Review Article

Potential Anticancer Properties and Mechanisms of Action of Formononetin

Dongjun Jiang,1 Azhar Rasul,1,2 Rabia Batool,2 Iqra Sarfraz,2 Ghulam Hussain,3 Muhammad Mateen Tahir,2 Tian Qin,1 Zeliha Selamoglu,4 Muhammad Ali,5 Jiang Li,6 and Xiaomeng Li1

1The Key Laboratory of Molecular Epigenetics of MOE, Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China
2Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad (GCUF), 38000, Pakistan
3Department of Physiology, Faculty of Life Sciences, Government College University Faisalabad (GCUF), 38000, Pakistan
4Department of Medical Biology, Faculty of Medicine, Nigde Omer Halisdemir University, Nigde, Campus 51240, Turkey
5Quaid-e-Azam University, Islamabad 45320, Pakistan
6Dental Hospital, Jilin University, Changchun 130021, China

Correspondence should be addressed to Xiaomeng Li: lixm441@nenu.edu.cn

Received 3 March 2019; Accepted 9 May 2019; Published 28 July 2019

1. Introduction

Natural products have served as an infinite reservoir of various diversified chemical compounds, driving pharmaceutical industries for many years [1]. In the discipline of cancer therapeutics, natural products hold a great potential. It has been reported that, from 1981 to 2014, about 1562 drugs were approved out of which 1211 drugs were derived from small molecules that are nonsynthetic [2]. About 50% of the medicines and 48.6% of anticancer drugs are derivatives of natural products [3]. Chinese encyclopedia that dates back to 2000 BC has reported that 1898 herbal products have been used as medicines [4]. In accordance to World Health Organization, about 80% of population depends upon plant-derived traditional medicines. This drug discovery approach exhibits far-reaching domain where large-scale research could provide novel leads against cellular or molecular targets [5].

Different pharmacological studies on natural products have proclaimed their authenticity as potent anticancer [6], antioxidant [7], and anti-inflammatory agents [8].

Triterpenoid saponins and isoflavones belong to family of amphiphilic glycosides which are naturally present in medicinal plants, herbs, and marine organisms. Saponins and isoflavones have major role in folk medicine due to their biological and pharmacological properties [9]. These secondary metabolites possess various anticancer, anti-inflammatory, and antioxidant properties. Epidemiological studies recommend that intake of food enriched with isoflavonoids reduces the risk of various cancers [9].
Formononetin, an isoflavone isolated from soy bean and red clover, has been known to be endowed with numerous pharmacological attributes such as anticancer [10], anti-inflammatory, [11], and antioxidant attributes [12].

To date, there is no review on the biological potential of formononetin. This article intends to focus on the researches relevant to the biological and pharmacological activities of Formononetin. The literature is searched via different e-sites like PubMed, Elsevier Science Direct, Springer Link, and related journals. Key words which are used for searching are “Formononetin”, “Formononetin and its biological activities”, “anticancer”, and “natural products”.

2. Natural Sources of Formononetin

Formononetin (Figure 1) has been reported to be isolated from different plants of bean family “Fabaceae” which is the 3rd largest family of land plants. Genus *Trifolium* (Fabaceae) contains 250 species, most of which have been documented as rich source of formononetin [13]. In addition to this family, formononetin is also present in different plants including *Trifolium pratense* [14], *Glycine max* [15, 16], *Sophora flavescens* [17], *Pycnanthus angolensis* [18], *Spatholobus suberectus* [19], *Cicer arietinum* [20], *Dalbergia odorifera*, *Pueraria thunbergiana* [21], *Actaea racemosa* [22], *Ononis spinosa L.* [9], *Dalbergia ecastaphyllum* [23], *Callerya speciosa* [24], *Astragalus membranaceus* [25], and *Astragalus mongholicus* as shown in Figure 1. The list of plants having Formononetin, their parts, and pharmacological properties are elaborated in Table 1.

3. Biological Activities of Formononetin and Mechanisms of Its Action

The biological and pharmacological activities of bioactive compound "Formononetin" are well-documented as anti-tumor activity, [23] antiproliferative activity [14], growth inhibitory activity [55], vasorelaxant action [56], neuroprotective effect [57], antiapoptotic activity [48], cardioprotective activity [58], mammary gland proliferative activity [59], antioxidant activity [60], antimicrobial activity, and anti-inflammatory activity [15] as provided in Figure 2. Numerous in vivo/in vitro investigations have been done on Formononetin to uncover its biological attributes and mechanisms of actions.
3.1. Anticancer Activity. Cancer is uncontrolled proliferation of cells which occurs due to genetic or epigenetic modifications and signaling defects in cells [61]. Different synthetic drugs are used for the treatment of this deadly disease [2]. Due to the limited success of synthetic drugs, it is necessary to identify novel natural products having the ability to induce apoptotic cell death and arrest cell cycle in tumor cells without toxic effect on normal cells [62]. Various studies have declared that formononetin can block, delay, or inhibit the initiation and progression of cancer. This review intends to focus on the studies relevant to anticancer potential of formononetin which will allow the researchers to further investigate this novel chemical entity as a potential anticancer drug candidate.

