



# Targeting Microtubules by Phytoestrogens for the Treatment of Cancer: An Overview

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## Abstract

Phytoestrogens are naturally occurring compounds that have similar molecular structure to estrogen hence able to exert estrogenic activities by binding to estrogen receptors (ERs), ER $\alpha$  and ER $\beta$ . Phytoestrogens have been reported to suppress cancer cell survival and growth in various types of cancers including lung cancer, breast cancer and prostate cancer. Moreover, phytoestrogens have been shown to inhibit cancer cell migration and invasion by targeting multiple pathways. These cellular processes are closely regulated by microtubule cytoskeleton, hence it is interesting to gain more understanding regarding the effects of phytoestrogen on microtubules structure and dynamics. In this review, we summarize reported effects of some phytoestrogens that bind directly to tubulin at various binding site and disrupt the microtubule dynamics, either by promoting microtubule polymerization or enhancing microtubule depolymerization. We also discuss the effects of phytoestrogens in combination with established chemotherapeutic drugs, including the MTAs. Evidence found is crucial for the development of phytoestrogens to be used as monotherapy or combination therapy (as adjuvant).

**Significance** | Phytoestrogens are potential proliferation and migration inhibitory microtubule targeting agents.

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## 1. Introduction

Eukaryotic cells undergo many essential cellular processes for survival, from cell growth and motility to intracellular trafficking. The cellular processes rely on interactions of a variety of cellular components such as microtubules, actin filaments and growth factors. Cancer begins when the normal processes that regulate cell behaviour are altered and causes abnormal capabilities such as excessive cell proliferation, uncontrolled invasion and metastasis, unregulated angiogenesis and avoiding destruction by the immune system (Pickup et al., 2014). Globally, cancer is a leading cause of death, such that it becomes one of the most significant public health burdens of the century that has spurred development of various therapeutic drugs that can keep patients alive and well for a longer time.

In most cancers, chemotherapy is adopted as the first-line treatment although its efficacy is far from satisfying due to its side effect in damaging surrounding healthy tissues, leading to tissue inflammation and maladaptive remodelling (Octavia et al., 2012). Other limitations of conventional chemotherapy include difficulty in selecting dosage to use and development of drug resistance by the cancer cells (Debela et al., 2021). An alternative option to overcome the lack specificity of chemotherapy that leads to collateral damage is by targeted drug therapy.

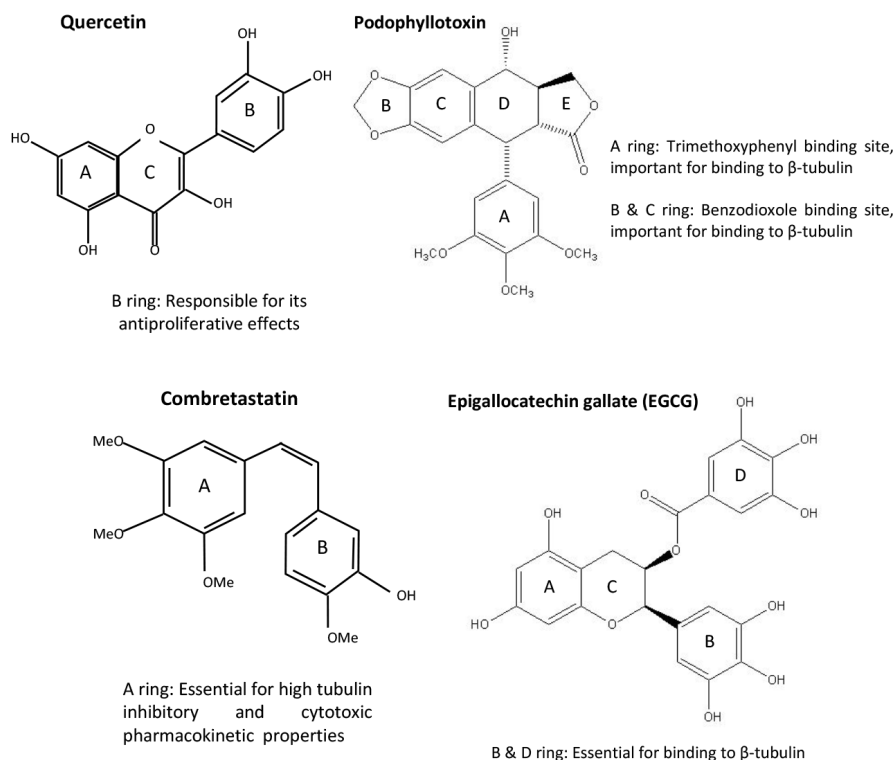
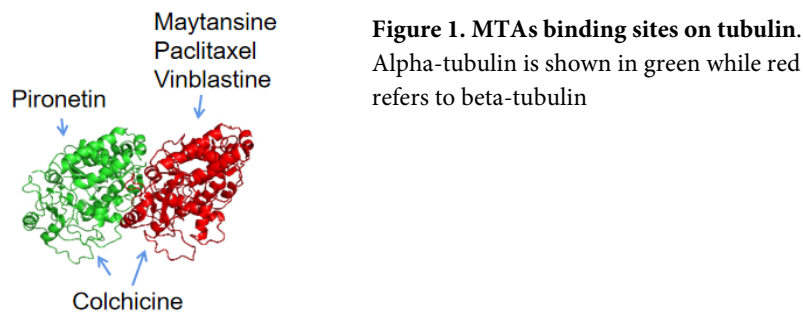
Selectively treating cancers with targeted drug has shown to improve survival rate in breast cancer and lung cancer patients (Mener and Aggarwal, 2015; Musika et al, 2021). The mechanism of action of targeted therapies is based on their interaction at the target site. Monoclonal antibodies therapy, for example, allows binding of engineered monoclonal antibodies to cancer cell surface.

