A Pharmacological Overview of Alpinumisoflavone, a Natural Prenylated Isoflavonoid

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Over the last decade, several studies demonstrated that prenylation of flavonoids enhances various biological activities as compared to the respective nonprenylated compounds. In line with this, the natural prenylated isoflavonoid alpinumisoflavone (AIF) has been explored for a number of biological and pharmacological effects (therapeutic potential). In this review, we summarize the current information on health-promoting properties of AIF. Reported data evidenced that AIF has a multitherapeutic potential with antosteoporotic, antioxidant and anti-inflammatory, antimicrobial, anticancer, estrogenic and antiestrogenic, antidiabetic, and neuroprotective properties. However, research on these aspects of AIF is not sufficient and needs to be reevaluated using more appropriate methods and methodology. Further series of studies are needed to confirm these pharmacological effects, and this review should lay the basis for the design of respective investigations. Overall, despite the drawbacks of studies recorded, AIF exhibits a potential as drug candidate.

Keywords: alpinumisoflavone, therapeutic potential, natural product, prenylated isoflavonoid, structure–activity relationship

INTRODUCTION

In the drug discovery process, plants still remain an invaluable source of drugs and drug leads. They possess enormous structural and chemical diversity that is not matched by any synthetic libraries of small molecules (Shen, 2015). As pharmacological activities of chemicals are generally structure dependent, the structural and chemical diversity is obviously an advantage. Over the last decade, the interest in (iso) flavonoids strongly increased. Especially the prenylated forms moved into the focus because of their versatile and promising pharmacological properties and health benefits on multitarget tissues (Kumar and Pandey, 2013; Chen et al., 2014). Prenylated isoflavonoids have increased lipophilicity as compared to nonprenylated forms, leading to high affinity with cell membranes and enhanced biological activities or significant pharmacological effects (Chen et al., 2014; Sherif et al., 2015). These compounds offer a multitude of biological activities, which justify major and much deeper pharmacological investigation (Botta et al., 2009). Accordingly, there is a recent in-depth investigation of prenylated flavonoids as promising anticancer, anti-inflammatory, antioxidant, and neuroprotective nutraceuticals (Yang et al., 2015; Venturelli et al., 2016), with the prenyl substituent playing a key role in the molecular activity. Prenylated flavonoids are found predominantly in the Leguminosae family, although the
phenyl-propanoid pathway—necessary for their production—is ubiquitously present in plants including nonleguminous families (Reynaud et al., 2005; Lapčík, 2007).

Alpinumisoflavone (AIF) or [5-hydroxy-7-(p-hydroxyphenyl)-2,2-dimethyl-2H-6H-benzo-[1,2-b:5,4-b]dipyran-6-one] is a dimethylpyrano derivative prenylated at ring A of genistein (Figure 1). It is a major constituent of Derris eriocarpa F.C. How, commonly referred as “Tugancao” in “Zhuang” and “Dai” ethnomedicine in Guangxi and Yunnan Province of China (Guangxi Institute of Chinese Medicine, 1986). A high content of AIF was reported in fully mature fruits (mandarin melon berry) of Cudrania tricuspidata Bur. ex Lavallee (syn. Maclura tricuspidata Carrière) (Shin et al., 2015), a crop cultivated in East Asia (Xiong et al., 1993; Shi, 2010), Europe and America (Markovski, 2016) for its fruits and timber, and with an immense medicinal and economic value (Xin et al., 2017). Isolated for the first time by Jackson et al. (1971), AIF was identified in many medicinal plants widely used over the world (Table 1). Although data depicted in this table are not exhaustive, the global trend is in accordance with Botta et al. (2005) who reported that prenylated flavonoids occur mostly in Leguminosae and Moraceae, with few detected in other families. Over the last two decades, the body of literature of AIF and its pharmacological potential is steadily growing. This review summarizes and gives a critical look on the current knowledge of the biological activities, therapeutic potential, and mechanism of action of AIF.

**PHARMACOLOGICAL ACTIVITIES**

Over the last decade research indicates that prenylation usually renders (iso)flavonoids with improved bioactivities (Yang et al.,...
| Family       | Plant species          | Plant parts       | Origin/city (country) | Biological and/or pharmacological activity                                                                 | References                      |
|--------------|------------------------|-------------------|-----------------------|-----------------------------------------------------------------------------------------------------------|---------------------------------|
| **Leguminosae** | **Crotalaria bracteata** | Roots and stems   | Roi-Et (Thailand)     | Cytotoxicity against MCF-7 and NCI-H187 cell lines (inactive)                                              | Sudanich et al., 2017          |
|              | **Erythrina caffra**    | Stem bark         | KwaZulu-Natal (South Africa) | Anti-bacterial activity against Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia and Escherichia coli | Chukwujeukwu et al., 2011      |
|              | **Erythrina indica**    | Stem bark         | Ibadan (Nigeria)      | Cytotoxicity against KB cells                                                                             | Nkengfack et al., 2001          |
|              | **Erythrina lystemon**  | Stem bark         | –                     | Estrogen-like effects in a menopause model of ovariectomized Wistar rats                                  | Mvondo et al., 2011; Mvondo et al., 2012; Mvondo et al., 2015 Na et al., 2006 |
|              | **Erythrina mildbraedii** | Root bark       | Buea (Cameroon)      | Inhibition of protein tyrosine phosphatase-1B (PTP1B)                                                    | Tjahjandarie and Tanjung, 2015a |
|              | **Erythrina orientalis** | Stem bark         | Kunir Kidul (Indonesia) | Cytotoxicity against murine leukemia P-388 cells Radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) | Tjahjandarie and Tanjung, 2015b |
|              | **Erythrina ovalifolia** | Stem bark         | Kunir Kidul (Indonesia) | Antiplasmodial activity against Plasmodium falciparum                                                   | Dijoge et al., 2009; Dijoge et al., 2010 |
|              | **Erythrina poeppigiana** | Stem bark         | Sancta Cruz (Bolivia) | Estrogen-like effect in U2OS human osteosarcoma cells through ERs-dependent reporter gene activity         | Lee et al., 2009a; Oh et al., 2005 |
|              | **Erythrina senegalensis** | Stem bark         | Foumban (Cameroon)    | Inhibition of the HIV-1 Protease Phospholipase Cy1inhibitory activity (inactive) Inhibition of acyl CoA-diacylglycerol acyltransferase | Lee et al., 2009a; Oh et al., 2009 |
|              | **Erythrina stricta**   | Stem bark         | Nagaland (India)      | Antimicrobial and radical scavenging (DPPH) activities                                                  | Akter et al., 2016              |
|              | **Erythrina suberosa**  | Stem bark         | Jammu (India)         | Cytotoxicity against human myeloid leukemia cell lines HL-60 and K-562 and T lymphoblastic cell line MOLT-4 | Kumar et al., 2013              |
|              | **Erythrina variegata** | Stem bark         | Dhaka (Bangladesh)    | Apoptotic potential in HL-60 cells Radical scavenging (DPPH) activity                                    | Rahman et al., 2010             |
| **Leguminosae** | **Dennis eriocarpa**    | –                 | –                     | Inhibition of osteoclast differentiation in vitro and antiosteoporotic effect in ovariectomized mice Suppression of tumor growth and metastasis of clear-cell renal cell carcinoma | Cong et al., 2017; Wang et al., 2017a |
|              | **Laburnum alpinum**   | Twigs             | Salford (England)     | –                                                                                                         | Jackson et al., 1971           |
|              | **Lonchocarpus glabrescens** | –               | Punchana, (Peru)     | Inhibition of the hypoxia-inducible factor-1 (HIF-1) activation in human breast tumor T47D cells No estrogenic activity on the β-galactosidase activity in a yeast two-hybrid assay | Liu et al., 2009; Okamoto et al., 2006; Ito et al., 2000 |
|              | **Milletia pachycarpa** | Stem and leaves   | –                     | Inhibition of the Epstein–Barr virus activation with no cytotoxicity against Raj cells Anti-fungal activity against wild-type Candida albicans and the reference strain ATCC18804C Antischistosomal activity against Schistosoma mansoni | Ayine-Tora et al., 2016; Lyddiard et al., 2002; Khalid et al., 1986 |
|              | **Milletia taiwaniana** | Twigs and leaves  | (Singapore)           | –                                                                                                         |                                 |
|              | **Millettia thonningii**| Seeds             | Legon-Accra (Ghana)   | Antifungal activity against wild-type Candida albicans and the reference strain ATCC18804C Antischistosomal activity against Schistosoma mansoni |                                |
|              | **Sophora moorcroftiana** | Aerial parts     | (Ghana)               | Antibacterial effects on Metcillin-resistant Staphylococcus aureus Anti-proliferative activity against leukemia [CCRFCEM, MOLT-4, and HL-60(TB)], renal SN12C, and breast MCF-7 cancer cells Anti-inflammatory activity in carrageenan-induced rat paw edema model | Wang et al., 2014; Amen et al., 2013 |
|              | **Tipuana tipu**       | Leaves            | Mansoura (Egypt)      |                                                                                                           |                                 |

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2015; Mukai, 2018), suggesting that prenylated compounds have a higher potential to be developed and utilized (Chen et al., 2014). Focusing on AIF, the following activities have been demonstrated and claimed to be promising by the authors (Table 2).