Currently, it is documented that about 60% drugs that are used for cure of cancer are derived from natural sources [63]. Secondary metabolites isolated from natural products encompassing terpenes, alkaloids, isoflavones, and polyphenols have been reported as anticancer agents [64, 65]. Anticancer properties of formononetin have been documented
against several types of cancer [66] such as breast [67], colon [41], glioma [54], osteosarcoma [68], multiple myeloma [52], adrenal medulla [51], nasopharyngeal [50], prostate [49], bladder [45], laryngeal [23], lung [43], and cervical cancer [42] (Figure 3).

3.1.1. Formononetin and Cell Cycle Arrest. Uncontrolled divisions of cells are key trait of cancer cells [69]. Natural compounds have ability to prohibit the functions of cyclin dependent kinases and various other cell cycle regulatory proteins, thus causing cell cycle arrest [70].

Formononetin has been documented to arrest the cell cycle at various stages [44]. In human ovarian cancer, Formononetin leads cancer cells towards apoptosis and arrested cell cycle at G0/G1 phase in ES2 and OVC10 cell lines [10]. Formononetin downregulated cyclin D1 which further
arrested the cell cycle at the phase of G0/G1 in HCT-116 cells [41]. Formononetin arrested cells at G0/G1 phase in HepG2 cancerous cells [23]. In lung cancer cells, it caused the arresting of cell cycle at G1 phase via alteration of the p21, cyclin A, and cyclin D expression [44]. In PC-3 and DU145 cells, this compound has the ability to arrest the cells at G0/G1 phase via reducing AKT/cyclin D1/CDK4 expression [49] (Figure 4).

It can be summarized that Formononetin causes the arrest the cells at G0/G1 phase but it needs to be investigated whether it has arrested the cellular cycle at G0 or G1 phase. Furthermore, it is interesting for researchers to investigate whether Formononetin could arrest the cells at G2/M or S-phase. Thus, further mechanistic investigations are yet obligatory to understand the mechanisms by which Formononetin arrests the cell cycle in various cancers.

3.1.2. Formononetin and Apoptosis. Apoptosis is a systematic and synchronized way of cell death which is peculiarized by different morphological features including formation of blebs on cell membrane, condensation of chromosomes, and fragmentation of nucleus [71, 72]. It is reported that certain cellular signals are necessary to regulate the growth of cells but mutation in these signals prevent the cells to undergo...
apoptosis [73, 74]. Accumulated data indicated that various chemopreventive agents have been identified that can lead cancer cells towards apoptosis [75, 76]. Formononetin has the ability to inhibit or block the growth of cancerous cells via intrinsic or extrinsic apoptosis pathways [49].

Formononetin has emerged as novel agent for its chemotherapeutic activity [52]. Anticancer activity of Formononetin has been known to be associated with induction of apoptosis via activation of caspase family, ROS, activation of Bax, downmodulation of antiapoptotic protein Bcl-2, [53], upregulation of p-AKT [37], inhibiting the activation of NF-κB, reduction of ERK1/2 [10], inhibition of MMP-2/-9, upregulation of p38, p21, and p53 [10, 44], increase of the level of P450 1A1 [36], and blockage of PI3K/AKT, STAT3 signaling pathways [41] (Table 2) (Figure 4).

(1) Formononetin and MAPK and PI3K/AKT Pathway. MAPK, mitogen-activated protein kinase pathway, performs significant function in cell division, differentiation, proliferation, and cellular apoptosis [77]. MAPK pathway has four distinct signaling domains such as ERK, JNK, BMK-1, and p38 MAPK. Extracellular signals conducted by these kinases regulate the process of cellular apoptosis and growth [78, 79]. MAPK pathway has been reported as novel target to combat cancer. The PI3K/AKT pathway also performs a significant role in tumor development. This activated pathway is affiliated with several cancer types. Therefore, targeting this pathway has the ability to combat cancer [80].

Isoflavonoids have been documented as anticancer agents that inhibit cancer cell proliferation and have antimitomastic effects [35, 81]. Formononetin has ability for the induction of apoptosis in breast cancerous cells via activating Ras/p38 MAPK pathway in a dose dependent mode [40].

Formononetin reduced the p-AKT, p-P90RSK, p-S6, p-ERK1/2, and p-P70S6K proteins as well as enhancing the levels of p-p38 MAPK to mediate cellular proliferation and apoptosis in OV90 and ES2 cells [10]. Treatment with formononetin enhanced the levels of ERα and p-AKT in HUVEC and MCF-7 cancer cells [37]. This natural compound Formononetin suppresses proliferation and invasive capability of cells by inhibiting MMP-2/-9 via inactivation of phosphorylation of AKT and PI3K in HCT-116 and SW1116 cells [37, 41]. Formononetin also exerts its anticancer effects due to downmodulation of p-AKT, miR-21 expression, and upregulation of PTEN in T24 cells [45]. Formononetin inhibited the proliferation via inactivation of PI3K/ AKT pathway, enhancing the Bax, and downmodulating the Bcl-2 expression [68]. Formononetin and its derivatives such as dithiocarbamate with IC_{50} value of 1.9 μM possess inhibitory potential against PC3 cells [46] (Table 2).

