SYSTEMATIC REVIEW

Molecular mechanisms of flavonoids and their modulatory effects against breast cancer: A scoping review [version 1; peer review: 2 approved with reservations, 1 not approved]

Mary Murithi[1,2], Esbon Wambugu[3], Steven Nyanjom[1], Victor Mobegi[4], Sayed Shahin[5], Francis Makokha[6]

[1]Pre-Clinicals, School of Pharmacy, Kabarak University, Nakuru, Private Bag, 20157, Kenya
[2]Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, 62000-00200, Kenya
[3]Pharmacology and Pharmacognosy, School of Pharmacy, Kabarak University, Nakuru, Private Bag-20157, Kenya
[4]Biochemistry, University of Nairobi, Nairobi, 30197-00100, Kenya
[5]Pathology, Aga Khan University Hospital, Nairobi, Nairobi, 30270-00100, Kenya
[6]Directorate of research and Innovation,, Mount Kenya University, Thika, 342-01000, Kenya

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Abstract

Background: Breast cancer is the most prevalent malignancy among women. It is a disease whose incidence and mortality rates are on the upsurge globally. Debilitating effects, cost and resistance to available chemotherapeutic interventions render them unideal. Dietary phytochemicals have been shown to have preventive and therapeutic effects. Research continues to affirm the role of flavonoids as potential chemotherapeutic agents in combating the disease. Understanding modulation of key cellular signalling pathways by flavonoids presents promising molecular targets that may be leveraged to develop better chemotherapeutic agents for breast cancer.

Methods: To describe the *in vitro* and *in vivo* modulatory effects of flavonoids on molecular anti-cancer mechanisms we searched three databases. We included original articles describing modulation of cell signalling processes such as; cell cycle, apoptosis, autophagy, angiogenesis, invasion and migration which are involved in tumorigenesis. The search guidelines such as; year of publication, search strategy, study design and language informed article selection.

Results: Thirty-six articles were reviewed. Modulatory effects of six subclasses of flavonoids on breast cancer tumorigenic pathways were reported. The effects included enhanced apoptosis, attenuation of; angiogenesis, cell cycle, invasion, migration and metastasis. For instance, pectolinarigenin inhibited signal transducer and activator of transcription 3 (stat3) signalling pathway in triple negative breast cancer. Whereas, sideritoflavone caused cell cycle arrest and inhibited migration in trastuzumab resistant breast cancer cells. Additionally,

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1. **Muthi Ikawati**, Universitas Gadjah Mada, Yogyakarta, Indonesia
2. **Sandip Patel**, L.M. College of Pharmacy, Ahmedabad, India
3. **Nupur Mukherjee**, ICMR-National Institute for Research in Reproductive and Child Health, Mumbai, 400012, India

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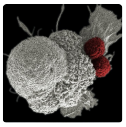
quercetin and ampelopsin inhibited stemness features in triple negative breast cancer cells.
Conclusion: Evidently flavonoids showed significant modulatory effects on cellular signalling pathways crucial for breast cancer progression. The ability of flavonoids to act on a wide range of mechanisms as well as on aggressive breast cancer types presents an array of hope. We recommend that further studies be done to ascertain the applicability of these compounds in treatment of breast cancers.

Keywords
Breast cancer, flavonoids, cellular molecular mechanisms, modulatory effects, chemotherapy



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Corresponding author: Mary Murithi (murithimary11@gmail.com)

Author roles: Murithi M: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Wambugu E**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Nyanjom S**: Conceptualization, Data Curation, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Review & Editing; **Mobegi V**: Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Review & Editing; **Shahin S**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing; **Makokha F**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

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Introduction

Breast cancer is the most commonly diagnosed disease in females worldwide (Sung et al., 2021). Of all the cancer cases in females, breast cancer leads with incidences and mortality rates of 2,261,419 (24.5%) and 684,996 (15.5%) respectively. According to the global cancer observatory (GLOBOCAN) 2020, these figures are projected to rise (Sung et al., 2021). This, therefore, calls for the urgent unearthing of interventions to help curb the disease.

Breast cancer is categorized into four molecular subtypes namely; Luminal A (estrogen receptor positive (ER+), progesterone receptor positive (PR+) and human epidermal growth factor 2 positive (HER2+)), Luminal B (ER+, PR+ and variable expression of HER2), HER2+ (ER-, PR- and HER2+) and basal like/triple negative (ER-,PR- and HER2-) (Eliyatkin et al., 2015). Treatment regimen of breast cancers is dependent on the type and stage of the disease. It incorporates either; surgery, radiotherapy or chemotherapy (targeted therapy) (Brufsky & Dickler, 2018). Luminal A constitutes 50% of the breast cancer cases, responds favourably to endocrine treatment and has good prognosis. Luminal B constitutes 20%, responds to endocrine treatment (tamoxifene and aromatase inhibitors). However, it has poor prognosis compared to luminal A. HER2+ constitutes 15%, responds to trastuzumab and anthracyclin therapy, and has unfavourable prognosis. The rare TNBC constitutes about 15%, does not show any response to either endocrine or trastuzumab therapy and has the worst prognosis (Eliyatkin et al., 2015). Alarmingly, up to date, no specific treatment has been developed for TNBC (Carels et al., 2016). Grave challenges with regards to disease metastasis, drug resistance and tumor relapse necessitates the search for alternative therapies to aid in combating the disease (Israel et al., 2018).

Breast cancer occurs in a multifaceted fashion. A range of risk factors may contribute to the development and progression of the disease. Among these are; demographics (female age and advanced age), reproductive (early menarche, late age of menopause, pre-term delivery, nulliparity, older age at first full term pregnancy), hormonal (oral contraceptives and post menopausal hormone replacement therapy), hereditary (genetic factors, history of breast cancer in first degree relatives), breast related (benign breast tumors), lifestyle (obesity and overweight, alcohol consumption, smoking, diet) and others (air pollution, higher socioeconomic status and exposure to radiation) (Eliyatkin et al., 2015; Kikuchi et al., 2019).

The mechanisms through which normal cells progress to carcinogenicity as outlined by Hanahan and Winberg in their review of the hallmarks of cancer are bountiful (Hanahan & Weinberg, 2011). Potentially, the hallmark processes present possible target mechanisms that can be leveraged for treatment. Healthy dietary choices such as regular consumption of fruits and cruciferous vegetables which are rich sources of phytochemicals have been reported to protect against various cancers including breast cancer (Vrhovac Madunić et al., 2018).