### Estrogenic and Antiestrogenic Activities

Estrogenic plant-derived products act via binding to human estrogen receptors (ERs). AIF was found to be a weak ERα and ERβ binder with conflicting results concerning the preference for ERβ versus ERα (Djioque et al., 2009; Magne Nde et al., 2012; Mvondo et al., 2012). The authors used the same estrogen receptor competitor assay based on fluorescence polarization in the same laboratory and according to the instructions of the same manufacturer. The discrepancies can probably be ascribed to the purity of compound. The ER competitive ligand binding assay cannot distinguish between estrogenic and antiestrogenic substances and does not provide insight into the ability of a substance to initiate the molecular cascade leading to altered gene expression (Legler et al., 1999). To overcome this disadvantage, reporter gene assays such as the ER-mediated chemically activated luciferase gene expression assay (ER-CALUX) and the yeast estrogen screen (YES) based on stably transfected cell lines are usually applied. In an ER-CALUX assay using human osteosarcoma U2OS cells stably transfected with ERα and transiently transfected with ERβ, AIF stimulated the endogenous reporter gene, β-galactosidase (Okamoto et al., 2006). Although ER-CALUX and YES assays rely on the same principle and use the same receptors, the yeast cell wall is usually less permeable to compounds compared to mammalian cell membranes (Legler et al., 1999). This makes the ER-CALUX assay robust, more sensitive and more predictable than the YES assay (Leusch et al., 2010). In MCF-7 cells, AIF upregulated ERα target genes such as proliferating cell nuclear antigen (PCNA), cyclin D1, cyclin E1, cMyc (myelocytomatosis viral oncogene homologue), and liver receptor homologue 1 (LRH-1), and downregulated growth pathway in the presence or absence of E2 is not promising as ERβ is known to counteract the proliferative responses of ERα involved in estrogen-related cancers, osteoporosis, and cardiovascular diseases.

In in vivo studies AIF induced estrogen-like effects by increasing uterine wet weight as well as uterine and vaginal epithelial height in ovariectomized Wistar rats (Mvondo et al., 2011, Mvondo et al., 2012). In this model, AIF also reduced the hot flush index by increasing the FSH/LH ratio. It displayed atheroprotective effects by an augmentation of HDL-cholesterol levels, a reduction in the atherogenic index of plasma (Legler et al., 2011), and by upregulating the expression of estrogen-sensitive genes associated with bile acid formation (Cyp7a1) (Mvondo et al., 2015). Taken together, the in vitro and in vivo systems/models used to study estrogenic effects of AIF are quite suitable. The investigations demonstrated that AIF,

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### TABLE 1 | Continued

| Family      | Plant species               | Plant parts           | Origin/city (country) | Biological and/or pharmacological activity                                                                 | References            |
|-------------|----------------------------|-----------------------|-----------------------|-----------------------------------------------------------------------------------------------------------|-----------------------|
| Moraceae    | Chlorophora tinctoria      | Leaves and twigs      | Maynas (Peru)         | Fatty acid synthase inhibitory and antifungal activities (inactive)                                        | Li et al., 2002       |
|             | (L.) Gaud.                 |                       |                       |                                                                                                            |                       |
|             | Cudrania tricuspidata      | Fruits                | Cheongju, (Korea)     | Inhibition of the mouse brain monoamine oxidase activity                                                  | Han et al., 2005      |
| (Karr.)     |                            |                       |                       |                                                                                                            |                       |
|             | Ficus bengalensis          | Aerial roots          | Sahiwal (Punjab, India)| Tyrosinase inhibition                                                                                     | Zheng et al., 2013    |
|             | Ficus benjamina var. nuda  | Fruits                | Honolulu (Hawaii)     | –                                                                                                          | Riaz et al., 2012     |
| (Mcq.) Barrett |                         |                       |                       |                                                                                                            | Dai et al., 2012      |
|             | Ficus chlamydocarpa        | Root bark             | Bahohan (Cameroon)    | Antimycobacterial, antibacterial and antifungal activities                                                | Kuete et al., 2008    |
| Mildbraed and Burrett |                     |                       |                       |                                                                                                            |                       |
|             | Ficus glumosa              | Stem bark             | Makenene (Cameroon)   | Cytotoxicity against prostate cancer PC-3 cell line                                                      | Nana et al., 2012     |
|             | Ficus nervosa              | Fruits                | Pingtung (Taiwan)     |                                                                                                            | Chen et al., 2010     |
| Heyne ex Roth. |                         |                       |                       | Inhibition of protein tyrosine phosphatase-1B (PTP1B)                                                    | Trinh et al., 2017    |
|             | Ficus racemosa             |                       |                       | Radical scavenging (DPPH) and α-glucosidase inhibitory activities                                        | Fu et al., 2018       |
|             | Ficus tikouva Bur          | Rhizomes              | (Vietnam)             | Cytotoxicity against human neuroblastoma SH-SYSY cell line                                              | Hong et al., 2018     |
| Maclura tricuspidata |                         |                       |                       |                                                                                                            |                       |
| Carrière (syn. | Fruits                     |                       | Jinju (South Korea)   |                                                                                                            |                       |
| Cudrania tricuspidata |                     |                       |                       |                                                                                                            |                       |
| Dilleniaceae | Tetracea scandens          | Branch                | –                     | Glucose-uptake induced activity in basal and insulin-stimulated L6 myotubes                               | Lee et al., 2009b     |
|             | Azorella madreporica       | Whole plant           | Valde Nevado (Chile)  | Antimycobacterial and antibacterial activities (inactive)                                               | San-Martin et al., 2015|
| Apiceae     |                            |                       |                       |                                                                                                            |                       |

Note: –, not indicated.