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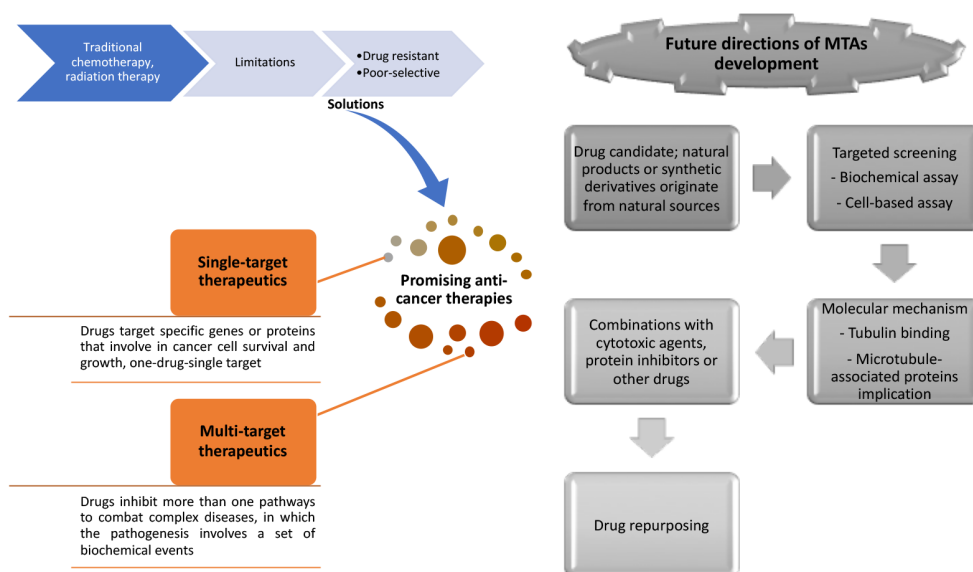
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**Figure 2. Structure activity relationship of phytoestrogens that bind tubulin.** Quercetin, podophyllotoxin and combretastatin bind to colchicine site on  $\beta$ -tubulin while ECGG bind  $\alpha$ -subunit of tubulin near colchicine site



**Figure 3. Future research strategies for MTAs development.** Research on molecular mechanism of phytoestrogens in disrupting microtubule dynamics may be directed towards developing phytoestrogen as promising therapeutic agent for monotherapy or combination therapy.

-e antigens to activate cell destruction through mechanisms including induction of apoptosis, activation of cellular phagocytosis or disruption of tumour blood vessel network (Coulson et al., 2014). While adverse effects of monoclonal antibodies therapy are milder compared with conventional chemotherapy, the production cost to produce such therapeutic monoclonal antibodies is expensive, estimated to be twice than required for conventional drugs (Craik et al., 2013).