Phytochemicals are secondary metabolites released by plants in response to environmental cues which may be either biotic or abiotic factors (Ashraf, 2020). Flavonoids are a class of phytochemicals belonging to a group of phenolic compounds. Over 4000 flavonoids have been documented, some of which are known to play a significant role in the prevention and treatment of breast cancer (Liu, 2004). Flavonoids are structurally classified into six sub-types namely; flavanones (hesperetin), flavanols (epichatechin), flavones (apigenin, wogonin, baicalein), flavonols (kaempferol, quercetin), anthocyanidins (cyanidin) and isoflavonoids (genistein) (Abotaleb et al., 2019). They have been reported to modulate signalling mechanisms known to enhance development of breast cancer cells such as; proliferation, cell cycle, anti-apoptosis, angiogenesis, invasion and metastasis (Nkwe et al., 2021).

Although many flavonoids have been widely reported to exert anticancer effects, their mechanisms of action have not been fully elucidated (Kopustinskiene et al., 2020). Therefore, understanding these mechanisms is critical as they can be leveraged as targets for effective prevention and treatment of breast cancer. Secondly, flavonoids may be used to develop novel plant-based chemotherapeutic agents which are believed to harbour less side effects, are less toxic and more effective compared to the conventional regimens (Nkwe et al., 2021).

This review therefore, reports a number of flavonoids shown to have chemotherapeutic properties and profiles their mechanism(s) of action in different breast cancer cell lines and animal models.

Methods

Protocol and Registration

The results are reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). Scoping reviews purposes to; establish evidence available, elucidate key ideas, establish how research is done and determine knowledge gaps for a certain topic (Munn et al., 2018).

Eligibility criteria

Articles were included based on the following criteria;

1. The studies primarily focuses on the molecular mechanism of flavonoids in breast cancer
2. The studies to have employed either *in vitro* and/or *in vivo* approaches
3. The studies were published from 1st January 2017 up to 31st December 2021
4. The studies were in English language
5. The studies' full texts were available

Articles were excluded based on the following criteria;

1. The studies were reviews, short communications, editorials
2. The flavonoids were laboratory modified or synthetic
3. The studies were cohort, case-control or *in silico*
4. The studies were fractions from plant extracts
5. The studies were co-chemotherapies (combined flavonoids with conventional anti-cancers)

Information sources

Articles relevant to the study topic were searched and retrieved electronically from PubMed (https://pubmed.ncbi.nlm.nih.gov/advanced/), Hinari (https://portal.research4life.org/content/ hinari) and Google Scholar (https://scholar.google.com/) using the advanced search builders. The search from the databases was lastly done on 6th January 2022

Search strategy

An advanced search in three databases; PubMed, Google Scholar and Hinari were searched to identify peer-reviewed articles on breast cancer, breast cancer therapy using flavonoids and mechanism of action of flavonoids on breast cancer. Specifically, the search queries consisted of relevant medical subject headings (MeSH) and key words relevant to the topic. The search terms included;

Breast cancer AND flavonoids OR flavanones OR flavanols OR flavones OR flavonols OR anthocyanidins OR isoflavonoids

Selection of sources of evidence

Using the search strategy and filters outlined in the search strategy section, the articles obtained were further vetted. Assessment of the resulting articles was done independently by all the reviewers. Disagreements between them were resolved through consensus. First, articles from the initial search were obtained. Duplicate references were removed through manual deduplication. The titles and abstracts of the retrieved articles were screened for relevance to the study topic. Full-text reports were examined for compliance with eligibility criteria.

Data chatting process

Data chatting from the sources of evidence was first assessed independently and then discussed by the team to reach a consensus. The information abstracted was as shown in the table.

Data items

Selection of the review articles based on the molecular mechanism of action as the main outcome domain was guided by the following items;

1. Title of study
2. Year of publication – 2017-2021

3. Study objectives – in vitro or in vivo mechanisms of action of flavonoids in breast cancer

4. Study design – Experimental

5. Results – summary on findings on molecular mechanisms

6. Discussion – detailed explanation of the results and limitations of the review

7. Conflict of interest – Authors declare no conflict of interest

Synthesis of results

Results from the selected articles were tabulated in the summary of findings (Table 1). The methodologies and molecular mechanisms of action (interventions) were summarized. This enhanced comparison of interventions for the different breast cancer cells.

Results

Search results

Preliminary search done on the three databases yielded 370 articles. Following deduplication, 270 articles were screened through reviewing their titles and abstracts. A total of 175 articles were retrieved for full text review. The other 15 were not retrieved. Full text review against inclusion and exclusion criteria led to elimination of 139 articles. Reasons of exclusion are outlined in the PRISMA flow diagram of the scoping review.

Characteristics of sources of evidence

The characteristics for which data was abstracted from each source of evidence are described in Table 1. They include; article citation, flavonoid name, cell lines and/or animal models, key findings and postulated mechanism of action.

Synthesis of results

Thirty-six studies evaluating the modulatory effects of flavonoids on different breast cancer cell types were reviewed. The majority of them used *in vitro* assays (n=35). Most studies utilized MDA-MB-231 cell lines (n=24) followed by MCF-7 cell lines (n=20). Other cell lines used included; BT 474 (n=3), 4T1 (n=3), MDA-MB-468 (n=2), BT 549 (n=2), T47D (n=2), JIMT 1 (n=2) MDA-MB-435 (n=1), MDA-MB-231/IR (n=1), MCF-10A (n=1), SKBR3 (n=1) and MDA-MB-453 (n=1). A few of the studies used *in vivo* methods such as nude mouse model (n=2), xenograft mouse model (n=2), sub-cutaneous homotransplant mouse model (n=1) and mice bearing MCF-7 or SKBR3 xenografts (n=1). Overall, most studies reported induction of apoptosis as the most common molecular mechanism through which different flavonoids exerted their anticancer effects against breast cancer (n=18). Other mechanisms proposed included; inhibition of migration and invasion (n=10), cell cycle arrest at G1 phase (n=4), inhibition of angiogenesis (n=4), inhibition of metastasis (n=4), reduction of cell viability (n=4), reduction of cell proliferation (n=4), cell cycle arrest at G2/M phase (n=3), induction of autophagy (n=2), inhibition of migration and enhancement of motility (n=1), inhibition of intravasation (n=1), inhibition of breast cancer stem cell proliferation (n=1), inhibition of growth (n=1), enhanced pyroptosis (n=1) and reduction of breast cancer stemness (n=1).