(2) Formononetin and JAK/STAT Signaling Pathway. JAK/STAT pathways mediate the transduction of various signals involving cell division, immunity, tumor development, and cell death. Disruption in JAK/STAT signaling pathways may cause several diseases including cancer and immune disorders [82]. Formononetin decreased the activation of STAT5 and STAT3 by suppressing the nuclear translocation of p-STAT5 and p-STAT3 and also inhibited the activation JAK1 and JAK2 in U266 cells. Formononetin also inhibited the IL-6-induced STAT3 activity which ultimately inhibits the cell viability and activates apoptosis [52]. Formononetin suppresses the cellular invasion and proliferation by inhibition of MMP-2/-9 via inactivation of p-STAT3 pathway in colon carcinoma cells [41].

4. In Vivo Studies and Biosafety Profile

Formononetin with IC_{50} value 2-6 μM has the ability to promote the expression of p-AKT, miR-375, and Bcl-2 in in vivo mice model [37]. Treatment with Formononetin suppresses growth of tumor in in vivo tumor mouse model at the dose of 60 mg/kg [49]. An in vivo investigation demonstrated that Formononetin combined with other compounds reduced allergic inflammation in mice model via downregulating NF-κB activation [83]. Administration of Formononetin reduced the size, volume, and weight of HeLa tumor in in vivo mice model induced by injection of HeLa cells in the posterior flanks of mice [84]. As Formononetin is the imperative components of Chinese folk medicines Radix Astragalus and Fukeqianjin which are mostly used as antioxidant and anticancer agents, respectively, therefore, it might serve as safe chemotherapeutic drug candidate [85, 86]. Formononetin turns out to be a fascinated bioactive entity as it combines active chemotherapeutic effect with less toxicity in comparison to other isoflavones. However, safe doses and effectiveness of formononetin in targeted therapeutic fields still need to be executed in future.

5. Other Biological Functions

Formononetin is extracted from different plants such as Dalbergia odorifera in which Formononetin along with other compounds showed antiallergic and anti-inflammatory activities [87]. Formononetin together with other known compounds acts as active inhibitor of EV-A71 infection [88]. Brazilian Red propolis extract containing isoflavonoids such as Formononetin exhibits anti-inflammatory potential in a rat model of inflammation [89]. Isoflavones such as Formononetin isolated from soy bean possess antimicrobial and antioxidant activities with IC_{50} values from 10.6 to 22.6 μg/mL [15]. A study indicates that a methoxy Isoflavone, Formononetin, has potential for bone healing process in mouse model and has promising role in fracture-repair process [90]. Hydroalcoholic extract of Red propolis containing Red propolis has the ability to repair axon after sciatic nerve injury in rat model [91].