Ablation cancer therapy refers to treatment technique that destroys tumours without removal of the cancer cells. This technique offers minimal invasive procedure to surgery, which is performed using methods involving thermal energy, high-intensity ultrasound beam and high-energy laser (Kang and Rhim, 2015). However, tumour ablation is not indicated for certain cases where tumours present near major blood vessels or major bile ducts since the ablation technique may destroy some of the normal tissues near the tumour. Besides, complications such as haemorrhage, hepatic and extrahepatic organ injuries as well as skin burn have been reported following ablation therapy (Koda et al., 2012).

Microtubule-targeting agents (MTAs) are chemical compounds that specifically bind to tubulin and interfere with the properties and functions of microtubules. Because microtubules have distinct functions in proliferation and survival processes, targeting microtubule dynamics becomes a promising anticancer therapy. MTAs are antimetabolic agents that suppress cell proliferation, either by inhibiting microtubule polymerization (such as vinblastine, colchicine and combretastatins) or stimulating microtubule polymerization (such as paclitaxel, docetaxel).

Exploration on small organic molecules to be developed as targeted therapy drugs for cancer is constantly emerging, owing to the fact that their structures can be easily modified to suit clinical needs and low-cost production. Phytoestrogens are polyphenolic small molecules that exhibit molecular structure similarity to estrogens, hence allows them to bind to estrogen receptors and exert estrogenic activity (Sayed et al., 2018). While some phytoestrogens have been reported to bind directly to tubulin and alter microtubules dynamics, there are other phytoestrogens that have indirect effects on microtubule stability. This review focuses on microtubule-targeting activity by several phytoestrogens and the future development as new anticancer strategies.

## 1 Microtubule composition and dynamics

### 2.1 Composition

Microtubules are made up of one polymerized dimer,  $\alpha$  and  $\beta$  tubulin, weight around 55 kDa and fold in double helix structure. Microtubules extend and grow to form into long hollow cylinders (Sun et al., 2021). Apart from  $\alpha$  and  $\beta$  tubulin, there are  $\gamma$ ,  $\delta$  and  $\epsilon$  tubulin families that mainly present in the centrosome.

The best-known role of microtubules in eukaryotes is in the formation of cytoskeleton. They control vital cellular activities ranging from cell division, cell movement and intracellular transport (Čermák et al., 2020). Spindle microtubules extend from centrioles to segregate chromosomes during cell division. While in protozoans, microtubules are the structural components that form flagella and cilia.

### 2.2 Dynamics

Microtubules have two main dynamic states, polymerization and depolymerization. Such change of balance between these states allow microtubules to dynamically alter their organization according to the needs of respective cellular processes, for example in cell cycle and cell division, which involve chromosomes movement and cell division to produce daughter cells. Polymerization of microtubules occurs with addition of  $\alpha$ - and  $\beta$ -tubulin dimers at both ends of microtubules that leads to microtubule growth while dissociation of the tubulin dimers at the ends, known as depolymerization, results in microtubule shrinkage (Horio and Murata, 2014).

The simultaneous presence of both microtubules elongation and shrinkage is required to carry out specialized cell functions. These events are regulated by microtubule-associated proteins (MAP) including Tau, MAP2 and MAP4 that promote stabilizing effect, and depolymerizing MAPs like stathmin, katanin and spastin that regulate microtubule destabilization (Goodson and Jonasson, 2018). The microtubules dynamic activities are demonstrated experimentally by evaluating their growth rate, shortening rate and transition frequency (Mukhtar et al., 2014).

## 2 Mechanism of action of microtubule-targeting compounds

Several naturally-derived compounds, such as vinca alkaloids and colchicine, are found to inhibit microtubule polymerization while compounds such as taxanes and laulimalides have been shown to encourage microtubule assembly. These compounds are known as microtubule targeting agents (MTAs) and interrupt microtubule stability by binding tubulin at respective sites that have been well characterized as shown in Figure 1 (Barbier et al., 2014). For example, vinblastine, taxol and maytansine bind to the  $\beta$ -tubulin subunit in microtubules whereas colchicine binds at the  $\alpha$  and  $\beta$  interphase of tubulin. The only natural product shown through crystallographic research that binds  $\alpha$ -tubulin is pironetin, a metabolite originally isolated from *Streptomyces* species (Yang et al., 2016).