Discussion

Summary of evidence

Thirty six studies published between 2017 and 2021 were reviewed. Our findings showed that flavonoids exert anticancer effects through modulation of key cellular signaling mechanisms. The reported mechanisms include; induced apoptosis and pyroptosis, inhibition of angiogenesis, inhibition of invasion and metastasis, cell cyle arrest, reduction of cell viability and proliferation, induction of autophagy, inhibition of inflammation, inhibition of proliferation of breast cancer stem cells, inhibition of growth. Knowledge on the effects of flavonoids on the modulation of cellular signaling mechanisms can be exploited to develop effective chemotherapeutic agents for breast cancer.

Cell viability

Cell viability using the MTT assay seemed to form a baseline against which a metabolite qualifies for further investigation. The studied flavonoids mainly displayed a dose and time-dependent effect on the variant breast cancer cell lines tested (Anaya-Eugenio et al., 2021; Attari et al., 2021; Go et al., 2018; Hallman et al., 2017; Hart et al., 2018; Hwang et al., 2020; Jia et al., 2018; Karimi et al., 2017; Li et al., 2017, 2018; Liang et al., 2021; Moradi et al., 2020; Qiu et al., 2019; Wang et al., 2021; Yu et al., 2018). The sensitivity of the flavonoids differed based on flavonoid type, concentration and cell line used. For instance, although both MCF-7 and MDA-MB-231 were sensitive to apigenin in a dose-dependent manner, MCF-7 cells were slightly more sensitive (Vrhovac Madunić et al., 2018).

Table 1. Results of individual sources of evidence.

	References	Findings	Proposed mechanism(s)
1.	(Moradi et al., 2020)	**Calycopterin** was tested in MDA-MB-231, MCF-7, HUVEC (control) cell lines Calycopterin reduced proliferation and cell viability in a dose and time dependent manner. No effects were seen on the HUVEC normal endothelial cells. It decreased colony formation. However, that of MCF-7 was more evident. Calycopterin inhibited cell migration There was increased sub G1 population Calycopterin increased apoptosis - Anti-apoptotic Bcl2 genes were down-regulated, pro-apoptotic Bax and caspase-3 genes were augmented in MDA-MB-231 cell lines while caspace-8 was increased in both cell lines	**Induction of apoptosis:** Apoptosis in MCF-7 cells was due to extrinsic pathways while that in MDA-MB-231 cells was due to both intrinsic and extrinsic pathways
2.	(Vrhovac Madunić et al., 2018)	**Apigenin** was tested on MCF-7, MDA-MB-231cell lines Dose-dependent morphological changes which were more pronounced in MDA-MB-231 cells Induced apoptosis - All concentrations of apigenin induced early apoptosis in MDA-MB-231 cells and conversely induced late apoptosis in MCF-7 cells. Induced lipid peroxidation in a dose-dependent manner albeit more pronounced in MDA-MB-231 cells. Induced genotoxicity in a dose-dependent manner but was more pronounced in MDA-MB-231 cells Treatment of normal cells with apigenin did not have any effect	**Induction of apoptosis:** Apigenin effected anticancer properties on MCF-7 and MDA-MB-231 cells via induced apoptosis as evidenced by neuron-like morphological changes, DNA damage, increased oxidative stress and cleavage of PARP-1
3.	(Harrath et al., 2021)	**Kaempferol-3-O-apiofuranosyl-7-O-rhamnopyranosyl (KARP)** was tested on MCF-7 cell lines KARP inhibited growth of MCF-7 cells in a dose-dependent fashion. It showed a dose dependent morphological changes consistent with apoptosis such as shrinkage, impaired cell density and loss of adhesion. It induced apoptosis - The percentage of early and late apoptotic cells increased proportionately to increase in KARP concentration It reduced quantities of ER1 and ER2 mRNA in a concentration-dependent manner	**Induction of apoptosis and autophagy**: There was increased generation of ROS in a concentration-dependent fashion hence increased cellular oxidative stress Reduced expression of ER1 hindered tumorigenesis of the breast cancer cell lines
4.	(Ko et al., 2020)	**Tangeretin** was tested on MDA-MB-231, MCF-7 cell lines *in vitro* and Nude mouse model *in vivo* Tangeretin impeded proliferation, migration and colony formation as well as mammosphere formation It enhanced late apoptosis It also inhibited mammosphere growth. It significantly suppressed the sub-population of CD44+/CD24- expressing cells among MCF-7 and MDA-MB-231 cells (breast cancer stem cells (BCSCs) Treatment of nude mouse model with tangeretin reduced both tumor weight and volume	**Induction of apoptosis:** Tangeretin suppressed tumour growth through enhancement of late apoptosis. Additionally, it retarded signal transducer and activator of transcription 3 (Stat3) signaling pathway and induced breast cancer stem cell (BCSC) death

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
5.	(Xiong et al., 2021)	**Rhoifolin (RFL)** was tested on MDA-MB-231, MDA-MB-468 cell lines RFL reduced proliferation in a dose and time-dependent manner It inhibited vertical migration of both cells in a dose-dependent manner It inhibited horizontal migration of both cells in a dose-dependent manner It disrupted the F actin arrangement inside the cell lines It exhibited reduced podocalyxin-Ezrin (PODXL-Ezrin) interaction. It reduced PODXL and Ezrin contents It reversed the expression of epithelial-mesenchymal translation (EMT) markers marked by upregulation of E-cadherin and downregulation of Vimentin, Twist, Slug and Sip1 Together with Ezrin-siRNA transfection of breast cancer cells inhibited cell motility	**Inhibition of migration:** RFL inhibited PODXL-Ezrin interaction hence promoting reduced EMT
6.	(Sotillo et al., 2021)	**Sideritoflavone** was tested on JIMT-1 cell lines Sideritoflavone reduced cell proliferation It reduced the migration of JIMT-1 cells into the wounded region It increased JIMT-1 cell mortility It did not have any colony forming effects It increased Wnt, Myc/Max and TGF-β It causedG2 phase accumulation of cells	**Cell cycle arrest at G2/M phase** as a result of c-Myc/Max pathway activation through double-strand break by sideritoflavone **Inhibition of migration and enhancement of motility** through enhancement of the TGF-β signaling pathway and an increased level of p65/NF-κB in sideritoflavone-treated JIMT-1 cells
7.	(Attari et al., 2021)	**Xanthomicrol** was tested on MDA-MB-231,MCF-7, 4T1 cell line *in vitro* and mouse fibroblast cells *in vivo* Xanthomicrol diminished the viability of in a concentration-dependent fashion. It increased the percentage of cells in early and late apoptosis compared to control It increased the percentage population of cells in G1 phase It reduced colony numbers There was a significant decrease in expression of miR21and miR27 genes. However, the expression of miR29 and miR34 genes was enhanced Treatment of mouse fibroblast cells with xanthomicrol resulted to a significant reduction in tumor volume. Lungs and livers of the animal models were normal after 14 days of treatment There was reduced tumor grade compared to control.	**Induction of apoptosis and cell cycle arrest:** Xanthomicrol induces early and late apoptosis and also causes arrest of the cell cycle at G1 Phase. Cell proliferation is also inhibited *in vivo.* **Inhibition of angiogenesis and metastasis:** Xanthomicrol also enhanced expression of tumor suppressor genes (miR29b and miR34) while repressing expression of tumor promoting genes like miR21, miR27 and miR125b. upregulation of caspase 9 and Bax protein