| Pharmacological activities | Experimental model | Dose/concentration | Mechanism of action | References |
|----------------------------|--------------------|--------------------|---------------------|------------|
| Estrogenic activity        | ER competitor binding assay | | Weak ERα and ERβ binder; higher selectivity for ERα | Mvondo et al., 2012; Magne Nde et al., 2012 |
|                           | ER competitor binding assay | | Weak ERα and ERβ binder; higher selectivity for ERβ | Djogue et al., 2009 |
|                           | U2OS-ERα, U2OS-ERβ human osteosarcoma cells | 10⁻⁸–10⁻⁶ M | Induction of luciferase reporter gene activity | Djogue et al., 2010; Magne Nde et al., 2012 |
|                           | MCF-7 breast cancer cells | 10⁻⁸–10⁻⁶ M | Up-regulation of the expression of estrogen α receptor target genes PCNA, cyclin D1, cyclinE1, cMyc, and LRH-1; downregulation of GREB1 at 10⁻⁹ M | Magne Nde et al., 2012 |
|                           | Ovariectomized Wistar rats | 0.01, 0.1, and 1 mg/kg daily for 3 days i.p. | Increase in uterine wet weight, and uterine and vaginal epithelial height | Mvondo et al., 2012 |
|                           | Ovariectomized Wistar rats | 1, 10 mg/kg daily for 28 days i.p. | Increase in uterine and vaginal epithelial height; increase in FSH/LH ratio; reduction in atherogenic risks | Mvondo et al., 2011 |
|                           | Ovariectomized Wistar rats | 0.1, 1, and 10 mg/kg daily for 3 days i.p. | Down-regulation of Esr1 mRNA expression; upregulation of Cyp7a1 mRNA expression. | Mvondo et al., 2015 |
| Antiosteoporotic activity  | RAW264.7 osteoclast precursor | 2.5 and 5 μM | Suppression of osteoclast differentiation and proliferation by inhibiting RANKL-induced p38, ERK and JNK activation | Cong et al., 2017 |
|                           | Ovariectomy-induced osteoporosis | 10, 25 mg/kg daily for 6 weeks p.o. | Prevention of OVX-induced bone loss by increasing BV/TV ratio, Tb.Th and Tb.N while decreasing Tb.Sp in OVX mice | Cong et al., 2017 |
|                           | Dexamethasone-induced osteoporosis | 20, 40 mg/kg daily for 8 weeks p.o. | Increase in bone mineral density and mineral content of the proximal femur bone in rats; increase in BV/TV ratio, Tb.Th and Tb.N; decrease in Tb.Sp | Wang et al., 2017b |
|                           | MC3T3-E1 and MLO-Y4 osteoblasts and osteocytes | 5–20 μM | Reverse of proapoptotic and antiproliferative effects of dexamethasone via suppressing Nox2-dependent ROS generation | Wang et al., 2017b; Yin et al., 2018 |
| Antioxidant activity       | DPPH assay | IC₅₀: 8.30 μg/ml, IC₅₀: 708.5 μM, IC₅₀: 54.80 μg/ml, IC₅₀: 54.02 μg/ml | DPPH scavenging activity of differing degree | Rahman et al., 2010; Tjahjandarie and Tanjung, 2015a; Fu et al., 2018; Börquez et al., 2013; Börquez et al., 2013; Li et al., 2018 |
|                           | Ferric Reducing Antioxidant Power (FRAP) assay | 35.55 μM trolox equivalents/1.5 mM, 5, 10 μg/ml | Free radical-scavenging activity | Li et al., 2018 |
|                           | LPS-stimulated RAW264.7 cells | | Increase in the FRAP reducing power | |
| Anti-inflammatory activity  | LPS-stimulated acute lung injury in mice | 1, 5, 10 mg/kg i.p. 1 h before LPS challenge | Alleviated lung lesions, pulmonary edema, and hemorrhages: inhibition of myeloperoxidase activity | Li et al., 2018 |
|                           | LPS-stimulated RAW264.7 cells | 5, 10 μg/ml | Decreased production of TNF-α, IL-6, IL-1β, ICAM-1, and NO; suppression of NF-κB, MAPKs, and NLRP3 pathways | Li et al., 2018 |
|                           | Carrageenan-induced rat paw edema | 25 mg/kg i.p. 30 min before λ-carrageenan (unique dose) | Inhibition of edema formation | Amen et al., 2013 |
| Antimicrobial activity     | Mycobacterium smegmatis MC2 155 Enterobacter cloacae LMP1104G Escherichia coli LMP0101U Morganella morgani LMP0904G Proteus mirabilis LMP0504G Staphylococcus aureus LMP0206U Bacillus stearothermophilus LMP0104G | | Growth inhibition | Kuete et al., 2008 |
|                           | Staphylococcus epidermidis LMP0110G Lactobacillus casei LMP0304G Microbacterium luteus LMP0404G | | Growth inhibition | Kuete et al., 2008 |

(Continued)
### Table 2: Pharmacological Activities of Alpinumisoflavone

| Pharmacological activities | Experimental model                                          | Dose/concentration | Mechanism of action                                                                 | References                        |
|----------------------------|-------------------------------------------------------------|--------------------|-------------------------------------------------------------------------------------|-----------------------------------|
| **Antimicrobial activity** | *Candida albicans* wild type                                | MIC = 0.25 μg/ml   | Growth inhibition                                                                   | Ayine-Tora et al., 2016          |
|                            | ATCC18804                                                  | MIC = 0.50 μg/ml   |                                                                                     | Sathishkumar and Tharani, 2017   |
|                            | 3D structure of CdsD protein of Chlamydial T3SS            |                    |                                                                                     |                                   |
|                            | *Bacillus subtilis* ATCC6051                               | MIC = 3.9 μg/ml    | Growth inhibition                                                                   | Chukwujekwu et al., 2011         |
|                            | *Staphylococcus aureus* ATCC12600                          |                    |                                                                                     |                                   |
|                            | *Klebsiella pneumoniae* ATCC 13883                         |                    |                                                                                     |                                   |
|                            | Escherichia coli ATCC11775                                 |                    |                                                                                     |                                   |
|                            | *Staphylococcus aureus* SA1199B                            | MIC = 64 μg/ml     | Growth inhibition                                                                   | Wang et al., 2014                |
|                            | *Staphylococcus aureus* R4220                              | MIC = 128 μg/ml    |                                                                                     |                                   |
|                            | *Staphylococcus aureus* EMRSA-15                           | MIC = 128 μg/ml    |                                                                                     |                                   |
|                            | *Staphylococcus aureus* XU212                              | MIC = 128 μg/ml    |                                                                                     |                                   |
|                            | *Staphylococcus aureus* EMRSA-16                           | MIC = 128 μg/ml    |                                                                                     |                                   |
|                            | *Staphylococcus aureus* ATCC25923                          | MIC = 128 μg/ml    |                                                                                     |                                   |
| **Antimicrobial activity** | *Staphylococcus aureus* MISSA                              | MIC = 15 μg/ml     | Growth inhibition                                                                   | Akter et al., 2016               |
|                            | *Staphylococcus aureus* MRSA                              | MIC = 30 μg/ml     |                                                                                     |                                   |
|                            | *Staphylococcus aureus* MDRSA                             | MIC = 30 μg/ml     |                                                                                     |                                   |
| **Anticancer activity**    | KB oral epidermoid carcinoma cells                         | ED_{50} = 4.13 μg/ml | Inhibition of cell proliferation                                                    | Nkengfack et al., 2001           |
|                            | P-388 leukemia cells                                       | IC_{50} = 4.31 μg/ml | Inhibition of cell proliferation                                                    | Tjahjandarie and Tanjung, 2015a |
|                            | HL-60, MOLT-4, K-562 leukemia cells                        | 50 μM              | Inhibition of cell proliferation; induction of apoptosis via both intrinsic and extrinsic pathways (activation of caspase-3, -8, -9; PARP cleavage; release of cytochrome c; Bax; downregulation of Bcl-2 expression) and inhibition of NF-kB (p65)/Stat3 tango in HL-60 cells | Kumar et al., 2013               |
|                            | Full NCI 60 cell panel                                     | 10^{-5} M          | Inhibition of proliferation of CCRF-CEM, MOLT-4, and HL-60(TB) leukemia cells, SN12C renal cancer cells and MCF7 breast cancer cells | Amen et al., 2013                |
|                            | H2108, H1299, MRC-5 lung cancer cells; LPS-stimulated RAW264.7 cells | 30, 60 μM          | Inhibition of cell viability; induction of apoptosis (activation of caspase 3/7; repression of AP-1 and NF-kB-dependent transcription; inhibition of ERK/MAPK pathway); Suppression of (LPS)-induced NO production | Namkoong et al., 2011            |
|                            | Eca109, KYSE30 esophageal squamous carcinoma cells (ESCC); Eca109 xenograft mouse model | 5, 10, 20 μM; 20 mg/kg daily for 20 days | Inhibition of cell proliferation; increase in radiosensitivity of ESCC; enhanced irradiation-induced DNA damage, apoptosis, G2/M cell cycle arrest; increase in irradiation-induced ROS generation by suppressing Nrf2 and target genes HO-1 and NQO-1; in vivo suppression of tumor growth and expression of Ki-67 and PCNA; more profound in combination with irradiation | Zhang et al., 2017               |