The MTAs are grouped into microtubule-stabilizing agents (MSAs) and microtubule-destabilizing agents (MDAs), of which reflects their activities at high concentrations in influencing microtubule mass. MSAs promote microtubules stability while MDAs trigger microtubule disassembly into tubulin dimers. Changes in microtubule dynamics may perturb the formation of mitotic spindle, arrest cell mitosis, restrict cell motility and induce

cell death. More researchers have adopted these approaches to investigate better therapy to combat cancer. MTAs that have been approved and introduced into the clinic for the treatment of various cancers include paclitaxel, epothilone and maytansine. Current knowledge on the molecular mechanisms of action of MSAs and MDAs to hijack the microtubule cytoskeleton is emerging and drives the future cancer treatment directions.

Taxane-site ligands; such as paclitaxel, epothilone, zampanolide and taccalonolide, are MSAs that bind to the same pocket on  $\beta$ -tubulin (Steinmetz and Prota, 2018). The taxane site consists of hydrophobic residues of helix H7, strand S7 and  $\beta$ -tubulin loops; H6-H7, the M-loop and S9-S10. Upon binding to taxane site, the ligands establish hydrophobic and polar interactions with the structural elements thus promote microtubule assembly and stability. Perturbation on microtubule dynamics is owing to the role of M-loop as the main element that forms lateral contacts in the microtubules.

However, in the case of paclitaxel, it does not exhibit a sidechain to interact with the M-loop but still promotes microtubules stabilization. Following high-resolution cryo-electron microscopy (cryo-EM) studies carried out by Alushin and co-workers (2014), it has been suggested that major mechanism contributes to the microtubule stabilization may not be through a particular M-loop conformation. This is supported by their findings in which the microtubule-stabilizing effect of paclitaxel involves modulation of longitudinal contacts and conformational strain within the tubulin monomers. Collectively, although binding to the same pocket on  $\beta$ -tubulin, different taxane-site ligands may have different molecular mechanism to exert their microtubule-stabilizing effect.

On the other hand, molecular mechanisms of MDAs involve either inhibition of native tubulin contacts formation or through prevention of tubulin curved-to-straight conformational change that accompanies microtubule formation. Vinca alkaloids are potent MDAs that target vinca site of tubulin. The vinca site comprises of a core zone and a pocket that protrudes into exchangeable GTP-binding site of  $\beta$ -tubulin (Wang et al., 2016). Vinca-site ligands trigger microtubule destabilization by establishing a 'wedge' at the tips of microtubules that prevents correct positioning of  $\alpha$ -tubulin hence causes GTP hydrolysis on  $\beta$ -tubulin and protofilaments disassembly (Ranaivoson et al., 2012). Other well-known MDAs are compounds targeting the colchicine site, a pocket buried deeper in the intermediate domain of  $\beta$ -tubulin and located at the interface between  $\alpha$ - and  $\beta$ -tubulin subunits. Upon binding to the colchicine site, the ligands form hydrophobic and polar interactions that prevents the movement of intermediate domain of  $\alpha$ - and  $\beta$ -tubulin subunits (Wang et al., 2022). Consequently, the conformational changes of tubulin from

a curved conformation to a straight structure, as required for tubulin assembly, is inhibited.

### 3 Phytoestrogens and microtubule dynamics

Phytoestrogens can be found in diverse food sources such as soy products, fruits and cereals (Torrens-Mas et al., 2020; Kashyap et al., 2017). Medicinal plants, which have been used in traditional practices to treat various diseases, have also been reported to contain phytoestrogens, especially those classified as flavanoids. Flavonoids comprise of several main subgroups, namely prenylflavonoids, coumestans, isoflavones, flavones, chalcones and anthocyanidins. While for the non-flavonoids such as stilbenes, lignans and tannins, they are commonly found in nuts, legumes and whole grains.

Phytoestrogens have been reported to potentially combat cancer by reducing tumour cell survival and growth as well as triggering cell death. For instance, silibinins may prevent migration of highly metastatic breast cancer cells (Lashgarian et al., 2020) while some lignans have been found to suppress mitotic spindle formation and tubulin polymerization in lung adenocarcinoma, ovarian cancer, and neuroblastoma-derived cells (Esfandiari et al., 2017). Kaempferol, one of the extensively studied phytoestrogens, have been reported to diminish 5-fluorouracil drug resistance in LS174 cells (Riahi-Chebbi et al., 2019). Some phytoestrogens with anticancer properties have been found to target microtubules dynamics as their mechanism of action (Mukhtar et al., 2015). Given the important roles of microtubules in most cell physiological events, altering microtubules dynamics may contribute to deleterious impacts to cancer cells to the extent that it often results in cell death.