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
8.	(Sp et al., 2017)	**Nobiletin** was tested on MCF-7, T47D, MDA-MB-231, SKBR3, HUVEC (control) cell lines Nobiletin inhibited cell proliferation in all the test cell lines apart from the control It inhibited VEGF-dependent angiogenesis in a dose dependent manner. It inhibited expression of EGFR expression in a dose-dependent manner in MCF-7 cells. It inhibited Src/FAK/STAT3 signalling in MCF-7 and HUVEC cells. It inhibited expression of VEGF and bFGF angiogenic factors It downregulated transcription of the angiogenic factors in MCF7 and T47D cells It inhibited DNA/STAT3 complex expression in MCF-7 cells It inhibited migration of the MCF-7 cells It inhibited invasion in the MCF-7 cells Knockdown of STAT3 followed by treatment of nobiletin showed complete inhibition of STAT3 expression. There was decreased expression of phospho STAT3 and PXN	**Inhibition of angiogenesis** through inhibition of Src/FAK/STAT3/PXN, inhibition of angiogenic factors VEGF and bFGF. There was also inhibition of nuclear translocation of phosphorylated STAT3, Src, FAK and hence reduced STAT3/DNA binding **Inhibition of tumor cell migration and invasion** through reduced expression of MMP2, MMP3 and MMP9 to block new capillary formation
9.	(Anaya-Eugenio et al., 2021)	**5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (PMF)** was tested on MCF-7, MDA-MB-231, MCF-10A cell lines PMF reduced cell viability in a dose-dependent manner in the order of MCF7>MCF-10A and MDA-MB-231 Treatment of on MCF-7 cell lines with PMF induced loss of MMP, induced ROS overproduction, Decreased intrinsic Bcl2 and increased Bax and increased Bax, cytc, caspase 3 and 7 as well as PARP-1, induced cell cycle arrest at the G1 phase, Upregulated P53 and P21, reduced migration of PMF treated MCF-7 cells, inhibited NK-kB p65 PMF treated zebrafish did not show any toxicity	**Induction of apoptosis:** Activation of intrinsic apoptotic pathway **G1 cell cycle arrest** through upregulation of p53 and p21 which reduces cyclin E/CDK2 **Inhibition of migration** through inhibition of NF-kB
10.	(Zhu & Xue, 2019)	**Kaempferol** was tested on MDA-MB-231, BT474 cell lines Kaempferol showed a dose dependent anti-proliferative effect on both cell lines. However, the MDA-MB-231 cells were more responsive It decreased cell population in G1 and increased in the G2 phase. It also nduced apoptosis in a time-dependent manner	**Cell cycle arrest** at the G2/M **Induction of apoptosis:** Kaempferol induced DNA damage proteins ATM, inhibited DNA repair through phosphorylating H2AX in MDA-MB-231 cells and induced apoptotic proteins caspase 9 and 3
11.	(Chen et al., 2018)	**Delphinidin (anthocyanin)** was tested on MDA-MB-453, BT474 cell lines Delphinidin reduced proliferation in a dose-dependent manner It increased fragmentation, increased transformation of GFP-LCI to GFP-LCII. It upregulated expression of atg5-atg12 conjugate protein complex. There was decreased phosphorylation of AKT/mTOR/eIF4e/p70s6k proteins and activated expression of LKBI/AMPK/ULK1/FOXO3a It enhanced formation of autophagosomes in the cytoplasm It increased amount of GFP-LC3 with increase in delphinidin which increased autophagosomes	**Protective autophagy** occurred through suppression of AKT/mTOR/eIF4e/p70s6k & activation of LKBI/AMPK/ULK1/FOXO3a

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
12.	(Yao et al., 2017)	**Wogonoside** was tested on MDA-MB-231, MDA-MB-435, BT-474, MCF-7 cell lines Wogonoside; Did not cause any significant cytotoxicity Decreased adhesive capabilities of TNF-α induced MDA-MB-231, MDA-MB-435, and BT-474 cells and TNF-α + TGF-β1-induced MCF7 cells Inhibited cell migration in TNF-α induced MDA-MB-231, MDA-MB-435, and BT-474 cells and TNF-α + TGF-β1-induced MCF7 cells Inhibited cell invasion of TNF-α induced MDA-MB-231, MDA-MB-435, and BT-474 cells and TNF-α + TGF-β1-induced MCF7 cells Increased mRNA expression levels of E-cadherin while those of vimentin, MMP-9 and Twist1 were decreased. Inhibited TRAF2 expression Increased expression of E-cadherin and decreased expression of MMP-9, MMP-2, CD44v6, vimentin in TNF-α induced MDA-MB-231 and MDA-MB-435 cells and TNF-α + TGF-β1-induced MCF7 cells which resulted to a decreased expression of Twist1 Proteins Inhibited NF-κB signaling Decreased expression of proteins associated with metastasis including; Twist1, vimentin, MMP-9, TNF-α, TRAF2 and TRAF4 in the late stage of metastasis while that of E-cadherin was increased. Decreased tumor weight in the wogonoside-treated group similar to that of the gemcitabine-treated control group. Wogonoside-treated MDA-MB-231 cells suppressed metastasis to the lungs, brain, liver and bone.	**Inhibition of migration** through suppression of EMT and inhibition of invasion and migration. Wogonoside treatment inhibited NF-kB signalling pathway and suppressed Twist1 transcription through downregulation of TRAF2 and TRAF4 expression. Proteins favoring metastasis such as MMP-9, MMP-2, CD44v6, vimentin and TNF-α were decreased while anti-metastatic E-Cadherin was increased
13.	(Go et al., 2018)	**Wogonin** was tested on MDA-MB-231 cell lines Wogonin down-regulated expression of IL-8, MMP9, BLT2 mRNA It inhibited the formation of BLT2 ligands. Depletion of BLT2 decreased induction and protein expression of IL-8 and MMP9 Wogonin inhibited invasion of MDA-MB-231 cells Wogonin inhibited the formation of IL8 and LTB4 in a dose dependent manner MDA-MB-231 cells pre-treated with wagonin, implanted into the mammary fat pads of mice showed a reduced number of nodules in the small bowel 14 weeks post-implantation	**Inhibition of invasion and metastasis** through inhibition of 5-LO/BLT2/ERK/IL-8/MMP-9 cascade
14.	(Terabayashi et al., 2018)	**Baicalein** was tested on MDA-MB-231cell lines Treating MDA-MB-231 cells with human mammary epithelium-derived cell line (MCF10A CM) resulted in elongated shapes in the breast cancer cell lines. There was increased migration of MDA-MB-231 cells when treated with MCF10A CM MS of the fractions that showed induced migration and morphological changes consisted of laminin (LN) α- 3, β- 3, and γ- 2 chains Immunoblotting confirmed the presence of LN γ- 2 chain MDA-MB-231 cells when treated with MCF10A CM cells exhibited quick formation of lamellipodia, increased migration and elongated morphology. Baicalein pre-treatment reduced the elongated morphology of these cells	**Inhibition of morphological changes and migration**. Baicalein suppressed the elongated morphological changes induced by conditioned medium from MCF10A and LN-332 on MDA-MB-231 cells. This is achieved through lamellipodia formation