6. Conclusion and Future Perspectives

In this review article, we have suggested that Formononetin is a good drug candidate with promising pharmacological activities. Various researches have documented the potential applications of Formononetin both in vivo and in vitro. Being an important bioactive constituent of edible foods such as soybean, chickpeas, and alfalfa beans, Formononetin might turn up as a safe chemotherapeutic drug candidate. Many studies have revealed that formononetin is an inducer of
| Cancer types | Cell lines | Treatment time | IC$_{50}$ | Molecular targets | Cell cycle arrest | References |
|--------------|------------|----------------|----------|-------------------|------------------|------------|
| Ovarian      | ES2,OV90   | 48 h           | 40 μM    | p38↑, ERK1/2↑,   | G0/G1            | [10]       |
|              |            |                |          | P90RSK, AKT↑,    |                  |            |
|              |            |                |          | P70S6K↑, ROS↑↑   |                  |            |
|              | SKOV3      | 24 h           | 283.5 μM | caspase3/9↑,     |                  | [34]       |
|              |            |                |          | Bax/Bcl2↑, MMP2L↓|                  |            |
|              |            |                |          | MMP9L↓, p-ERK↓   |                  |            |
|              | A2780      | 48 h           | 209.3 μM |                   |                  |            |
|              |            |                |          |                   |                  |            |
|              | MDA-MB-231,| 24 h           | 200 μM   |                   |                  |            |
|              | MCF-7      |                |          |                   |                  |            |
|              | SK-BR-3    |                |          |                   |                  |            |
|              | MCF-7 WS8  | 48 h           | 310.0 μM |                   |                  |            |
|              |            |                |          |                   |                  |            |
|              | 4T1, MDA-MB-231 | 24 h | 160 μM | TIMP-2↑, TIMP-1↑, | G0/G1            | [38]       |
|              | MDA-231, MDA-435 | 24, 48h   | 50 μM   |                   |                  |            |
|              |            |                |          |                   |                  |            |
|              | MCF-7      | 24, 48h        | 100 μM   |                   |                  | [39]       |
|              |            |                |          |                   |                  |            |
| Breast       | MCF-7 WS8  | 48 h           | 10 μg/ml | CYP1A1↑, p53↑,    |                  | [36]       |
|              |            |                |          | ROS↓, p21↑,       |                  |            |
|              |            |                |          | ERα↑, miR-375↓,  |                  |            |
|              |            |                |          | Bcl-2↑, p-AKT↑↑   |                  |            |
|              | 4T1, MDA-MB-231 | 24 h | 10 μg/ml |                    |                  |            |
|              | MDA-231, MDA-435 | 24, 48, 72h | 100 μM   |                   |                  | [40]       |
|              |            |                |          |                   |                  |            |
| Colon        | SW1116     | 24 h           | 200 μM   | P53↑, IGF1R↑,    | G0/G1            | [41]       |
|              | HCT116     |                |          | PARP-1act, miR-375↓|                  |            |
|              |            |                |          |                   |                  |            |
| Hepatoma     | HuH-7      | 24 h           | 20 μM    | p-38MAPK↑,       | G0/G1            | [18]       |
|              |            |                |          |                   |                  |            |
|              |            |                |          |                   |                  |            |
| Cervical     | HeLa, SiHa, CaSk  | 24 h | 25 Mm | ROS↓, JNK↓, caspase-8 act, | -- | [42] |
|              |            |                |          | caspase-3 act, Bax/Bcl2↑, |                  |            |
|              |            |                |          | PTEN↑, p-AKT↓,    |                  |            |
|              |            |                |          |                   |                  |            |
| Laryngeal    | Hep-2      | 24, 48 h       | 50 and 75 μg/ml | ROS↑, CdCl2↑↑ | G0/G1            | [23]       |
|              |            |                |          |                   |                  |            |
| Lung         | A549       | 48 h           | 60 mg/ml | p-Smad 2/3↑, TGF-β↑, EMT↓ | -- | [43] |
|              | A549, NCI-H23 | 24 h | 100 μM | p53↑↑, p21↑↑, cyclin A↓, | G1 | [44] |
|              |            |                |          | cyclin D↓↑,      |                  |            |
| Bladder      | T24        | 24 h           | 200 μM   | T24↑, PTEN↑, p- |                 | [45]       |
|              |            |                |          | AKT↓, miR-21↑↑, |                  |            |
| Gastric      | MGC-803    | --             | --       |                   |                 | [46]       |
| Esophagus    | EC-109     | --             | --       |                   |                 | [46]       |
| Prostate     | PC3        | 48 h           | 1.97 μM  | p-ERK↑, p-INK↓,   | G1               | [46]       |
|              |            |                |          | c-Myc↑, p-p38↑, cyclin |                  |            |
|              |            |                |          | B↓, Bcl↓, cyclin |                  |            |
|              | DU145, PC3 | 48 h           | 200 μM   |                   |                  | [47]       |
|              | PC3        | 48 h           | >12.5 μM | Bcl-2↑↑, RASD1↑↑, | -- | [48] |
|              | PC-3, DU145 | 48 h | 80 μM | AKT↓, cyclin D↓/CDK4 | G0/G1 | [49] |
| Nasopharyngeal| CNE2       | 24 h           | 1 μM     | Bax↑, bcl-2↑↑,   | --               | [50]       |
|              |            |                |          | p-ERK↓/2↑↑        |                  |            |
| Adrenal medulla| PC12      | 24 h           | 20 μg/ml | ROS↑, MDA↓, GSH↑ | --               | [51]       |
apoptosis in many cancerous cells, but still mechanism of its action is not fully clarified. After the analysis of data, we have found that Formononetin is most active against nasopharyngeal cell line CNE2 with IC_{50} value of \mu M; so, further mechanistic studies should be conducted in future because nasopharyngeal carcinomas are one of the most prevalent cancers in Asia. This review also elucidates the potential role of Formononetin for the cure of several other diseases. Reported studies acclaim multiple biological properties of Formononetin but further experimentations on mechanism of its action, medicinal chemistry, and preclinical researches are yet necessary to figure out full array of its biological and pharmacological potential.

**Conflicts of Interest**

Authors declare that there are no conflicts of interest.

**Authors’ Contributions**

Dongjun Jiang and Rabia Batool made contribution to writing different parts of the manuscript. Azhar Rasul and Ghulam Hussain have made substantial contribution to integration of the data and drafting of the manuscript. Muhammad Mateen Tahir has contributed to acquisition of data. Iqra Sarfraz and Tian Qin designed and generated figures of manuscript. Zeliha Selamoglu and Muhammad Ali have reviewed the manuscript. Xiaomeng Li and Jiang Li have read and approved the final manuscript.