(Continued)
### TABLE 2 | Continued

| Pharmacological activities | Experimental model | Dose/concentration | Mechanism of action | References |
|---------------------------|--------------------|--------------------|---------------------|------------|
| **Anticancer activity** | 786-O, RCC4 clear-cell renal cell carcinoma (ccRCC); 786-O xenograft mouse model | 2.5, 5, 10 µM; 40, 80 mg/kg daily for 24 days | Suppression of cell growth; induction of apoptosis; inhibition of cell invasion; increased miR-101 expression; repression of RLIP76 expression; inhibition of Akt in vivo suppression of tumor growth and pulmonary metastasis | Wang et al., 2017a |
| | HCT-116, SW480 colorectal cancer (CRC) cells; HCT-116 xenograft mouse model | 5, 10 µM; 25, 50 mg/kg daily for 24 days i.p. | Inhibition of cell proliferation; induction of apoptosis; increased DNA double-strand breaks by inhibiting DNA repair via RAD51 downregulation; suppression of CRC tumor growth without adverse effects on normal tissues; downregulation of in situ levels of Ki-67, Bcl-2 and RADS1; increased cleaved caspase-3 and Bax in tumor tissues | Li et al., 2019 |
| | PC-3 prostate cancer cells | IC_{50} > 30 µM | Inhibition of cell proliferation | Nana et al., 2012 |
| | A375, SK-MEL-1 melanoma cells; B16-F10 mouse model of lung metastasis | 5, 10 µM; 20, 50 mg/kg daily for 24 days (intragastric route) | Inhibition of cell proliferation; impaired metastatic potential by downregulating COX-2 via the miR-124/SPHK 1 axis; decreased number of lung metastases; decreased COX-2 and SPHK1 expression and increased miR-124 expression in metastatic tissues | Gao et al., 2017 |
| | EC9706, KYSE30 ESCC cell lines; KYSE30 xenograft mouse model | 10, 20 µM; 50, 100 mg/kg daily for 30 days | Suppression of cell proliferation and tumor growth; induction of apoptosis by upregulating the miR-370/PIM1 signaling | Kuete et al., 2016 |
| | MDA-MB-231-pcDNA3, MDA-MB-231- BCRP clone 23 breast cancer cells | Moderate inhibition of cell proliferation (degree of resistance = 0.62); induction of GO/G1 cell cycle arrest and apoptosis in CCRF-CEM cells through caspase 3/7 activation, mitochondrial membrane potential loss, and ROS production | Kuete et al., 2016 |
| | HCT116 (p53+/+), HCT116 (p53−/−) colon cancer cells | Moderate inhibition of cell proliferation (degree of resistance = 0.86) | Kuete et al., 2016 |
| | U87MG, U87MG.ΔEGFR glioblastoma cells | Moderate inhibition of cell proliferation (degree of resistance = 0.90) | Kuete et al., 2016 |
| | T47D, MDA-MB-231 breast cancer cells | Inhibition of hypoxia-induced and iron chelator-induced HIF-1 activation in T47D cells; inhibition of MDA-MB-231 cell migration and chemotaxis | Liu et al., 2009 |
| | α-glucosidase | IC_{50} = 73.3 µM | Inhibition of α-glucosidase activity | Fu et al., 2018 |
| | Protein tyrosine phosphatase-1B (PTP1B) | IC_{50} = 42.0 µM | Inhibition of PTP1B activity | Na et al., 2006 |
| | PTP1B | IC_{50} = 21.2 µM | Inhibition of PTP1B activity | Trinh et al., 2017 |
| | L6 myotubes; PTP1B | 1, 10, 25 µM | Stimulation of basal and insulin-treated glucose-uptake in L6 myotubes by increasing AMPK activation, glucose transporters mRNA expression; moderate inhibition of PTP1B (IC_{50} ~ 37.52 µM) | Lee et al., 2009b |
| | Acyl-CoA:diacylglycerol acyltransferase (DGAT) | 12.5 µg/ml | Inhibition of DGAT activity | Oh et al., 2009 |
| **Antidiabetic activity** | Monoamine oxidases (MAOs) | IC_{50} = 25.8, 52.6, 16.8 µM, respectively | Inhibition of mixed mouse total brain MAO, MAO-A and MAO-B activity | Han et al., 2005 |
| | SH-SY5Y neuroblastoma cells | IC_{50} > 25 µM | Attenuation of 6-hydroxydopamine-induced neurotoxicity and ROS generation | Kim et al., 2017 |
through activation of ERs and modulation of estrogen-sensitive genes, exhibited estrogenic activities on uterus and vagina and by influencing several factors reduced the atherogenic risk. Nevertheless, further in vivo studies are necessary to get deeper insight into its potential.

**Antiesteoporotic Activity**

Isoflavonoids are increasingly considered as a promising first-line prophylaxis for osteoporosis in clinical settings (Ma et al., 2008; Lambert and Jeppesen, 2018). The therapeutic strategies globally emphasize the inhibition of “osteoclast-mediated bone resorption” and/or the prevention of the apoptosis of osteoblasts and osteocytes.

Using experimental protocols of postmenopausal or glucocorticoid-induced osteoporosis, AIF exhibited an antiosteoporotic activity both in vitro and in vivo. A 6-week oral treatment with AIF (10 and 25 mg/kg) prevented ovariectomy-induced osteoporosis in mice by suppressing osteoclast differentiation (Cong et al., 2017). Despite its limits (Egermann et al., 2005; Lelovas et al., 2008), the ovariectomized rat/mouse model is the most widely used animal model in research on postmenopausal osteoporosis. Long-term exposure to glucocorticoids, e.g., in the treatment of chronic autoimmune and pulmonary disorders, cancers of the lymphoid system, as well as in the prevention of transplant rejection (Oakley and Cidlowski, 2013), is the primary cause of the secondary osteoporosis (Tanaka, 2014). In dexamethasone-induced osteoporosis in rats, 20 and 40 mg/kg AIF p.o. prevented bone loss (Wang et al., 2017b). In vitro, 5–20 μM AIF abrogated the dexamethasone-induced cytotoxicity and proapoptotic effects on osteoblasts and osteocytes (MC3T3-E1 and MLO-Y4 cells) through the inhibition of ROS production as well as through the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and AMPK-dependent NAD(P)H oxidase 2 (Nox2) signaling pathways (Wang et al., 2017b; Yin et al., 2018). Osteoporosis remains an important target of research (Hendrickx et al., 2015), and, despite some limits, the two described animal models are appropriate and closer to the human situation than other models. Although further investigation is needed, the available studies showed that AIF, by suppressing osteoclast differentiation or osteoblasts and osteocytes apoptosis, could have beneficial effects on postmenopausal- and glucocorticoid-induced bone damage.

**Antioxidant and Anti-Inflammatory Activities**

Through free radical-scavenging and antioxidative effects, antioxidants constitute the first line of defense against the pathogenesis of several diseases (Rani, 2017).

AIF showed radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Rahman et al., 2010; Börquez et al., 2013; Tjahjandarie and Tanjung, 2015a; Akter et al., 2016; Fu et al., 2018). IC50 values of 8.30 μg/ml (Rahman et al., 2010), 54.02 μg/ml (Börquez et al., 2013), 54.80 μM (Fu et al., 2018), and 708.50 μM (Tjahjandarie and Tanjung, 2015a) were determined. In lipopolysaccharide (LPS)-stimulated murine macrophages RAW264.7 and in mice with LPS-stimulated acute lung injury (ALI), 5 and 10 μg/ml AIF significantly increased the production of antioxidative enzymes such as catalase, heme oxygenase-1 (HO-1), glutathione peroxidase, and superoxide dismutase (Li et al., 2018).

There is a long and ever-growing list of in vitro antioxidant assays. In the DPPH free radical scavenging assay, quite different IC50 values were obtained with AIF probably due to the differences in assay conditions (Table 3). Variable DPPH concentrations, incubation times, sample volumes, solvent systems, and pH clearly result in large differences in IC50 values (reviewed by Tan and Lim, 2015). To standardize the methodology and ensure comparability between studies or laboratories, a DPPH concentration of 50 μM (for good accuracy), an incubation time of 30 min, and methanol as solvent for less polar samples or buffered methanol for more polar samples have been proposed (Sharma and Bhat, 2009; Mishra et al., 2012). Results expressed in different units additionally impede cross-comparison in many cases. The DPPH assay does not actually measure the antioxidant activity but the reducing capacity of the sample (Benzie and Strain, 1999). Moreover, there is no linear relationship between the antioxidant concentration and the radical scavenging activity. The numerous drawbacks of this assay underline its ineptitude to evaluate the antioxidant capacity. Among the other single electron transfer (SET)-based assays such as the trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and thiobarbituric acid reactive substances (TBARS), TEAC assay is most popular due to its convenient application which better reflects the antioxidant activity (Floegel et al., 2011). More recently, hydrogen atom transfer (HAT)-based

| TABLE 2 | Continued |
| Pharmanaceutical activities | Experimental model | Dose/concentration | Mechanism of action | References |
| --- | --- | --- | --- | --- |
| Antiplasmodial activity | Plasmodium falciparum | IC50 = 1.98 μg/ml | Inhibition of parasite proliferation | Tjahjandarie and Tanjung, 2015a |
| Anti-HIV | HIV-1 protease | IC50 = 30.1 μM | Inhibition of HIV-1 protease activity | Lee et al., 2009a |