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
15.	(Li et al., 2017)	**Calycosin** was tested on MCF-7, T47D cell lines There was dose-dependent effect on the viability of both cell lines on treatment with calycosin Upon treatment with calycosin the cell lines showed reduced migration, motility and invasion in a dose and time-dependent manner Decreased FoxP3 mRNA transcripts in a dose-dependent manner Decreased FoxP3 protein levels in a dose-dependent manner	**Inhibition of migration** and invasion of the breast cancer cells through down-regulating the expression of forkhead box P3 (Foxp3) transcription regulators. Reduced Foxp3 inhibits MMP-9 and VEGF activities
16.	(Mu et al., 2017)	**Glabridin (GLA)** was tested on MDA-MB-231 cell lines There was no significant effect of GLA on MDA-MB-231 cells compared to control cells. There was decreased mRNA expression of lymphoid enhancer factor/T-cell factor 4 (LEC/TCF4) upon treatment of MDA-MB-231 cells with GLA There was decreased protein expression of Wnt1 and active β-catenin upon treatment of MDA-MB-231 cells with GLA β-catenin which was distributed in both the nucleus and cytoplasm in MDA-MB-231 cells translocated to cytosolic membranes upon treatment with GLA. Treatment of MBA-MD-231 cells transfected with anti-miR-148a caused a further decrease in Wnt/β-catenin signalling pathway molecules	**Inhibition of angiogenesis** through dysregulation of Wnt/β-catenin signalling pathway molecules such as LEC/TCF4 on treatment with GLA. The GLA treatment also reduced VEGF secretion
17.	(Lee et al., 2020)	**Aviculin** was tested on MCF 7 cells Aviculin suppressed MCF-7 cells in a dose dependent manner Aviculin incubated cells were characterized with blebbed, shrunk and condensed nucleus Cells incubated with aviculin had increased levels of caspases9 and 7, Bax and decreased levels of Bcl-2	**Induction of intrinsic mitochondria mediated apoptosis** resulting from increased expression of pro-apoptotic proteins caspase-9, -7, PARP and Bax and simultaneous decreased expression of anti-apoptotic protein Bcl-2
18.	(Hallman et al., 2017)	**Epigallocatechin-3-gallate (EGCG)** was tested on T47D cell lines EGCG suppressed the expression of estrogen receptor (ERα) protein and progesterone receptor (PR) protein in a dose dependent fashion EGCG led to remarkable decrease in viable T-47D cells	**Reduced cell viability** mediated through steroid receptors, (ERα) and PR
19.	(Hong et al., 2018)	**Apigenin and Luteolin** were tested on MDA-MB-231 cells Both compounds inhibited circular chemorepellent induced defect (CCID) of MDA-MB-231 cells in LEC monolayers in a dose-dependent manner. Luteolin effect was stronger. In addition, both compounds synergized to inhibit CCID formation They both inhibited MMP1 expression and CYP1A1 activity When combined with BAPTA-AM (intracellular Ca2+ inhibitor), they both inhibited CCID When combined with U73122 (PLC inhibitor), they both inhibited CCID formation Both compounds also inhibited phosphorylation of Tyr397-FAKin LEC	**Inhibition of intravasation** of MDA-MB-231 cells through interference of MMP1 expression and CYP1A1 activity as well as inactivation of FAK hence reduced CCID formation