**Acknowledgments**

This study was supported by Ministry of Science and Technology, China (no. 2016YFE0128500), National Natural Science Foundation of China (no. 31870758), Jilin Provincial Science and Technology Department (20180414057GH, 20170204009YY), Changchun Science & Technology Department, China (17YJ006; 17YJ001), University S & T Innovation Platform of Jilin Province for Economic Fungi (#2014B-1), the Program for Introducing Talents to Universities (no. B07017), The Nagai Foundation Tokyo, Japan (NFT-R-2018), TWAS-COMSTECH Research Grant (no.17-180 RG/PHA/AS-C), and NRPU Research Grants (8381/Punjab/NRPU/R&D/HEC/2017, 8382/Punjab/NRPU/R&D/HEC/2017). The authors would also like to thank Higher Education Commission (HEC), Pakistan, for providing access to related papers from various journals.

**References**

[1] B. B. Mishra and V. K. Tiwari, "Natural products: an evolving role in future drug discovery," European Journal of Medicinal Chemistry, vol. 46, no. 10, pp. 4769–4807, 2011.

[2] I. Sarfraz, A. Rasul, F. Jabeen et al., “Fraxinus: a plant with versatile pharmacological and biological activities,” Evidence-Based Complementary and Alternative Medicine, vol. 2017, Article ID 4269868, 12 pages, 2017.

[3] D. J. Newman and G. M. Cragg, "Natural products as sources of new drugs over the period 1981 to 2010," Journal of Natural Products, vol. 75, no. 3, pp. 311–335, 2012.

[4] W. H. Gerwick and A. M. Fenner, "Drug discovery from marine microbes," Microbial Ecology, vol. 65, no. 4, pp. 800–806, 2013.

[5] D. J. Newman, G. M. Cragg, and K. M. Snader, “Natural products as sources of new drugs over the period 1981–2002,” Journal of Natural Products, vol. 66, no. 7, pp. 1022–1037, 2003.

[6] N. Bauereiss, S. Obermaier, S. E. Özüal, and H. Baumeister, "Effects of existential interventions on spiritual, psychological, and physical well-being in adult patients with cancer: Systematic review and meta-analysis of randomized controlled trials," Psycho-Oncology, vol. 27, no. 11, pp. 2531–2545, 2018.

[7] Y. L. Qiu, X. N. Cheng, F. Bai, L. Y. Fang, and H. Z. Hu, "Aucubin protects against lipopolysaccharide-induced acute pulmonary injury through regulating Nrf2 and AMPK pathways," Biomedicine pharmacotherapy = Biomedecine pharmacotherapie, vol. 106, pp. 192–199, 2018.

[8] C. Veeramani, M. A. Alsaiif, and K. S. Al-Numair, "Lavatera critica controls systemic insulin resistance by ameliorating adipose tissue inflammation and oxidative stress using bioactive compounds identified by GC–MS," Biomedicine & Pharmacotherapy, vol. 106, pp. 183–191, 2018.

[9] N. Gampe, A. Darcsi, L. Kursinszki, and S. Béni, "Separation and characterization of homopipeolic acid isoflavonoid ester derivatives isolated from Ononis spinosa L. root," Journal of Chromatography B, vol. 1091, pp. 21–28, 2018.

[10] S. Park, F. W. Bazer, W. Lim, and G. Song, "The O-methylated isoflavone, formononetin, inhibits human ovarian cancer cell
proliferation by sub G0/G1 cell phase arrest through PI3K/AKT and ERK1/2 inactivation,” Journal of Cellular Biochemistry, vol. 119, no. 9, pp. 7377–7387, 2018.

[11] D. Wu, K. Wu, Q. Zhu et al., “Formononetin administration ameliorates dextran sulfate sodium-induced acute colitis by inhibiting nlrp3 inflammasome signaling pathway,” Mediators of Inflammation, vol. 2018, Article ID 3048532, 2018.

[12] Y.-W. Chin, H.-A. Jung, Y. Liu et al., “Anti-oxidant constituents of the roots and stolons of licorice (Glycyrrhiza glabra),” Journal of Agricultural and Food Chemistry, vol. 55, no. 12, pp. 4691–4697, 2007.

[13] J. Dluhošová, J. Lštíněk, J. Nedělková, and J. Řepková, “Red clover (Trifolium pratense) and zigzag clover (T. medium) – a picture of genomic similarities and differences,” Frontiers in Plant Science, vol. 9, p. 724, 2018.

[14] A. Brandli, J. Simpson, and S. Ventura, “Isoflavones isolated from red clover (Trifolium pratense) inhibit smooth muscle contraction of the isolated rat prostate gland,” Phytotherapy Research, vol. 17, no. 11, pp. 895–901, 2010.

[15] T. Wang, Y. Liu, X. Li, Q. Xu, Y. Feng, and S. Yang, “Isoflavones from green vegetable soybean and their antimicrobial and antioxidant activities,” Journal of the Science of Food and Agriculture, vol. 98, no. 5, pp. 2043–2047, 2018.