*Axk, protein kinase B; BV/TV ratio, bone volume/total volume ratio; cMYC, myelocytomatosis viral oncogene homolog; COX-2, cyclooxygenase-2; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ER, estrogen receptor; EFR, extracellular signal-regulated kinase; GRIEB1, growth regulation by estrogen in breast cancer 1; HIF-1, hypoxia-inducible factor-1; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule-1; IZ, inhibition zone; JNK, c-Jun N-terminal kinases; LRH-1, liver receptor homologue 1; MAPKs, mitogen-activated protein kinases; MCF, minimum inhibitory concentration; NIF, nuclear factor-kappa B; NLPR3, nucleotide-binding domain-like receptor protein 3; NOQ-1, NADPH:quinone oxidoreductase-1; PARP, poly-ADP Ribose polymerase; PCNA, proliferating cell nuclear antigen; PIM1, Pim family kinases 1; RANKL, receptor activator of nuclear factor kappa-B ligand; ROS, reactive oxygen species; SPHK1, sphingosine kinase 1; Tb.N, trabecular number (linear bone density of the trabecular bone); Tb.Sp, trabecular separation (distance between the edges of the trabecular bone); Tb.Th, trabecular thickness.
assays like oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), and crocin-bleaching assays provide better analogies to in vivo action (Prior et al., 2005). The main limits of TRAP (which relies only on the lag phase of the kinetic curve for quantitation) and the crocin-bleaching (easily disturbed by compounds absorbing at the monitored wavelength of 450 nm, crocin is not sold as pure compound but as an extract of saffron) assays are probably the reason why the ORAC assay is currently preferred in food and pharmaceutical industries (Huang et al., 2005; Čiž et al., 2010; Power et al., 2013). According to Tan and Lim (2015), a mix of SET and HAT-based assays, encompassing several different radical types is recommended to better estimate the overall antioxidant activity of a sample. In summary, the DPPH assay is not appropriate to evaluate the antioxidant activity of a sample. Moreover, the studies recorded in this review did not use the standardized protocols. However, results from the LPS-induced ALI protocol, well known to be associated with the production of ROS and oxidative stress (Su et al., 2014; Yeh et al., 2014), indicate that, via an activation of antioxidative enzymes, AIF could be beneficial in the treatment of diseases associated with oxidative stress.

The overproduction of free radicals is usually associated with excessive or sustained inflammatory reactions (Dandekar et al., 2015). Administered 1 h before LPS challenge, AIF (10 mg/kg i.p.) alleviated LPS-induced lung lesions, pulmonary edema, and hemorrhages by reducing the activity of myeloperoxidase (Li et al., 2018). In this model and in LPS-induced RAW264.7 cells, 5 and 10 μg/ml AIF inhibited the production of proinflammatory mediators including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1b, intercellular adhesion molecule-1 (ICAM-1), and nitric oxide (NO). The mechanisms underlying these activities include the suppression of nuclear factor-kappa B (NF-kB), mitogen-activated protein kinases (MAPKs), the nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome, and IL-17 signaling pathways (Li et al., 2018). A similar effect on LPS-induced NO production in RAW264.7 cells was observed by Namkoong et al. (2011) with an IC_{50} value of 15.97 μM. In GOLD docking fitness, AIF displayed a fitness of 78.12 μg/ml (only against M. morganii), with an IC_{50} of 15.97 μM. In GOLD docking fitness, AIF displayed a fitness of 78.12 μg/ml (only against M. morganii), with an IC_{50} of 15.97 μM. AIF displayed a minimum inhibitory action (MIA) of 78.12 μg/ml (only against M. morganii) and a minimum bactericidal concentration (MBC) of 39.06 μg/ml against M. smegmatis. AIF displayed a minimum inhibitory action (MIA) of 78.12 μg/ml (only against M. morganii) and a minimum bactericidal concentration (MBC) of 39.06 μg/ml against M. smegmatis. This is why the ORAC assay is currently preferred in food and pharmaceutical industries (Huang et al., 2005; Čiž et al., 2010; Power et al., 2013). According to Tan and Lim (2015), a mix of SET and HAT-based assays, encompassing several different radical types is recommended to better estimate the overall antioxidant activity of a sample. In summary, the DPPH assay is not appropriate to evaluate the antioxidant activity of a sample. Moreover, the studies recorded in this review did not use the standardized protocols. However, results from the LPS-induced ALI protocol, well known to be associated with the production of ROS and oxidative stress (Su et al., 2014; Yeh et al., 2014), indicate that, via an activation of antioxidative enzymes, AIF could be beneficial in the treatment of diseases associated with oxidative stress.

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## Antimicrobial Activity

Over the last decade, the antimicrobial properties of AIF have been evaluated against several drug-resistant and drug-susceptible strains using agar disk diffusion (Kuete et al., 2008), broth microdilution (Kuete et al., 2008; Chukwujekwu et al., 2011; Wang et al., 2014; Ayine-Tora et al., 2016) and macrodilution assays (San-Martin et al., 2015), thin layer chromatography (TLC) bioautography (Akter et al., 2016), and computer-based (virtual) (Sathishkumar and Tharani, 2017) methods.

Tuberculosis (TB) is the leading human infectious-related cause of death (WHO, 2017). Usually, nonpathogenic mycobacterial species such as Mycobacterium smegmatis are used as model systems (Altaf et al., 2010; Namouchi et al., 2017). M. smegmatis displays an identical susceptibility to that of multidrug-resistant (MDR) clinical isolates of Mycobacterium tuberculosis for the two frontline anti-TB drugs isoniazid and rifampicin (Chaturvedi et al., 2007). AIF displayed a minimum inhibitory concentration (MIC) of 19.53 μg/ml and a minimum bactericidal concentration (MBC) of 39.06 μg/ml against M. smegmatis MC2 155 (32-fold less active than ciprofloxacin) while showing no activity against M. smegmatis ATCC14468 (San-Martin et al., 2015) and M. tuberculosis H37Rv (Kuete et al., 2008).

AIF also prevented the growth of Gram-negative (Enterobacter cloacae, Escherichia coli, Morganella morganii, and Proteus mirabilis) and Gram-positive (Staphylococcus aureus and Bacillus stearothermophilus) bacteria with inhibition zones (IZ) of 15.5–18.5 mm. Only the effects against E. coli (IZ = 7.0 mm) were weaker. MIC values of 39.06 μg/ml (against E. cloacae, M. morganii, S. aureus, and B. stearothermophilus) and an MBC of 78.12 μg/ml (only against M. morganii) were determined. In this series, AIF was less active than the reference gentamycin (IZ = 23.8–31.7 mm; MIC = 2.44–9.76 μg/ml) (Kuete et al., 2008). In a study by Chukwujekwu et al. (2011), AIF displayed MICs of 3.9 μg/ml (against S. aureus, E. coli, and Klebsiella pneumoniae) and 7.8 μg/ml (against Bacillus subtilis), while those of the reference neomycin ranged between 0.78 and 1.6 μg/ml. Weak inhibitory activity (MIC ≥ 64 μg/ml) was observed against drug-resistant (SA1199B, RN4220, EMRSA-15, XU212, and EMRSA-16) and wild-type strains of S. aureus (Wang et al., 2014). Using a TLC bioautography assay, Akter et al. (2016)
reported minimum inhibitory quantities of 15 μg for methicillin-sensitive \textit{S. aureus} and 30 μg for a methicillin-resistant and an isolated multidrug resistant strain of \textit{S. aureus}. By contrast, AIF did not show antibacterial activity against the clinical isolates of \textit{S. aureus}, \textit{M. morganii}, \textit{E. coli}, and \textit{Klebsiella granulomatis} in a study by San-Martín et al. (2015). This might be explained by differences in the methods and microbial strains.

\textit{Chlamydia trachomatis} is the most common infectious cause of trachoma. By significantly interacting (G.score of −2.5 kcal/mol) with the active site residue GLU-626(O-H) of contact-dependent secretion D (CdsD) protein \textit{in silico}, AIF might disrupt the assembly of the type III secretion system (T3SS) involved in differentiation, replication, and dissemination \textit{C. trachomatis} (Sathishkumar and Tharani, 2017).

AIF was fungistic against wild (MIC = 0.25 μg/ml) and ATCC18804 (MIC = 0.50 μg/ml) strains of \textit{Candida albicans} (Ayine-Tora et al., 2016). At the concentration of 50 μg/ml, AIF was not able to inhibit the activity of fatty acid synthase (a potential antifungal target) and the growth of \textit{C. albicans} ATCC90028 and \textit{Cryptococcus neoformans} ATCC90113 (Li et al., 2002). It did not display any activity against \textit{Candida glabrata} (Kuete et al., 2008).