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
20.	(Hwang et al., 2020)	**Geristin (GS)** was tested on MCF7 and MDA-MB 231 cells Gs significantly decreased cell viability in MCF-7 cells, an effect which was negligible in MD-MB-231 cells Gs led to cell cycle arrest at G1 phase GS impeded nuclear translocation of ERα and also inhibited DNA-ERα binding Enhanced apoptosis observed among GS-treated ER+ MCF-7 cells GS suppressed expression of antiapoptotic proteins (Bcl-2, Bcl-xL, Survivin), proliferative protein (COX 2), cell cycle regulatory protein (Cyclin D1), angiogenic protein (VEGF) and metastatic protein (MMP-9) in MCF-7 cells	**Induction of of apoptosis in MCF-7 cells only** through activation of caspases 8 and 9 in addition to induction of PARP cleavage Inhibition of ER signalling pathway in ER+ MCF-7 cells by inhibiting translocation of ERα into the nucleus and ERα-DNA binding
21.	(Jia et al., 2018)	**Quercetin** was tested on MCF-7 and MDA-MB-231 cells *in vitro* and xenograft mouse model *in vivo* It decreased cell viability in a dose dependent manner It suppressed cell invasion and mobility through reduction of MMP-2, MMP-9 and VEGF It inhibited glycolysis through inhibition of glucose uptake, reduced lactic acid production, reduced key glycolytic enzymes; pyruvate kinase M22 (PKM2) and lactate dehydrogenase A (LDHA) as well as reduced glucose uptake protein (GLUT1) Quercetin also suppressed glycolysis and cell mobility through Akt-mTOR pathway mediated autophagy Inhibition of tumor metastasis and glycolysis *in vivo* was evidenced by reduced tumor growth, reduced expression of VEGF in tumor tissue, down regulation of PKM2, suppression of Beclin 1 (autophagy marker protein) and decreased p-AKT/AKT ratio	**Inhibition of tumor growth and metastasis** through attenuation of glycolysis by quercetin and induction of Akt-mTOR pathway mediated autophagy
22.	(Karimi et al., 2017)	**Silibinin** was tested on MCF-7 cells Silibinin reduced cell viability of MCF-7 in a concentration and time dependent fashion Expression of ERα gene and maspin was suppressed in silibinin treated cells	**Reduced cell viability** mediated through reduced expression of ERα which can serve as a transcriptional factor
23.	(Li et al., 2019)	**Pectolinarigenin (Pec)** was tested on 4T1, MDA-MB-231 and MCF-7 cells in *vitro* and tumor xenograft mice model incubated with 4T1 cells *in vivo* Pec inhibited metastasis in vivo Pec inhibited migration and invasion of breast cancer cells in vitro through downregulation of p-stat3, MMP-2, MMP-9 and upregulation of TIMP2 Pec reduced breast cancer cell proliferation and triggered cell apoptosis through decreased mitochondrial membrane potential, decreased Bcl-2, increased Bax and cleaved caspase-3	**Reduced cell proliferation, invasion, metastasis and increased apoptosis** brought about by inhibition of signal transducer and activator of transcription 3 (stat3) signalling pathway

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
24.	(Li et al., 2018)	**Quercetin was tested on MCF-7 cell lines** *in vitro* and **nude mice xenografts** *in vivo* Quercetin induced G1 phase cell cycle arrest in MCF-7 cells Quercetin retarded the viability, clone formation and mammosphere formation in CD44+/CD24− cancer stem cells (CSCs) subpopulation of MCF-7 cells There was suppression of tumorigenicity and metastasis of CD44 +/CD24− cancer stem cells in nude mice models Expression of m-TOR, p-m-TOR, PI3K, p-PI3K, Akt, and p-Akt in quercetin treated CSCs was depressed Quercetin treated MCF-7 cells showed diminished mRNA expression of ERα, Cyclin D1, and B cell lymphoma 2 (Bcl-2) in quercetin-treated MCF-7 cells and remarkably increased Bcl-2-like protein 4 (Bax) expression	**Reduced cell viability** through cell cycle arrest at G1 phase and increased apoptosis cell cycle arrest was as a result of reduced expression of Cyclin D1 **Inhibition of CSCs proliferation** through inhibition of PI3K/Akt/mTOR-signaling pathway
25.	(Liang et al., 2021)	**Liquiritigenin** was tested on TNBC cell lines MDA-MB-231 and BT549 The compound decreased cell viability and colony formation of both cells in a dose dependent manner Liquiritigenin inhibited invasion, migration and EMT in the breast cancer cell lines. There was reduced expression of E-cadherin and increased expression of N-cadherin, vimentin and MMP-9 proteins It increased mRNA and protein expressions of BRCA1 There was increased expression of p21 and growth arrest and DNA-damage-inducible 45 alpha (GADD45A) There was decreased DNA methyl trensferase (DNMT) activity as well as decreased BRCA1 methylation activity	**Reduced cell proliferation, invasion and migration as well as increased apoptosis** may be as as a result of decreased DNMT activity and increased BRCA1 *
26.	(Liu et al., 2021)	**Corylin** was tested on MCF-7 and MDA-MB-231 cells Corylin decreased cell proliferation, invasion and migration Decreased EMT inducing gene expression of vimentin and SNA1 Increased CDIH gene expression Increased miR-34c gene expression hence reduced long non coding RNA LIN00963	**Reduced migration, invasion and EMT** of breast cancer cells through miR-34c/LIN00963 target
27.	(Pham et al., 2021)	**Apigenin** was tested on MCF-7 cells It reduced proliferation of MCF-7/Akt cells It also decreases expression of cyclin B1, MCF7/Akt cells hence G2/M arrest	**Inhibition of cell proliferation, increased G2/M phase cell cycle arrest and increased apoptosis.** This resulted from inhibition of AKT/FOXM1 signalling pathway
28.	(Qiu et al., 2019)	**Hyperoside** was tested *in vitro* on MCF-7 cells and 4T1 cells and in vivo on subcutaneous homotransplant mouse model Decreased cell viability was observed among hyperoside treated MCF-7 and 4T1 cells Apoptosis was enhanced in dose dependent fashion in hyperoside treated cells Upregulation of Bax, cleaved caspase-3 and cleaved PARP Reduced intracellular ROS levels in 4T1 cells thus attenuating activation of the NF-κB signalling cascade The average tumour size was decreased in hyperoside treated subcutaneous homotransplant mice group compared to control	**Induction of apoptosis** through inhibition of NF-κB signalling cascade caused by reduced intracellular ROS levels. This leads to downregulation of XIAP, Bcl-2 and upregulation of Bax