Quercetin has been found to bind tubulin at colchicine site to promote microtubule depolymerization hence causes cell cycle arrest at G/M phase in prostate carcinoma and breast cancer cells (Almatroodi et al., 2021). Upon binding to tubulin, quercetin stimulates the GTPase activity of soluble tubulin that later perturbs microtubule polymerization dynamics (Gupta and Panda, 2002). Previous study by Novo and co-workers (2015) showed that Wnt/ $\beta$ -catenin signalling was hindered following quercetin treatment in B-1 lymphocytes, leading to alterations in cell proliferation and differentiation. Since there is evidence to support Wnt/ $\beta$ -catenin signalling as a crucial antitumor effect of MTAs (Kumari et al., 2021), perhaps anticancer properties of quercetin may also be due to alteration in the Wnt/ $\beta$ -catenin signalling.

As a naturally occurring isoflavone, podophyllotoxin shows a great potential against diverse types of cancer such as non-small cell lung cancer, breast cancer and hepatocellular carcinoma (Choi et al., 2015; Zhang et al., 2020; Li et al., 2019). Podophyllotoxin-treated lung cancer cells show suppressed cell growth and migration *in vitro* (Yang et al., 2019). This is consistent with their

immunofluorescence data that show disorganization of microtubule structure and spindle formation as well as rearrangement of vimentin filaments following podophyllotoxin treatment in A549 cells. The microtubule disorganization is due to inhibition of microtubule polymerization, which resulted from podophyllotoxin binding to the colchicine site of tubulin with the its 3,4,5-trimethoxybenzoyl at ring-A buried deeply inside the colchicine binding site (Figure 2).

Other potential anticancer phytoestrogens that disrupt microtubules dynamics by promoting microtubule depolymerization are combretastatin, epigallocatechin gallate (EGCG) and theaflavin. While combretastatin binds to colchicine site on the  $\beta$ -tubulin subunit at its 3,4,5-trimethoxy substituted A ring and the 4-methoxy substituted B ring (Figure 2), EGCG has been found to bind  $\alpha$ -subunit of tubulin near colchicine site (Bukhari et al, 2017; Chakrabarty et al., 2015). Although EGCG binding to tubulin does not overlap with the colchicine site, it is more potent than colchicine in promoting microtubule depolymerization. Intriguingly, the mechanism for microtubule depolymerization of theaflavin occurs through vinblastine binding site (Chakrabarty et al., 2019). In the presence of theaflavin, fluorescence intensity of BODIPY FL vinblastine, a fluorescent analogue of vinblastine, is reduced suggesting inhibition of BODIPY FL vinblastine binding to tubulin.

Of phytoestrogens studied for their effects on tubulin, fisetin has been reported to interrupt the dynamic behaviour of microtubules through stimulating microtubules polymerization and stabilizing microtubules at high concentrations (Mukhtar et al., 2015). Fisetin treatment in cancer cells inhibits cellular processes that are closely regulated by cytoskeleton such as cell proliferation and migration. Finding from *in vitro* tubulin polymerization assay shows that fisetin enhances microtubule polymerization higher than paclitaxel and increases microtubule network stability. Binding evaluations through surface plasmon resonance (SPR) reveals that fisetin interacts with  $\beta$ -tubulin pocket within paclitaxel binding site. Moreover, expression of microtubule associated proteins (MAPs), MAP-2 and MAP-4, has been found to be increased following fisetin treatment in prostate cancer cells (Mukhtar et al., 2015). These proteins are best known for their important role in microtubule network regulation through microtubule-stabilizing activity (Mohan and John, 2015).

**Figure 2: Structure activity relationship of phytoestrogens that bind tubulin.** Quercetin, podophyllotoxin and combretastatin bind to colchicine site on  $\beta$ -tubulin while EGCG bind  $\alpha$ -subunit of tubulin near colchicine site

#### 4 Discussion

Naturally-derived compounds that disrupt the normal function of microtubule have been deemed to be one of chemotherapeutic agents work against for various tumour.