The antibacterial activity of flavonoids is often widely conflicting mainly due to the use of different nonstandardized techniques. To overcome this issue, the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial susceptibility testing (EUCAST) have approved and published some guidelines over the last two decades (CLSI, 1998; CLSI, 2002; CLSI, 2004; CLSI, 2008; CLSI, 2010a; CLSI, 2010b; CLSI, 2012a; CLSI, 2012b; EUCAST Definitive Document, 2000; EUCAST Discussion Document, 2003). However, despite these guidelines for agar dilution, broth microdilution, and broth microdilution, results from nonstandardized protocols are still published even in highly reputable journals. Although the disk diffusion technique is easy to apply without specialized equipment, and cheap, the determined IZ value is not related to the antibacterial activity but depends on polarity, concentration, and molecular weight of compounds (Tan and Lim, 2015). Thus, highly polar compounds display a high IZ, and many compounds with the same diffusion rate result in quite different antimicrobial activities. This method is only useful for a simple qualitative screening. It does not allow the quantification of the amount of the antimicrobial agent diffused into the agar, impeding the determination of MICs and MBCs (Ncube et al., 2008; Balouriri et al., 2016). The broth macrodilution or microdilution assays are among the most appropriate methods to determine MIC and MBC values despite the fact that they are unsuitable for highly nonpolar compounds (Tan and Lim, 2015). The reproducibility and the low price due to small amounts of reagents are the main advantages of the microdilution assay over the macrodilution assay. The latter is tedious to perform, requires a lot of manual handling, and is associated with a risk of errors in the preparation of antimicrobial solutions (Jorgensen and Ferraro, 2009). Accordingly, the microdilution method appears to be more accurate. In general, the interpretation of the efficacy depends on the profound knowledge of the model and the used protocol. Nevertheless, stringent endpoint criteria have been set to MIC values of <10 μg/ml or <25 μM for promising plant compounds (Ríos and Recio, 2005; Cos et al., 2006). According to this criterion, AIF could be considered promising only against \textit{S. aureus} ATCC12600, \textit{E. coli} ATCC11775, \textit{K. pneumonia} ATCC13883, \textit{B. subtilis} ATCC6051, and wild and ATCC18804 strains of \textit{C. albicans}. Overall, despite the limits of the used assays/protocols and the discrepancies in results, recorded data suggest the potential of AIF to act as an antimicrobial drug against few microorganisms.

### Anticancer Activity

Different studies reported promising anticancer activities of plant-derived (iso)flavonoids (Magne Nde et al., 2015; Nwodo et al., 2016; Patil and Masand, 2019) including the suppression of proliferation, migration/invasion, tumor angiogenesis and metastasis, and the promotion of apoptosis in various cancers.

In several studies the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was adopted to determine the cytotoxic effects of AIF: The compound exhibited strong cytotoxicity against human oral epidermoid carcinoma KB cells (IC\textsubscript{50} = 4.13 μg/ml) (Nkengfack et al., 2001) and murine leukemia P-388 (IC\textsubscript{50} = 4.31 μg/ml) cells (Tjahjandarie and Tanjung, 2015a). IC\textsubscript{50} values of 19, 34, and 41 μM, respectively, were observed against human leukemia HL-60, K-562, and MOLT-4 cell lines (Kumar et al., 2013). In human lung H2108, H1299, and MRC-5 cancer cell lines, AIF displayed moderate cytotoxicity with IC\textsubscript{50} values of 33.5, 38.8, and 52.5 μM, respectively (Namkoong et al., 2011). At the concentration of 10 μM, the growth and invasion of the human clear-cell renal carcinoma ccRCC 786-O and Caki1 cells were suppressed by 40 and 50–60%, respectively (Wang et al., 2017a). An IC\textsubscript{50} value of >25 μM was obtained against human prostate PC-3 (Nana et al., 2012) and neuroblastoma SH-SY5Y (Hong et al., 2018) cancer cells. At the concentration of 10 \textsuperscript{3} μM, AIF inhibited the growth of the renal SN12C cancer cells by 32.67% (Amen et al., 2013). AIF (10 μg/ml) displayed a low antiproliferative activity (30-40% inhibition) against the human melanoma A375 and SK-MEL-1 cells after a 24-h incubation and suppressed the migration and invasion of these cell lines (Gao et al., 2017). However, after 48 h of incubation, AIF did not exhibit inhibitory effects against SK-MEL-28 cells in a study by Hu et al. (2017). The degree of cytotoxicity in MTT assay increases with the cell number, the concentration of MTT, and the incubation time (van Tonder et al., 2015). The concentration of MTT was not indicated in the two latter studies, and the cell number (5 × 10\textsuperscript{3} cells/well) was only indicated by Hu et al. (2017). Comparison of the incubation times showed that the higher incubation time (Hu et al., 2017) was associated with lower antiproliferative activity. Although neglected in the vast majority of studies, long incubation times are often associated with the decomposition, metabolism, or precipitation of compounds (Ateba et al., 2018).

Under use of a cell counting kit-8 (CCK-8) assay, AIF exhibited a moderate antiproliferative activity against human
esophageal squamous carcinoma (ESCC) Eca109 and KYSE30 cells and at 5 µM enhanced the sensitivity of these cell lines to irradiation (Zhang et al., 2017). In the same model, it also reduced the viability of colorectal HCT-116 and SW480 cancer cells (IC_{50} of 10 and 5 µM, respectively) (Li et al., 2019). The enzyme-based methods including CCK-8 and MTT assays are easy to use, safe, and have a high reproducibility. However, the toxicity of MTT as well as interference of polyphenols with the tetrazolium MTT dye has to be taken into consideration (Wang et al., 2010). A further advantage of the CCK-8 method is its far higher sensitivity (https://www.dojindo.eu.com/Shared/Flyers/Flyer_CCK-8-Rev.pdf).

At 10⁻³ M, AIF inhibited the growth of leukemia CCRF-CEM, MOLT-4, and HL-60 cancer cells by 51.17, 26.15, and 15.49%, respectively. In this study, the type of assay was not specified (Amen et al., 2013).

Induction of apoptosis is a very important property of anticancer drug candidates. In 786-O and Caki cells, AIF led to apoptosis by modulating the miR-101/RLIP76 signaling pathway through the inhibition of Akt (Wang et al., 2017a). In addition to the induction of DNA damage and cell cycle arrest (Zhang et al., 2017), AIF induced apoptosis in ESCC cells by upregulating the miR-370/Pim family kinases 1 (PIM1) signaling (Han et al., 2016) and by suppressing the expression of Nrf2, HO-1 and NADPH:quinone oxidoreductase-1 (Zhang et al., 2017). In HL-60 leukemia cells, apoptotic cell death was observed via the suppression of NF-kB and the signal transducer and activator of transcription (STAT) signaling pathway (Kumar et al., 2013). AIF induced lung tumor apoptotic cell death by repressing both the ERK/MAPK and NF-kB pathways (Namkoong et al., 2011). In HCT-116 and SW480 cells, it triggered apoptosis by blocking DNA damage repair mediated by the DNA double-strand break repair gene RAD51 (Li et al., 2019) and in CCRF-CEM cells through the loss of MMP and production of ROS (Kuete et al., 2016).

Drug resistance constitutes a major impediment to effective cancer treatment. AIF displayed antiproliferative effects against several MDR cancer cell lines. Strong antiproliferative activities were obtained for both the drug-sensitive CCRF-CEM (IC_{50} = 9.6 µM) and the multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 (IC_{50} = 5.91 µM) cells (Kuete et al., 2016). In other drug-sensitive cell lines [breast MDA-MB-231-pcDNA3, colon HCT116 (p53⁺/−), glioblastoma U87MG] and their MDR counterparts [MDA-MB-231-BCRP clone 23, HCT116 (p53⁺/−) and U87MG.AEGFR], AIF displayed moderate effects with IC_{50} values of 42.4–46.7 and 36.4–65.6 µM, respectively. In comparison to normal AML12 hepatocytes, a selective index >3.13 was observed towards HepG2 liver cancer cells (Kuete et al., 2016).

The role of increased activity of hypoxia-inducible factor-1 (HIF-1), especially HIF-1α is well known in cancer progression (Massoud and Li, 2015; Schito and Semenza, 2016). Hypoxic cancer cells seem to be resistant to radiation and chemotherapy (Rohwer and Cramer, 2011; Zhang et al., 2015). Therefore, targeting HIF-1 is an important approach for cancer prevention and treatment. AIF suppressed both hypoxia-induced and iron chelator-induced HIF-1 activation in T47D human breast cancer cells as well as MDA-MB-231 cell migration (Liu et al., 2009).

The antiproliferative or cytotoxic activity associated with apoptosis in malignant cells is a highly important target in the screening of anticancer drugs. Given the severe adverse reactions in normal tissues by tumoricidal doses of chemotherapeutic agents, the cytotoxic activity of drug candidates should also be evaluated against normal cells. In addition to a strong antiproliferative activity (IC_{50} <4 µg/ml or <10 µM for a pure compound after 48–72 h incubation) (Boiik, 2001), a high selectivity (selectivity index ≥3) towards malignant cells is needed. The inclusion of positive controls in respective studies of natural compounds is indispensable in good experimental practice (Ateba et al., 2018). Among the 13 in vitro studies recorded in this review, only one investigated the effects against normal cells (Kumar et al., 2013) and four used a positive control (Nana et al., 2012; Tjahjandarie and Tanjing, 2015a; Hu et al., 2017; Hong et al., 2018). In addition to this deficit, human tumor cell lines as the workhorse of cancer research are cultured since decades and do not adequately mirror (different tumor environment) the biology of human tumors (Ben-David et al., 2017). Therefore, in vivo models with better and more clinically predictive power of human cancers are an imperative.