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
29.	(Abd Razak et al., 2020)	**Eupatorin** was tested on BALB/c mice 4T1 challenged mice *in vivo* Treatment of the mice with eupatorin caused a dose and time dependent inhibition of proliferation towards 4T1 cells Eupatorin increased the number of apoptotic cells post-treatment Eupatoring inhibited metastasis to the lungs in the triple negative mouse model Eupatolin increased production of 1.1 and CD3 NK cells, CD8 and reduced that of CD4+ cells	**Inhibition of inflammation and increased apoptosis** through down-regulation of pro-inflammatory cytokines such as TNF-α and IL-1β hence lowering NF-κB expression.
30.	(Truong et al., 2021)	**Ampelopsin** tested on MDA-MB-231/IR cell line, which is enriched in CSCs Ampelopsin reduced colony formation and proliferation of stem cell rich MDA-MB-231/IR cells Enhanced apoptosis of MDA-MB-231/IR cells Stemness features of MDA-MB-231/IR cells were decreased (reduced mammosphere generation, the CD44+/CD24−/stunted population, aldehyde dehydrogenase activity, and the amount of stem cell markers (CD44, MRP1, β-catenin, and KLF4) by treatment with ampelopsin Reduced migration, invasion ability and mesenchymal markers in addition to enhanced expression of E-Cadherin among ampelopsin treated cells Downregulation of OXPHOS-related genes in ampelopsin treated cells which impairs mitochondrial metabolism Ampelopsin blunted oxidative phosphorylation which resulted in decreased amount of phosphorylated IκBα and NF-κB p65	**Inhibition of invasion and migration** through diminished epithelial mesenchymal transition and Inhibition of TNF-α/NF-κB signalling pathway
31.	(Tian et al., 2017)	**Calycosin** was tested on MDA-MB-468, SKBR3, MCF-7, T47D, MDA-MB-231 and MCF10A cell lines *in vitro* and on mice bearing MCF7 or SKBR3 xenografts *in vivo* Calycosin inhibited cell proliferation in both ER+ (MCF-7, T47D) and ER- (MDA-MB-468, SKBR3) cell lines in a dose dependent manner. It had no effect on the normal MCF10A cells or MDA-MB-231 cells which are GPR30 deficient. Its effects were more pronounced in the ER+ cell lines. Calycosin inhibited phosphorylation of SRC, EGFR, ERK1/2 and Akt in GPR30 positive cell lines (MDA-MB-468, SKBR3, MCF-7, T47D) Calycosin also inhibited tumor growth and volume in mice bearing MCF7 or SKBR3 xenografts. Calycosin increased expression of WDR7-7 and reduced that of GPR30 in xenografts	**Inhibition of growth** in ER+ cell lines through downregulation of miR-375-ERα feedback loop. **Inhibition of proliferation** through inhibition of WDR7-7-GPR30 signalling pathway (esterogen independent mechanism)
32.	(Tsai et al., 2021)	**Luteolin** was tested on **MDA-MB-231 cells** The expression of stemness properties (ABCG2, CD44, Oct4, Sirt3, ALDH1, and also Cripto-1) of MDA-MB-231 cells treated with luteolin was reduced Expression of antioxidant proteins (Nrf2 and Sirtuin 3;Sirt3) was downregulated in luteolin treated cells	**Suppression of breast cancer cells stemness through** Nrf2-mediated pathway

Table 1. *Continued*

	References	Findings	Proposed mechanism(s)
33.	(Wang et al., 2021)	**Nobiletin** was tested on **MCF-7, BT-549 BC** cell lines and **MCF 10A** normal breast epithelial cells There was a concentration-dependent reduction in viability of BC cells treated with nobiletin Nobiletin remarkably enhanced expression of NLRP3, cleaved caspase 1, IL-1β, IL-18, and ASC Induction of pyroptosis and apoptosis in nobiletin treated BC cells Treatment with nobiletin resulted in significant downregulation of JAZF1 expression in nobiletin treated BT 549 cells Nobiletin suppressed nuclear translocation of IκB	**Enhanced pyroptosis and apoptosis** mediated through, modulation of miR-200b/JAZF1 pathway
34.	(Xu et al., 2019a)	**Isoquercitrin (IQ)** was tested on MDA-MB-231 cells Treatment of MDA-MB-231 cells with IQ inhibited lysine demethylase I (LSD-1) induced proliferation	**Enhanced apoptosis** resulting from decreased mitochondrial transmembrane potential and increased Bax/Bcl2 ratio
35.	(Yu et al., 2018)	**Baicalein** was tested on MCF-7 and MDA-MB-231 cells Baicalein activated lncRNA PAX8-AS1-N expression in breast cancer cells Increased PAX8-AS1-N expression decreased cell viability, inhibited cell cycle progression at G1 phase and enhanced apoptosis	**Decreased cell viability, G1 cell cycle arrest and enhanced apoptosis** was as a result of activated baicalein/PAX8-AS1-N/miR-17-5p/PTEN, CDKNIA,ZBTB4 regulation axis
36.	(Zhang et al., 2020)	**3-(4-methoxyphenyl) quinolin-4(1H)-one (MEQ)** was tested on MDA-MB-231 and 4T1 TNBC cell lines MEQ enhanced SerRS expression through interaction with MTA2 to inhibit expression of VEGFA and TNBC angiogenesis MEQ inhibited tumor growth when administered to bc allograft mouse model MEQ inhibited growth of MDA-MB-231 xenografts	**Inhibition of angiogenesis** through regulation of MTA2/SerRS/VEGFA axis *in vitro* as well as inhibition of proliferation and enhancement of apoptosis *in vivo*

Cell cycle arrest

Cell cycle arrest is a major mechanism through which some potent conventional anticancer drugs exert their cytotoxic action. The cell cycle is a complex molecular process that is closely regulated by several essential molecular compounds such as cyclin-dependent kinases (CDK) and CDK inhibitors such as p21 and p27 (Kikuchi et al., 2019; Lin et al., 2015). Downregulation of some CDK subfamilies and upregulation of p21, p27 and p53 has been associated with cell cycle arrest (Fan et al., 2014). Transcription factors such as Forkhead box transcription factor (FOXO3a) are also heavily involved in the modulation of cell cycle progression and apoptosis (Lin et al., 2015; Yuan et al., 2018). Recent research has revealed that upregulation of p21 and p27 causes increased expression of FOXO3a and subsequently, arrest of the MCF-7 cell cycle at G0/G1 phase (Yuan et al., 2018). Further results from studies suggest that FOXO3a is involved in the evolution of breast cancer and could even serve as a prognostic factor (Lin et al., 2015; Yao et al., 2017; Yuan et al., 2018). A few anticancer drugs have been shown to augment the expression of such factors. Cell cycle arrest at G1, G2/M and S can eventually result in apoptosis of the cancerous cells (Lin et al., 2015). Flavonoids such as; xanthomirol, calycopterin, PMF, genistin, quercetin and baicalein were shown to arrest the BC cell cycle at G1 phase (Anaya-Eugenio et al., 2021; Attari et al., 2021; Hwang et al., 2020; Li et al., 2018; Yu et al., 2018). On the contrary, other flavonoids caused arrest of cell cycle at G2/M phase. This observation was evident with sideritoflavone, kaempferol, apigenin (Pham et al., 2021; Sotillo et al., 2021; Zhu & Xue, 2019). These effects were determined using flow cytometry. Sideritoflavone induced DNA double-strand break which consequently led to activation of c-Myc/Max pathway and eventually led to arrest of the cell cycle at G2/M phase (Sotillo et al., 2021). The findings were further evidence of anti-proliferative efficacy of these flavonoids against breast cancer. Findings from the reviewed studies have affirmed that impeding the proliferative activity of cancerous cells ultimately suppresses the tumorigenic and malignant potential of these cells.

