-induced cell autophagy. Wang found that calycosin inhibited colorectal cancer proliferation and migration by enhancing BATF2 to target plasminogen activator inhibitor-1 [27]. Moreover, this molecule was able to abolish transforming growth factor β-(TGF-β)-induced epithelial-to-mesenchymal transition via altering Wnt mechanism [27]. In addition, calycosin robustly restricted HCT-116 cells viability and invasiveness by enhancing ERβ and phosphatase and tensin homolog (PTEN) expressions [28].

Osteosarcoma is the most common malignant bone tumor with potential for invasion and metastasis; however, the current chemotherapy for osteosarcoma is not yet perfect [29, 30]. Calycosin is evidenced to induce MG-63 apoptosis, reduce cell proliferation, and decrease matrix metalloproteinase 2 (MMP2) and proliferating cell nuclear antigen expression after 48-hour incubation [31]. In tumor-bearing nude mice study, the tumor size and weight were reduced in calycosin-treated group [31]. Protein expression levels of IκBα and interleukin-6 (IL-6) were attenuated after calycosin interference for 3 weeks [31]. The data was in line with Wang et al.’s work published in 2018; they found that calycosin suppressed PI3K/AKT/mTOR pathway. In the MG-63 xenografts nude mice, calycosin inhibited tumor growth and also regulated phosphorylations of PI3K/Akt [31–33]. Hence, Table 1 summarizes the anticancer functions of calycosin.

### 2.2. Antioxidative Properties

Oxidative stress is a phenomenon triggered by the excessive production and accumulation of reactive oxygen species (ROS) in cells and finally leads to dysfunction of tissues [34, 35]. Calycosin has been evidenced to protect doxorubicin-induced oxidative stress in cultured cardiomyocyte by inhibiting ROS generation via enhancing antioxidant enzymatic activities, that is, glutathione peroxidase, catalase, and superoxide dismutase (SOD) [Table 2] [36]. Moreover, the levels of sirtuin 1-NOD-like receptor protein 3 and related proteins were elevated after calycosin was incubated for 24 hours both in vitro and in vivo [36]. Liu found that calycosin could also attenuate H2O2-induced H9C2 cell apoptosis rate in a dose-dependent manner [38]. Pretreatment with ER antagonist, ICI 182,780, negated the protective effect of calycosin against H2O2-induced apoptosis [37, 38]. Elsherbiny et al. (2020) have reported that calycosin showed potential effects on type 2 diabetes mellitus treatment after 4-week administration [40]. The contents of IL33/ST2 mRNA were enhanced and levels of p65, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and TGF-β were downregulated in calycosin treatment mice [Table 2] [39, 40]. Interestingly, calycosin reduced oxidative stress in intracerebral hemorrhage mouse model by stimulating Nrf2 protein expression [42]. Oral administration of calycosin at 25 or 50 mg/kg/day was able to enhance amylase and lipase levels in serum in acute pancreatitis rat model [41]. The cytokine levels after calycosin treatment were mitigated [41]. Additionally, calycosin was able to decrease cerulein-induced pancreatic edema, inhibiting myeloperoxidase activity and stimulating SOD activity [41]. Studies have shown that calycosin can extend the lifespan of C. elegans, and this extension is related to the antioxidant capacity by enhancing stress resistance capacity and reducing the accumulation of ROS [43]. Lu group (2017) published that calycosin required insulin signaling involvement to promote lifespan extension [43]. On the other hand, they observed that calycosin can enhance the nuclear translocation of the core transcription factor DAF-16/
TABLE 1: Anticancer functions of calycosin

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Model</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Cleaved caspase 3/9 upregulation; enhanced Bax/Bcl-2 ratio</td>
<td>MCF-7 cells; MDA-MB231 cells</td>
<td>Tian et al. [14]; Tian et al. [15]</td>
</tr>
<tr>
<td>Reduced cell migration and invasion; enhanced apoptosis rate via blocking IGF-1R pathway; downregulated VEGF and MMP</td>
<td>MCF-7 cells; T47D cells</td>
<td>Li et al. [16]; Chen et al. [17]; Chen et al. [18]</td>
</tr>
<tr>
<td>Downregulation of Rab27B and β-catenin</td>
<td>MDA-MB-231 cells</td>
<td>Wu et al. [19]</td>
</tr>
<tr>
<td>Abnormal expression levels of ERα, ERβ, ATP-binding cassette subfamily G member 2, breast cancer type 1 susceptibility protein, p450, and EGFR</td>
<td>OMIM data</td>
<td>Huang et al. [22]</td>
</tr>
<tr>
<td>Colorectal cancer proliferation</td>
<td>SW480 cells</td>
<td>Zhao et al. [25]</td>
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<td>Bcl2-1 and LC-3II overexpression</td>
<td>HT-29 cells</td>
<td>El-kott et al. [26]</td>
</tr>
<tr>
<td>Upregulation of ERβ and PTEN</td>
<td>HCT-116 cells</td>
<td>Chen et al. [28]</td>
</tr>
<tr>
<td>Induced osteosarcoma apoptosis and decrease MMP2</td>
<td>MG-63 cells</td>
<td>Qiu et al. [31]</td>
</tr>
<tr>
<td>Reduced tumor size and PI3K/Akt phosphorylation</td>
<td>Nude mice</td>
<td>Wang et al. [32]; Qiu et al. [31]; Tian et al. [33]</td>
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TABLE 2: Antioxidative functions.