In vivo, AIF has been tested in various xenograft mouse models. After 30 consecutive days of treatment, the compound reduced tumor growth in KYSE30 (50 and 100 mg/kg/day) and Eca109 (20 mg/kg/day) xenograft mouse models (Han et al., 2016; Zhang et al., 2017). It also suppressed the tumor growth in an HCT-116 xenograft mouse model after 24 days treatment (25 and 50 mg/kg/day AIF i.p.) (Li et al., 2019). In a B16-F10 mouse lung model of metastasis, 24-day intrastragalis administration of 20 and 50 mg/kg/day AIF decreased the number of metastatic pulmonary nodules. The reduction in COX-2 through modulating miR-124/SPHK1 axis was the underlying mechanism involved (Gao et al., 2017). The dose of 40 and 80 mg/kg/day for 24 days suppressed the growth and pulmonary metastatic nodules in a 786-O xenograft mouse model by modulating miR-101/RLIP76 signaling (Wang et al., 2017a). In a study by Zhang et al. (2017), combination of AIF (20 mg/kg/day for 30 days) with irradiation induced a more profound tumor regression than single treatments. All these in vivo activities occurred without affecting the body weight of the mice. Despite the drawbacks of the majority of the current cell-line-derived or patient-derived mouse xenograft models reviewed by Landgraf et al. (2018), they have become a prominent cancer model system over decades. For research in pharmaceutical industry, the accurate description of materials and methodology is indispensable to assure that experiments can be accurately replicated. However, the route of administration of AIF, an extremely important parameter, is not mentioned in the studies by Zhang et al. (2017) and Wang et al. (2017a), published in “high-impact” journals. As a different route of administration leads to different results, this underlines the importance of an accurate review of such papers. Nevertheless, all data reported in this section demonstrate that AIF could have a potential to suppress some tumor growth in vivo.
Antidiabetic Activity
Adequate glycemic control remains the main foundation of managing diabetes mellitus (DM) (Chaudhury et al., 2017). Retarding the release of D-glucose from dietary carbohydrates and delaying its absorption through the inhibition of α-glucosidase is an attractive therapeutic target for the treatment of DM, obesity, and other related complications (van de Laar et al., 2005). In vitro, AIF exhibited a moderate α-glucosidase inhibitory activity with an IC₅₀ value of 73.3 ± 12.9 μM (Fu et al., 2018).

Protein tyrosine phosphatase 1B (PTP1B) is a negative key regulator of insulin signaling pathways that leads to insulin resistance. Thus, it is a promising molecular-level therapeutic target in the management of type 2 DM and obesity (Wang et al., 2015). In a study by Na et al. (2006), AIF exhibited in vitro PTP1B inhibitory activity with an IC₅₀ value of 42 μM as compared to the positive controls RK-682 (IC₅₀ = 4.5 ± 0.5 μM) and ursolic acid (IC₅₀ = 3.6 ± 0.2 μM) (Trinh et al., 2017). By increasing the AMPK activation and the expression of glucose transporters’ (GLUT-4 and -1) mRNA as well as by inhibiting the PTP1B activity (IC₅₀ = 37.52 μM vs. ursolic acid—5.13 μM), AIF significantly stimulated the uptake in L6 myotubes (Lee et al., 2009b). These differences in IC₅₀ values can be explained by the application of different experimental conditions. Using a nonkinetic method to estimate the amount of produced p-nitrophenol at 405 nm, Na et al. (2006) added 10 M NaOH to stop the reaction, while in the study of Trinh et al. (2017), the release rate of p-nitrophenol (kinetic method) was determined by measuring the absorbance at 405 nm every 30 s for 10 min. Moreover, these studies used different concentrations of PTP1B and the substrate p-nitrophenyl phosphate.

Acyl-CoA:diacylglycerol acyltransferase (DGAT) is a key enzyme in the synthesis of triglycerides, the imbalance of which usually leads to insulin resistance and type 2 DM. At the concentration of 12.5 μg/ml, AIF induced 23% inhibition of the activity of this enzyme, while the positive control displayed an IC₅₀ value of 4.8 μg/ml (Oh et al., 2009).

Overall, these preliminary results suggest that AIF could exhibit a potential for the treatment of type 2 DM by retarding the glucose absorption from small intestine, by increasing the insulin sensitivity and the glucose transport into cells, and by improving triglycerides’ profile. But most important, this hypothesis has to be confirmed by respective meaningful in vivo models.

Neuroprotective Activity
Elevation of the activity of brain monoamine oxidases (MAOs), especially MAO-B, contributes to chronic neurodegeneration and brain atrophy (Naoi et al., 2018; Tong et al., 2017). AIF inhibited the mixed type of mouse total brain MAO with an IC₅₀ value of 25.8 μM. Its activity on MAO-B (IC₅₀ = 16.8 μM) was 3.1-fold higher than that on MAO-A (Han et al., 2005). Globally, AIF was more active than the positive control amitriptyline on mixed MAO, MAO-A, and MAO-B. By destroying dopaminergic and noradrenergic neurons in the brain through excessive production of ROS such as superoxide radicals, the neurotoxin 6-hydroxydopamine (6-OHDA) induces neuronal cell death and Parkinson’s disease in rats (Heikkila et al., 1988; Perese et al., 1989; Schober, 2004). At nontoxic concentrations, AIF attenuated (IC₅₀ > 25 μM) the 6-OHDA-induced neurotoxicity and ROS generation in SH-SY5Y cells (Kim et al., 2017).

The relatively high MAO inhibitory activity of AIF compared to amitriptyline and its capacity to protect against 6-OHDA-induced neurotoxicity justifies further in-depth investigations of AIF for its potential in neurodegenerative diseases such as Parkinson’s and Alzheimer's.

Other Activities
With 216 million cases and 445,000 deaths in 2016, malaria remains a major cause of death worldwide, especially in Africa (http://www.who.int/malaria/en/). AIF has shown strong antiplasmodial properties against Plasmodium falciparum with an IC₅₀ value of 1.98 μg/ml as compared with the positive control chloroquine (IC₅₀ = 1.02 μg/ml) (Tjahjandarie and Tanjung, 2015b).

HIV-1 protease and reverse transcriptase are the most important targets in the search for anti-HIV agents. In vitro, AIF showed a low inhibitory activity against HIV-1 protease with an IC₅₀ value of 30.1 μM (Lee et al., 2009a).

STRUCTURE-ACTIVITY RELATIONSHIP
Numerous prenyl- (Hu et al., 2017), O-methyl- (Waffo et al., 2000; Han et al., 2005; Liu et al., 2009; Lim et al., 2012; Ndemangou et al., 2013; Ayine-Tora et al., 2016; Ocloo et al., 2017; Fu et al., 2018), and/or O-acetyl (Bórquez et al., 2013; Ayine-Tora et al., 2016) derivatives of AIF have been detected in various plants and studied for the impact on the biological activities. From studies comparing both the activities of AIF and those of one or more of its derivatives (Table 4), it can be deduced that:

i) The replacement of C4’-OH and/or C5-OH by −OMe or O-acetyl reduces the antifungal activity against C. albicans (Ayine-Tora et al., 2016).

ii) The 4’-O-methylated form of AIF promoted the inhibition of HIF-1 activation in T47D cells, the MDA-MB-231 cell migration (Liu et al., 2009), and the inhibition of urease (Ndemangou et al., 2013) and MAO-A (Han et al., 2005) activities, while no significant changes on the influence on MAO-B and α-glucosidase activities were observed (Han et al., 2005; Fu et al., 2018).

iii) In AIF, initially inactive, the introduction of a prenyl group at the C-8 position to obtain scandenolone or warangalone significantly increased the growth inhibitory activity (IC₅₀ < 5 μM) towards human melanoma SK-MEL-28 cells (Hu et al., 2017).

iv) The [1,2-b:5,4-b’]dipyran derivative derrone showed antiproliferative activity in human leukemia U937 cells in a similar magnitude like AIF (Matsuda et al., 2007). The same refers to the inhibition of PTP1 (IC₅₀ = 12.6 μM for derrone and 21.6 μM for alpinumisolavone) (Trinh et al., 2017). In contrast, derrone was moderately inhibiting phospholipase...
Cs1 activity and the formation of inositol phosphates in phospholipase Cs1-overexpressing NIH3T3 fibroblasts, whereas AIF remained without effect (Oh et al., 2005).