[16] X. Kong, F. Wang, Y. Niu, X. Wu, and Y. Pan, “A comparative study on the effect of promoting the osteogenic function of osteoblasts using isoflavones from Radix Astragalus,” Phytotherapy Research, vol. 32, no. 1, pp. 115–124, 2018.

[17] J. Hwang, S. A. Lee, S. S. Hong et al., “Monoamine oxidase inhibitory components from the roots of Sophora flavescens,” Archives of Pharmacal Research, vol. 28, no. 2, pp. 190–194, 2005.

[18] T. A. Mansoor, R. M. Ramalho, X. Luo, C. Ramalhete, C. M. P. Rodrigues, and M.-J. U. Ferreira, “Isoflavones as apopto- sis inducers in human hepatoma HuH-7 cells,” Phytotherapy Research, vol. 25, no. 12, pp. 1819–1824, 2011.

[19] S. H. Shim, “20S proteasome inhibitory activity of flavonoids isolated from Spatholobus suberectus,” Phytotherapy Research, vol. 25, no. 4, pp. 615–618, 2011.

[20] Q. Li, Y. Yang, Y. Zhao, D. Gu et al., “Comparative study on separation and purification of isoflavones from the seeds and sprouts of chickpea by HSCCC,” Journal of Liquid Chromatography & Related Technologies, vol. 32, no. 19, pp. 2879–2892, 2009.

[21] C. W. Choi, Y. H. Choi, M.-R. Cha et al., “Yeast α-glucosidase inhibition by isoflavones from plants of leguminosae as an in vitro alternative to acarbose,” Journal of Agricultural and Food Chemistry, vol. 58, no. 18, pp. 9988–9993, 2010.

[22] B. Avula, Y. H. Wang, T. J. Smillie, and I. A. Khan, “Quantitative determination of triterpenoids and formononetin in rhizomes of black cohosh (Actaea racemosa) and dietary supplements by using UPLC-UV/ELS detection and identification by UPLC-MS,” Planta Medica, vol. 75, no. 04, pp. 381–386, 2009.

[23] C. O. D. S. Frozza, D. A. Santos, L. C. Rufatto et al., “Antitumor activity of Brazilian red propolis fractions against Hep-2 cancer cell line,” Biomedicine & Pharmacotherapy, vol. 91, pp. 951–963, 2017.

[24] F. Qiao, X. Jiang, H. Cong, H. Sun, L. Li, and P. Nick, “Cell shape can be uncoupled from formononetin induction in a novel cell line from Callerya speciosa,” Plant Cell Reports, vol. 37, no. 4, pp. 665–676, 2018.

[25] Y. Liu, J. Liu, K.-X. Wu, X.-R. Guo, and Z.-H. Tang, “A rapid method for sensitive profiling of bioactive triterpene and flavonoid from Astragalus mongholicus and Astragalus membranaceus by ultra-pressure liquid chromatography with tandem mass spectrometry,” Journal of Chromatography B, vol. 1085, pp. 110–118, 2018.

[26] X. Ma, P. Tu, Y. Chen, T. Zhang, Y. Wei, and Y. Ito, “Preparative isolation and purification of two isoflavones from Astragalus membranaceus Bge. var. mongholicus (Bge.) Hsiao by high-speed counter-current chromatography,” Journal of Chromatography A, vol. 992, no. 1-2, pp. 193–197, 2003.

[27] Y. J. Park, A. A. Thwe, X. Li et al., “Triterpene and flavonoid biosynthesis and metabolic profiling of hairy roots, adventitious roots, and seedling roots of astragalus membranaceus,” Journal of Agricultural and Food Chemistry, vol. 63, no. 40, pp. 8862–8869, 2015.

[28] M. V. das Neves, T. M. da Silva, E. d. Lima, E. V. da Cunha, and E. d. Oliveira, “Isoflavone formononetin from red propolis acts as a fungicide against Candida sp,” Brazilian Journal of Microbiology, vol. 47, no. 1, pp. 159–166, 2016.

[29] N. L. Booth, C. R. Overk, P. Yao et al., “Seasonal variation of red clover (Trifolium pratense L., Fabaceae) isoflavones and estrogenic activity,” Journal of Agricultural and Food Chemistry, vol. 54, no. 4, pp. 1277–1282, 2006.

[30] D. Benedec, L. Vlase, I. Oniga, A. Toiu, M. Tâmaș, and B. Tiperciuc, “Isoflavonoids from Glycyrrhiza sp. and Ononis spinosa,” Farmacia, vol. 60, no. 5, pp. 615–620, 2012.

[31] L. T. Nguyen, U. T. Nguyen, Y. H. Kim, H. M. Shin, and I. J. Yang, “Astragalus Radix and its compound formononetin amelio- rate diesel particulate matter-induced skin barrier disruption by regulation of keratinocyte proliferation and apoptosis,” Journal of Ethnopharmacology, vol. 228, pp. 132–141, 2019.