Compared to biologics, phytoestrogens are relatively small molecules that have multiple biological activities with fewer toxic effects, which perhaps could potentially counteract the drug resistance problems of traditional chemotherapy (Pu et al., 2015). Phytoestrogen that target microtubules has also been shown to overcome cancer cell drug resistance when used in combination with established conventional chemotherapy drugs. For instance, EGCG reverses gefitinib drug resistance in lung cancer cells by elevating cell death through inhibition of ERK phosphorylation (Meng et al., 2019).

Phytoestrogens that do not directly target microtubules for their anticancer activity are also beneficial in improving efficiency of chemotherapeutic drugs. Kaempferol has been shown to reverse drug resistance of LS174 cells to 5-fluorouracil (5-Fu) when used in combination with the chemotherapy drug (Riahi-Chebbi, et al., 2019) while treatment of gastric cancer cells with genistein reduces chemoresistance to 5-Fu as well as cisplatin *in vitro* and *in vivo* (Huang et al., 2014). The reduced chemoresistance is owing to suppression of ERK 1/2 activity that significantly diminishes expression of chemoresistant gene ABCG2.

Moreover, the antitumour efficacy of cisplatin against triple negative breast cancer cells can be enhanced using combined treatment with apigenin, which results in inhibition of telomerase activity (Aziz et al., 2017). Resveratrol, a well-known polyphenol, increases chemosensitization effect of human breast cancer cells MCF-7 to melphalan by activating caspases 7 and 9 hence induces cells death (Casanova et al., 2012). Besides, sensitization of resveratrol leads to decreased expression of cyclin A, suggesting cell cycle arrest in the S phase.

Extensive investigations on co-treatment of phytoestrogens and established MTAs have been performed using drugs such as paclitaxel, docetaxel and vincristine. Combination therapy of resveratrol and paclitaxel leads to further increase of calcium ion ( $\text{Ca}^{2+}$ ) into DBTRG glioblastoma cells through activation of TRPM2 channel contributing to increased cancer cell death (Öztürk et al., 2019). Increased mitochondrial ROS production has also been observed, which causes cytotoxic effects to the cancer cells.

Chemoresistance and side effects of docetaxel can be overcome by using the drug together with EGCG and quercetin as reported by Wang et al., 2015 following treatment in prostate cancer cells. They found that EGCG and quercetin enhance therapeutic impact of docetaxel through various mechanisms including triggers antiproliferative activity, increases apoptosis and inhibits tumour cell invasion. Quercetin has also been shown to enhance oral cancer cells sensitivity to vincristine and significantly increases apoptosis rate (Yuan et al., 2015).



Given the promising anticancer properties of phytoestrogens, using phytoestrogens alone as potential MTAs or as an adjuvant with other chemotherapeutic drugs is an exceptional alternative to resolve limitations of traditional chemotherapy. Development of phytoestrogens as new treatment strategies that leads to lesser side effects and resolves drug resistance could provide a more effective therapeutic alternative for cancer patients. Here we propose future research strategies for combination therapies using phytoestrogens and established anticancer drugs in achieving a safe and effective treatment (Figure 3).

More studies are warranted to comprehend better on the mechanistic role of phytoestrogens to enhance their usage and effectiveness in combating cancer. Modern techniques such as cryo-EM, atomic force microscopy and X-ray crystallography can be adapted to understand detailed molecular mechanisms by which phytoestrogens alter microtubule dynamics. Regarding clinical study design to evaluate the benefit/risk ratio of combination therapy compared with single drug, it is important to report on responder percentages of patients benefit from combined treatment and patients expose to adverse effects in order to avoid overestimating benefits of combination therapy.

## 5 Conclusion

Collectively, phytoestrogens receive much attention for development as promising chemotherapeutic agents in clinical use due their simple structures, various biological activities and less toxic to healthy cells. With regard to microtubule targeting activities by some of the phytoestrogens, we suggest they could be developed as proliferation and migration inhibitory agents that aim multiple pathways to combat cancer cells survival and invasion. As more studies are being carried out to investigate the potential of phytoestrogens in overcoming drug resistance, it is crucial to design strategies to integrate phytoestrogens as an adjuvant with other chemotherapeutic agent.

## Author Contributions

XF Writing, Original Draft, NZS Writing, Review, Supervision. NMA Conceptualization, Writing, Review and Editing, Supervision.

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## Competing financial interests

The authors have no conflict of interest

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