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<th>Biomarkers</th>
<th>Model</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Enhance glutathione peroxidase, catalase, and superoxide dismutase enzymatic activities</td>
<td>H9C2 cells and male Kunming mice</td>
<td>Zhai et al. [36]</td>
</tr>
<tr>
<td>Reduced H2O2-induced cell apoptosis rate</td>
<td>H9C2</td>
<td>Chen et al. [37]; Liu et al. [38]</td>
</tr>
<tr>
<td>Downregulation of cytokine levels and IL33/ST2 mRNA level</td>
<td>Type 2 diabetes mellitus rat model</td>
<td>Wang &amp; Zhao [39]; Elsherbiny et al. [40]</td>
</tr>
<tr>
<td>Stimulating Nrf2 expression and SOD levels</td>
<td>Intracerebral hemorrhage mouse</td>
<td>Ma et al. [41]; Chen et al. [42]</td>
</tr>
<tr>
<td>Prolong lifespan and stimulate SOD levels</td>
<td>C. elegans</td>
<td>Lu et al. [43]</td>
</tr>
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</table>

2.3. Anti-Inflammatory Functions. The anti-inflammatory properties of calycosin were widely documented on lipopolysaccharide- (LPS-) induced RAW 264.7 cells [41, 44]. Calycosin significantly attenuated nitric oxide (NO), prostaglandin E2 (PGE2), TNF-α, IL-1β, and IL-6 releases, and the anti-inflammatory properties had been confirmed by NF-κB and MAPK signal pathways [44]. The effective dosage was from 30 nM to 5 μM, and the inhibitory function was dose-dependent. Besides, calycosin could also diminish inflammatory cell markers CD68 and F4/80 mRNA levels in a dose-dependent manner [45].

Calycosin was reported to possess renal protective functions in high-fat diet-induced type 2 diabetes mellitus rat model by altering SOD and TGF-β content in renal tissues as compared to the sham group [40]. Zhang et al. published similar data and reported that this molecule was able to effectively alleviate kidney injury in diabetic kidneys of db/db mice after treatment for 28 days (Table 3) [50]. The serum contents of inflammatory cytokines were reduced via suppressing IκBα and NF-κB p65 [50]. Additionally, fed glucose level in db/db obese mice was declined after calycosin administration which was proposed to be related to the anti-inflammatory effects [45]. Reduced serum triglyceride levels, alleviated insulin resistance, and glucose intolerance were observed in calycosin-treated mice compared with the vehicle-treated controls [45].

Xu et al. found that calycosin was able to relieve advanced glycation end products- (AGE-) induced inflammation both in vitro and in vivo [49]. AGEs act as the central role in vasculitis development by recruiting the receptor for AGE overexpression (Table 3) [49]. Calycosin was able to diminish vasculitis development by downregulating the AGEs-induced overexpression of receptor for advanced glycation end products (RAGE) and proinflammatory cytokines in both rat and HUVECs [46]. ERK1/2 and NF-κB pathways were involved and evidenced by Kim et al. and Cheng et al. after calycosin presence for 4 hours [47, 48].

2.4. Estrogenic-Like Properties

2.4.1. Osteogenic Functions of Calycosin. Women suffering from menopause have higher risk of getting osteoporosis. FOXO, rather than the conservative stress response transcription factor SKN-1/Nrf-2 [43].

Women suffering from menopause have higher risk of getting osteoporosis. The role of calycosin in preventing osteoporosis is widely reported [51, 52]. The proliferation and differentiation capacities of MG-63 were determined to be enhanced in both calycosin and EGFR expressions [50]. The results showed that calycosin was able to stimulate osteoblast differentiation by downregulating the RAGE and proinflammatory cytokines in both rat and HUVECs [46]. ERK1/2 and NF-κB pathways were involved and evidenced by Kim et al. and Cheng et al. after calycosin presence for 4 hours [47, 48].