[32] K. Kyogoku, K. Hatayama, and M. Komatsu, “ Constituents of chinese crude drug ‘kushen’ (the root of sophora flavescent ait.), isolation of five new flavonoids and formononetin,” Chemical Pharmaceutical Bulletin, vol. 21, no. 12, pp. 2733–2738, 1973.

[33] E. C. N. Nonoo, P. Mkounga, V. Kuete, K. Marat, P. G. Hultin, and A. E. Nkengfack, “Pycnanthulignenes A-D, antimicrobial cyclolignene derivatives from the roots of pycnanthus angolin- sis,” Journal of Natural Products, vol. 73, no. 2, pp. 213–216, 2010.

[34] J. Zhang, L. Liu, J. Wang, B. Ren, L. Zhang, and W. Li, “Formononetin, an isoflavone from Astragalus membranaceus inhibits proliferation and metastasis of ovarian cancer cells,” Journal of Ethnopharmacology, vol. 221, pp. 91–99, 2018.

[35] R. Zhou, H. Chen, J. Chen, X. Chen, Y. Wen, and L. Xu, “Extract from Astragalus membranaceus inhibit breast cancer cells proliferation via PI3K/AKT/mTOR signaling pathway,” BMC Complementary and Alternative Medicine, vol. 18, no. 1, p. 83, 2018.

[36] T. L. Dunlap, C. E. Howell, N. Mukand et al., “Red clover aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) agonists enhance genotoxic estrogen metabolism,” Chemical Research in Toxicology, vol. 30, no. 11, pp. 2084–2092, 2017.

[37] J. Chen, X. Zhang, Y. Wang, Y. Ye, and Z. Huang, “Formononetin promotes proliferation that involves a feedback loop of microRNA-375 and estrogen receptor alpha in estrogen receptor-positive cells,” Molecular Carniogenesis, vol. 55, no. 3, pp. 312–319, 2016.
human breast cancer by calycosin and formononetin,” *Cellular Physiology and Biochemistry*, vol. 32, no. 6, pp. 1790–1797, 2013.

[40] J. Chen and L. Sun, “Formononetin-induced apoptosis by activation of Ras/p38 mitogen-activated protein kinase in estrogen receptor-positive human breast cancer cells,” *Hormone and Metabolic Research*, vol. 44, no. 13, pp. 943–948, 2012.

[41] A. L. Wang, Y. Li, Q. Zhao, and L. Q. Fan, “Formononetin inhibits colon carcinoma cell growth and invasion by miRNA-p498-mediated TGFβ3 downregulation and inhibition of PI3K/AKT and STAT3 signaling pathways,” *Molecular Medicine Reports*, vol. 17, no. 6, pp. 7721–7729, 2018.

[42] H. Lee, D. Lee, K. S. Kang, J. H. Song, and Y.-K. Choi, “Inhibition of intracellular ROS accumulation by formononetin attenuates cisplatin-mediated apoptosis in LLC-PK1 cells,” *International Journal of Molecular Sciences*, vol. 19, no. 3, 2018.

[43] X. R. He, S. Y. Han, X. H. Li, W. X. Zheng, L. N. Pang et al., “Chinese medicine Bu-Fei decoction attenuates epithelial-mesenchymal transition of non-small cell lung cancer via inhibition of transforming growth factor beta signaling pathway in vitro and in vivo,” *Journal of Ethnopharmacology*, vol. 204, pp. 45–57, 2017.

[44] Y. Yang, Y. Zhao, X. Ai, B. Cheng, and S. Lu, “Formononetin suppresses the proliferation of human non-small cell lung cancer through induction of cell cycle arrest and apoptosis,” *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 12, pp. 8453–8461, 2014.

[45] Y. Wu, X. Zhang, Z. Li, H. Yan, J. Qin, and T. Li, “Formononetin inhibits human bladder cancer cell proliferation and invasiveness via regulation of miR-21 and PTEN,” *Food & Function*, vol. 8, no. 3, pp. 1061–1066, 2017.

[46] D. Fu, L. Zhang, J. Song et al., “Design and synthesis of formononetin-dithiocarbamate hybrids that inhibit growth and migration of PC-3 cells via MAPK/Wnt signaling pathways,” *European Journal of Medicinal Chemistry*, vol. 127, pp. 87–99, 2017.

[47] X. Liu, Y. Li, Q. Chen, S. Xiao, and S. Zeng, “Up-regulating of RAS and apoptosis of DU-145 human prostate cancer cells induced by formononetin in vitro,” *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 6, pp. 2835–2839, 2014.

[48] W. Huang, L. Bi, Z. Li, X. Zhang, and Y. Ye, “Formononetin induces the mitochondrial apoptosis pathway in prostate cancer cells via downregulation of the IGF-1/IGF-1R signaling pathway,” *Pharmaceutical Biology*, vol. 52, no. 4, pp. 466–470, 2014.