The differences in the activities of some compounds closely related to AIF, preclinically tested in comparable assays but not directly compared with AIF, are difficult to interpret due to the limitations as discussed above. Nevertheless, we include those results, which were obtained in the most similar experimental setups:

i) The 2′-OH derivative parvisoflavone B showed a stronger α-glucosidase inhibition (ICso = 12.2 µM; Dendup et al., 2014) than AIF (ICso = 73.3 µM; Fu et al., 2018). Nevertheless, due to the lack of a positive control in the study with AIF, a comparison of the results remains questionable. Parvisoflavone B resulted also in a better cytotoxic effect against MDA-MB-231 breast cancer cells (ECso = 16.9 µM) (Nyandoro et al., 2017). The weak antimycobacterial effect (MIC = 90.9 µM) of parvisoflavone B against M. tuberculosis H37rV (Nyandoro et al., 2017) differed from the inactive AIF (Kuete et al., 2008).

ii) Cudraisoflavon M—carrying an additional 2,3-di-hydroxy-prenyl group at C-8 of AIF—did not show any activity against 6-OHDA-induced cell death in SH-SY5Y cells (Hiep et al., 2017), whereas cudraisoflavon H with a prenyl group at C-8 and an additional hydroxy group at C-2′ of AIF resulted in an ICso value of 4.5 µM (Hiep et al., 2015).

iii) The comparison of the antimicrobial effects of derrone with AIF is extremely difficult due to the differences in the experimental setup as discussed above. Nevertheless, the activity of derrone against E. coli, S. aureus, and C. albicans seems lower than the one of AIF (Edziri et al., 2012). The antiproliferative potential of derrone and AIF differ in dependence of the cell line: In SW480 cells, AIF with an ICso value of 5 µM inhibits the proliferation (Li et al., 2019), whereas derrone remains inactive (Li et al., 2017). In MCF-7 cells, AIF was inactive (Sudanich et al., 2017) and derrone at 10 µM inhibited this cell line by 13.6% (Li et al., 2017). HepG2 cells seem to be similarly sensitive to the two compounds [AIF—ICso 37.99 µM (Kuete et al., 2016); derrone—23.7% inhibition at 10 µM (Li et al., 2017)].

iv) 2′-Hydroxyerythrin A with the OH group from C-5 shifted to C-2′ showed good activity against several Gram-positive and Gram-negative bacteria (Wang et al., 2018). The magnitude of the DPPH radical scavenging effect was in the same range (Wang et al., 2018) as in some studies with AIF (Rahman et al., 2010; Bórquez et al., 2013; Fu et al., 2018).

The infrequency of studies and the low number of different substitution patterns and of investigated activities are the main drawback in the deduction of structure–activity relationships (SAR) of AIF and its derivatives. Continued efforts are needed to further synthesize or isolate new derivatives of AIF to expand SAR. Nevertheless, published data indicate that the C4′-O-methylation and the C8-prenylation increase the activity of AIF in cancer and neurodegenerative conditions. This is in accordance with Bernini et al. (2011) who indicated that O-methylation of flavonoids ensures a superior anticancer activity as compared with the corresponding hydroxylated derivatives, since such compounds are more resistant to hepatic metabolism and show higher intestinal absorption. In addition, Walle et al. (2007) suggested that O-methylation enhances the stability of flavonoids to metabolic degradation and increases their bioavailability as well as a higher tissue distribution as compared to unmethylated forms.

### POINT OF VIEW AND FUTURE PERSPECTIVE

Prenylated (iso)flavonoids are attracting more and more attention due to a series of promising biological activities ascribed to their increased lipophilicity and a strong affinity to biological membranes as compared to the respective unprenylated compounds (Botta et al., 2005; Botta et al., 2009; Chen et al., 2014; Sherif et al., 2015; Mukai, 2018).

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**TABLE 4 | Impact of different substitutions on the activity of alpinumisoflavone.**

| Substituent             | Impact on the activity                                                                 | Experimental model                                           | References                |
|------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------|---------------------------|
| 4′-O-methyl            | ↑ Inhibition of hypoxia-inducible factor-1 (HIF-1) activation                          | Human breast tumor T47D cells                                | Liu et al., 2009          |
|                        | ↓ Inhibition of tumor cell migration and chemotaxis                                     | MDA-MB-231 cells                                             | Liu et al., 2009          |
|                        | ↓ Antiradical activity                                                                | DPPH assay                                                  | Fu et al., 2018           |
|                        | ↓ Antifungal activity                                                                 | α-Glucosidase enzyme model                                  | Fu et al., 2018           |
|                        | ↓ Monoamine oxidase-A (MAO-A) activity                                                | Candida albicans (wild and ATCC18804 strains)               | Ayine-Tora et al., 2016   |
|                        | ↓ MAO-B activity                                                                      | Mitochondrial fraction from mouse brain                     | Han et al., 2005          |
| O, O-dimethyl          | ↓ Antifungal activity                                                                 | Candida albicans (wild and ATCC18804 strains)               | Ayine-Tora et al., 2016   |
| 5-O-acetyl- and 4′-O-methyl | ↓ Antiradical activity                                                                 | Candida albicans (wild and ATCC18804 strains)               | Ayine-Tora et al., 2016   |
| 4′-O-acetyl            | ↓ Antiradical activity                                                                | DPPH assay                                                  | Bórquez et al., 2013      |
| 8-prenyl               | ↑ Antiproliferative activity                                                           | Human melanoma SK-MEL-28 cells                              | Hu et al., 2017           |

↑: increase, ↓: decrease, ↔: not different.
In this context, numerous pharmacological investigations of alpinumisoflavone, extracted from various medicinal plants, were carried out over the last decades. Data recorded in this review evidence a wide array of activities such as antiosteoporotic, antioxidant, anti-inflammatory, antimicrobial, anticancer, estrogenic and antiestrogenic, antidiabetic, and neuroprotective. Discrepancies between results were usually attributed to the purity of the tested compound, the experimental setup, the operator’s experience, or other experimental parameters (Ateba et al., 2018). Many of related pathologies or conditions such as antimicrobial resistance, cancer, diabetes mellitus, and neurodegenerative diseases are becoming pivotal concerns for public health over the world. However, although AIF might be considered a promising preventive and/or therapeutic agent for such ailments, these investigations are only at the beginning. Using suitable and well-designed standardized models or assays, further and thorough studies related to the above mentioned or other pathologies/conditions are needed to confirm this potential. In vitro evaluation is an important primary screen and due to its rapidity common practice in many research laboratories. Nevertheless, many in vitro studies are not necessarily optimal due to poor standardization, redundancy, and/or outdated methodology (Tan and Lim, 2015). Clearly, compounds exhibiting promising activity require further studies to validate or confirm their therapeutic potential (Kenny et al., 2015). Accordingly, the correlation with in vivo data using appropriate models is an indispensable prerequisite.

The analysis of structure–activity relationships provides information on the preferential conformation to maintain high activities. Studies of AIF until now revealed that the free –OH groups at C-4′ and C-5 are important for the fungicidal activity towards C. albicans (Ayine-Tora et al., 2016). 4′-O-Methylation and the presence of a prenyl group at C-8 enhanced anticancer activities (Liu et al., 2009; Hu et al., 2017). Studies with diversified substituents would be ideal for the investigation of SARs. They might allow the identification of important structures with reduced toxicity and increased therapeutic efficacy that can guide the design of novel leads or drug candidates. However, such studies on AIF and its derivatives are scarce until now, and this underlines the necessity of further well-performed investigations.

Besides the efficacy, extensive safety and pharmacokinetic data are required for potential drug candidates as an important aspect in the drug development process. However, till today, no study dealing with the toxicity or pharmacokinetics of AIF has been reported.

**FIGURE 2** | Overview over pharmacological activities of alpinumisoflavone and some of its derivatives.
CONCLUSION

This review evidences that AIF is a versatile compound with a wide array of possible health benefits. Wesummarize the current preclinical evidence of the antiosteoporotic, antioxidant and anti-inflammatory, antimicrobial, anticancer, estrogenic and antiestrogenic, antidiabetic, and neuroprotective activities (Figure 2). However, more persuasive and scientific evidence and detailed mechanistic studies are urgently needed for a therapeutic exploitation of AIF. Moreover, SAR of AIF and its derivatives indicates that 4′-O-methyl-AIF appears to be more promising than AIF, and these indications need to be investigated in-depth.

AUTHOR CONTRIBUTIONS

SA obtained literatures, wrote the first draft, and edited the manuscript; MM obtained literatures and wrote sections of the manuscript. SD, SZ, and DN gave ideas and critically reviewed the manuscript. LK gave ideas, critically reviewed and edited the manuscript. All authors read and approved the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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