<table>
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<tr>
<th>Biomarkers</th>
<th>Model</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Enhanced osteogenic functions of calycosin</td>
<td>Mouse osteoblast cells</td>
<td>Xu et al. [53]</td>
</tr>
<tr>
<td>Reduced tumor size and PI3K/Akt phosphorylation</td>
<td>Nude mice</td>
<td>Wang et al. [32]; Qiu et al. [31]; Tian et al. [33]</td>
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2.4.2. Hematopoietic Functions of Calycosin.

Time incubation [55]. Therefore, calycosin may be useful as a therapeutic agent for bone loss-associated diseases.

2.5. Neuroprotective Functions.

2.4.2. Hematopoietic Functions of Calycosin.

One symptom of estrogen deficiency is anemia [7]. Several lines of evidence suggested that calycosin could stimulate the expression of erythropoietin (EPO), the central regulator of red blood cell mass, in cultured human embryonic kidney fibroblasts (HEK293T) after exposure of calycosin for 24 hours [7, 8, 11, 35]. The calycosin-induced EPO expression was mediated by HIF-1α from western blotting results [56]. The in vitro experiments showed that calycosin could enhance the number of RBCs, WBCs, PLTs, and content of Hb in peripheral blood and the area of bone marrow hematopoietic tissue [57]. The serum contents of thrombopoietin, EPO, granulocyte-macrophage colony stimulating factor, colony of CFU-GM, CFU-MK, CFU-E, and BFU-E were also enriched after calycosin treatment [57]. The animal experiments showed that this agent reduced G0/G1 cells and increased G2/M cells in hematopoietic stem cells.

3. Pharmacokinetics of Calycosin

Because of hydroxyl groups found within the chemical structure of calycosin, they are metabolized to glucuronide by phase II metabolic enzymes such as UDP-glucuronosyltransferases from the intestine and liver after oral administration [65]. In addition to the role of metabolism, absorption, hydrolysis, efflux, and intestinal circulation in the intestinal tract also participate in the disposal of calycosin in the body, affecting their systemic and local bioavailability [66].

Studies have shown that, after oral administration of Astragalus water extract, the enriched content of calycosin-7-O-β-glucoside is detected in plasma, indicating that calycosin-7-O-β-glucoside can enter the intestinal cells in a prototype form and be metabolized [66, 67]. In the study, it was found that, after administration of calycosin by oral gavage, calycosin-7-O-β-glucoside can be detected in the plasma, which shows that calycosin-7-O-β-glucoside can penetrate the cell membrane in a prototype form and undergo further metabolism [68]. This indicates that calycosin-7-O-β-glucoside may directly pass through the cell membrane in a prototype form without hydrolysis [69]. Using Caco-2 cells to study the absorption and transport characteristics of calycosin and its glucoside, it was found that calycosin and calycosin-7-O-β-glucoside are mainly absorbed in the form of passive diffusion, and the absorption process will not be affected by inhibitors of MATEs such as P-gp and MRP2 [69, 70]. Calycosin can be metabolized in human liver microsomes to generate two glucuronides. UGT1A1 and UGT1A9 are the major metabolic enzyme

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<th>Biomarkers</th>
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<tbody>
<tr>
<td>Declined NO, PGE2, TNF-α, and other cytokine release activities</td>
<td>RAW 264.7 cells</td>
<td>Dong et al. [44]; Ma et al. [41]</td>
</tr>
<tr>
<td>Reduced CD68 and F4/80 mRNA levels</td>
<td>Raw 264.7</td>
<td>Hoo et al. [45]</td>
</tr>
<tr>
<td>Enhanced SOD and TGF-β contents</td>
<td>Type 2 diabetes mellitus rat model</td>
<td>Elsherbiny et al. [40]</td>
</tr>
<tr>
<td>Reduced IkBα and NF-xB p65 protein translational levels and alleviated insulin resistance</td>
<td>db/db obese mice</td>
<td>Hoo et al. [45]</td>
</tr>
<tr>
<td>Alleviated inflammatory responses and cytokine levels via attenuating Erk1/2 and NF-xB pathways</td>
<td>Hepatocyte cell line; HUVEC</td>
<td>Figarola et al. [46]; Kim et al. [47]; Cheng et al. [48]; Xu et al. [49]</td>
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subtypes that generate these two glucuronides, respectively [71]. On the other hand, after oral administration of calycosin, the calculated bioavailability of calycosin-7-O-β-glucoside was only 0.304%, indicating that hydrolyzing is an important process of metabolism in vivo [72, 73]. However, glucoside hydrolase is solely present in the intestine and liver of rats. In order to confirm the hydrolysis site of calycosin-7-O-β-glucoside, the pharmacokinetics of calycosin-7-O-β-glucoside injection in rats were investigated because the drug was directly absorbed by the hepatic portal vein after intraperitoneal injection [74]. The drug will not be processed through the intestine, so as to exclude the effect of the intestine on the calculated bioavailability of calycosin-7-O-β-glucoside treatment in the body [75]. The results show that the drug time curve of calycosin-7-O-β-glucoside and its metabolites is completely different from that of calycosin-7-O-β-glucoside after oral administration.