[49] T. Li, X. Zhao, Z. Mo et al., “Formononetin promotes cell cycle arrest via downregulation of akt/cyclin D1/CDK4 in human prostate cancer cells,” *Cellular Physiology and Biochemistry*, vol. 34, no. 4, pp. 1351–1358, 2014.

[50] Y. H. Guo, Y. Wang, and M. Xin, “Low concentration of formononetin stimulates the proliferation of nasopharyngeal carcinoma cell line CNE2 by upregulating bcl-2 and p-ERK1/2 expression,” *Pharmaceutical Biology*, vol. 54, no. 9, pp. 1841–1846, 2016.

[51] Q. Hu, J. Yu, W. Yang et al., “Identification of flavonoids from Flammulina velutipes and its neuroprotective effect on pheochromocytoma-12 cells,” *Food Chemistry*, vol. 204, pp. 274–282, 2016.

[52] C. Kim, S. Lee, W. M. Yang et al., “Formononetin-induced oxidative stress abrogates the activation of STAT3/5 signaling axis and suppresses the tumor growth in multiple myeloma preclinical model,” *Cancer Letters*, vol. 431, pp. 123–141, 2018.

[53] W. Hu and Z. Xiao, “Formononetin induces apoptosis of human osteosarcoma cell line U2OS by regulating the expression of Bcl-2, Bax and MiR-375 in vitro and in vivo,” *Cellular Physiology and Biochemistry*, vol. 37, no. 3, pp. 933–939, 2015.

[54] Q. Liu, Y. Sun, J.-M. Zheng et al., “Formononetin sensitizes glioma cells to doxorubicin through preventing EMT via inhibition of histone deacetylase 5,” *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 6, pp. 6434–6441, 2015.

[55] J. Chen, B. Ge, Y. Wang, Y. Ye, S. Zeng, and Z. Huang, “Biochanin A promotes proliferation that involves a feedback loop of microRNA-375 and estrogen receptor alpha in breast cancer cells,” *Cellular Physiology and Biochemistry*, vol. 35, no. 2, pp. 639–646, 2015.

[56] J.-H. Wu, Q. Li, M.-Y. Wu et al., “Formononetin, an isoflavone, relaxes rat isolated aorta through endothelium-dependent and endothelium-independent pathways,” *The Journal of Nutritional Biochemistry*, vol. 21, no. 7, pp. 613–620, 2010.

[57] D. Yu, Y. Duan, Y. Bao, C. Wei, and L. An, “Isoflavonoids from Astragalus mongholicus protect PC12 cells from toxicity induced by l-glutamate,” *Journal of Ethnopharmacology*, vol. 98, no. 1-2, pp. 89–94, 2005.

[58] G. Li, M. Yang, X. Hao, C. Li, Y. Gao, and J. Tao, “Acute toxicity of sodium formononetin-3-sulphonate (Sul-F) in Sprague-Dawley rats and Beagle dogs,” *Regulatory Toxicology and Pharmacology*, vol. 73, no. 2, pp. 629–633, 2015.

[59] W. Wang, Y. Tanaka, Z. Han, and C. M. Higuchi, “Proliferative response of mammary glandular tissue to formononetin,” *Nutrition and Cancer*, vol. 23, no. 2, pp. 131–140, 1995.

[60] B. L. Pool-Zobel, H. Adlerecreutz, M. Glei et al., “Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells,” *Carcinogenesis*, vol. 21, no. 6, pp. 1247–1252, 2000.

[61] M. Khan, A. Maryam, H. Zhang, T. Mehmood, and T. Ma, “Killing cancer with platycodin D through multiple mechanisms,” *Journal of Cellular and Molecular Medicine*, vol. 20, no. 3, pp. 389–402, 2016.

[62] A. Rasul, F. M. Millimouno, W. Ali Eltayb, M. Ali, J. Li, and X. Li, “Pinocembrin: a novel natural compound with versatile pharmacological and biological activities,” *BioMed Research International*, vol. 2013, Article ID 379850, 9 pages, 2013.

[63] S. Dall’acqua, “Natural products as antimitic agents,” *Current Topics in Medicinal Chemistry*, vol. 14, no. 20, pp. 2272–2285, 2014.

[64] D. S. Rawat and R. Singh, “Plant derived secondary metabolites as anti-cancer agents,” *Anti-Cancer Agents in Medicinal Chemistry*, vol. 13, no. 10, p. 1551, 2013.

[65] L. Yu, R. Ma, Y. Wang, and H. Nishino, “Potent anti-tumor activity and low toxicity of tubemiside I isolated from Bolbostemma paniculatum,” *Planta Medica*, vol. 60, no. 03, pp. 204–208, 1994.

[66] L. Navrátilová, L. Applová, P. Horký, P. Mladěnka, P. Pavek, L. Navrátil, L. Máša, V. Marleta, and A. Korpita, “Biochanin A promotes proliferation that involves a feedback loop of microRNA-375 and estrogen receptor alpha in breast cancer cells,” *European Journal of Pharmacology*, vol. 204, pp. 274–282, 2016.