4. Chemical Interactions

As the main bioactive molecule isolated from Astragali Radix, the pharmacological activity of calycosin is not performed alone but by the joint action of multiple chemical substances. Cotreatment of calycosin with other biochemicals identified from Astragali Radix, that is, formononetin, ononin and astragaloside, showed effective therapeutic functions as compared to single compound. The study found that the expression levels of drug-metabolizing enzymes, such as CYP3A4, CYP2B6, CYP2E1, UGT1A, and efflux transporters, that is, P-gp, MR2, BCRP, and MRP3, were increased in a dose-dependent manner in the drug combination group [76]. Zheng et al. found that cotreatment of calycosin with formononetin stimulated EPO expression in a dose- and concentration-dependent manner in cultured HEK 293 cells. The hematopoietic functions of these combinations were even stronger than the positive control [77, 78]. Furthermore, Zhang et al. reported that flavonoid combination containing formononetin and calycosin at weight ratio of 1:5 showed the best hematological functions on anemic rat after drug treatment [79].

The research of calycosin in modern medicine is no longer confined to a single compound or Astragali Radix. More and more scientists have discovered the combination of multiple substances enjoying a broad spectrum in disease treatment by “Fu Fang.” Astragali Radix and Angelicae Sinensis Radix are usually combined together clinically [35, 52]. Cotreatment of calycosin and Astragali Sinensis Radix-derived ferulic acid protected bleomycin-induced pulmonary fibrosis in rats, and this action was believed via blocking NOX4 expression [80]. Furthermore, combination of calycosin and ferulic acid showed better immune-modulatory pharmaceutical activities in Raw 264.7 and inducing blood vessels in HUVECs and Zebra fish [81–85]. Administration of calycosin and ferulic acid attenuates cytokine and inflammatory mediators’ releases in atopic dermatitis-like mouse [85].

Additionally, there are myriad of formulae containing calycosin at different dosage forms either alone or in combination with other bioactive molecules in market. The pharmaceutical functions of calycosin on anticancer, anti-oxidative, immune-modulatory, and estrogenic-like properties were summarized. We believe the potential pharmaceutical value of calycosin is still behind the veil, and which motivating us to discover more in the future. The in vitro and in vivo pharmaceutical functions do not directly translate into the clinic because of bioavailability and biotransformation influenced by gut microbiota. Considering various compositions of microbiota between individuals, the fluctuating process of bioavailability and biotransformation mediated by gut microbiota could have a consequential effect of calycosin and its metabolites in plasma, finally leading to diverse clinic functions. Hence, gut microbiota-induced bioavailability and biotransformation of calycosin and its metabolites should be taken into consideration before clinical application.

5. Conclusion

Calycosin serves as a common dietary flavonoid and is consumed in daily cuisine and/or TCM decoction. As the main bioactive molecule isolated from Astragali Radix, the pharmacological activity of calycosin is not performed alone but by the joint action of multiple chemical substances. Cotreatment of calycosin with other biochemicals identified from Astragali Radix, that is, formononetin, ononin and astragaloside, showed effective therapeutic functions as compared to single compound. The study found that the expression levels of drug-metabolizing enzymes, such as CYP3A4, CYP2B6, CYP2E1, UGT1A, and efflux transporters, that is, P-gp, MR2, BCRP, and MRP3, were increased in a dose-dependent manner in the drug combination group [76]. Zheng et al. found that cotreatment of calycosin with formononetin stimulated EPO expression in cultured HEK 293 cells. The hematopoietic functions of these combinations were even stronger than the positive control [77, 78]. Furthermore, Zhang et al. reported that flavonoid combination containing formononetin and calycosin at weight ratio of 1:5 showed the best hematological functions on anemic rat after drug treatment [79].

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Data Availability

The data used in this paper are available upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.
Authors’ Contributions
Guowei Gong and Yang Yang wrote the main text. Yuzhong Zheng and Zhen Wen were responsible for polishing the manuscript. Yang Yang and Yixuan Sui formatted the references. All authors read and approved the final version of manuscript.

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References
[26] A. F. El-Kott, M. A. Al-Kahtani, and A. A. Shati, “Calycosin induces apoptosis in adenocarcinoma HT29 cells by inducing cytotoxic autophagy mediated by SIRT1/AMPK-induced...
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accumulation of hypoxia-inducible factor-1α,” *Journal of Agricultural and Food Chemistry*, vol. 59, no. 5, pp. 1697–1704, 2011.