Inhibition of colony formation

A number of studies assessed the effect of specific flavonoids on colony formation of breast cancer cell lines such as MCF-7, MDA-MB 231, MDA-MB-468. Xanthomicrol, calycopterin and tangeretin exhibited inhibitory effects against colony formation of breast cancer cell lines (Attari et al., 2021; Ko et al., 2020; Moradi et al., 2020). However, sideritoflavone did not affect JIMT1 cell lines colony formation (Sotillo et al., 2021).

Tumour angiogenesis

Angiogenesis is integral to metastasis of breast cancer (De Palma et al., 2017; Ferrara, 2002). Most cancerous cells have the intrinsic potential of producing tumour angiogenic factors that promote the formation of new blood vessels. Tumour cell hypoxia is presumed to prompt the production of chemicals which enhance angiogenesis (Giverso & Ciarletta, 2016; Sp et al., 2017). Several flavonoids were shown to attenuate angiogenesis in breast cancer cell lines. Nobiletin was found to inhibit angiogenesis in ER+ breast cancer cell lines by blocking both the angiogenesis signalling cascade (Src/FAK/STAT3 pathway) and angiogenic factors such as VEGF and FGF. Further findings from the study revealed that nobiletin inhibited the expression of MMPs that are critical for angiogenesis (Sp et al., 2017). Glabridin was also shown to exert anticancer effects through a similar mechanism (Hsu et al., 2011). Glabridin has also been reported to have antiangiogenic effects on TNBC cell lines. For instance, Mu et al., established that glabridin harboured a significant antiangiogenic effect on MDA-MB-231 cell lines that was mediated through blockade of Wnt/β-catenin signalling pathway (Mu et al., 2017). Previously, Mu et al. (2015) had established glabridin to have antiangiogenic effect on MDA-MB-231 and Hs-578T cells. However, this effect was mediated through inhibition of NF-kB/IL-6/STAT-3 axis which reduced transcription of VEGF (Mu et al., 2015). Xanthomicrol was also found to have appreciable antiangiogenic effect that was exerted through attenuation of angiogenesis promoting factors VEGF and MMP9 as a result of overexpression of miRNA29b. This was modulated through reduced expression of miR27 that further led to inhibition of ZBTB10 and VEGF receptors and enhanced expression of miR29b which inhibits metastasis by modulating angiogenic and metastasis promoting factors (Attari et al., 2021). MEQ inhibited angiogenesis through regulation of MTA2/SerRS/VEGFA axis (Zhang et al., 2020).

Inhibition of migration and invasion (Anti-metastasis)

The ability of tumor cells to migrate and invade normal tissue has profound effects on the capacity of these cells to metastasize and thus affect distant organs which negatively influences the outcome of patients with cancer (Chen et al., 2014). Matrix metalloproteinases (MMPs) play an integral role of breaking down the surrounding extracellular matrix (ECM) that allows the cancer cells to access the circulatory system (Chen & Liu, 2018). Successful metastasis is dependent on the ability of cancer cells to migrate and invade surrounding tissue. This may be achieved through epithelial to mesenchymal phenotypic morphological changes (Xiong et al., 2021). The epithelial-mesenchymal transition (EMT) process can be demonstrated through the expression of biomarkers (E-cadherin, Vimentin, Snail, slug, Twist, FOXC2) and modulation of signalling pathways (NF-κB signaling). Rhoifolin was shown to cause inhibition of ezrin and subsequently decreased interaction between PODXL and ezrin which as a result led to decreased metastatic ability of the breast cancer cells. Moreover, rhoifolin decreased expression of E-Cadherin and also increased expression of vimentin (Xiong et al., 2021). Similarly, liquiritigenin inhibited migration and invasion through a similar mechanism

(Liang et al., 2021). Sideritoflavone inhibited JIMT 1 cell line migration but enhanced their motility by causing an increase in p65/NF-κB and activation of TGF-β signaling pathway (Sotillo et al., 2021). These findings can be compared to those of Anaya-Eugenio et al who also concluded that PMF inhibited migration of MCF-7 cells through reduced expression of p65/NF-κB (Anaya-Eugenio et al., 2021). Yao et al. established that wogonoside exhibited antimetastatic effect through an array of mechanisms which included suppression of EMT in addition to inhibition of invasion and migration of TNF-α induced MDA-MB-231, MDA-MB-435, and BT-474 cells and TNF-α + TGF-β1-induced MCF7 cells (Yao et al., 2017). This was achieved through inhibition of NF-κB signaling. Comparatively, ampelopsin inhibited invasion and migration of MCF-7 and MDA-MB-231 cells through diminished epithelial mesenchymal transition and inhibition of TNF-α/NF-κB signalling pathway (Truong et al., 2021). Wogonin reduced (Lipopolysaccharide) LPS induced metastasis of MDA-MB-231 cells by inhibiting 5-Lipoxygenase/leukotriene B4 Receptor 2 (5-LO-/BLT2) pathway (Go et al., 2018). Baicalein was reported to inhibit morphological changes of MDA-MB-231 cancer cells through interruption of lamellipodia formation (Terabayashi et al., 2018). Further findings by Li et al suggested that calycosin diminished migration and invasive ability of MCF-7 and T47 D cancer cells via down-regulation of FOXP3 and hence down regulation of VEGF and MMP-9 (Li et al., 2017). Consequently, targeting the migration ability of breast cancer cells could provide a critical breakthrough in the development of potent chemotherapeutic drugs. Some studies have further investigated the effects of flavonoids on migration of the cell lines which is an integral characteristic of malignant tumour cells using in vitro tests such as Scrape wound healing assay (Sotillo et al., 2021), AP 48 chamber system to assess vertical migration and Oris™ cell migration assay (Xiong et al., 2021), Gap closure and transwell migration assay (Loung et al., 2019). A similar effect of inhibition of migration of the malignant cell lines was observed in these studies. Pec inhibited migration and invasion of MDA-MB-231, MCF-7 and 4T1 cells in vitro through down-regulation of p-stat3, MMP-2 and MMP-9 hence inhibiting stat3 signalling pathway (Li et al., 2019). Corylin reduced migration, invasion and EMT of MCF-7 and MDA-MB-231 cells through miR-34c/LIN00963 target (Liu et al., 2021).

Autophagy

Autophagy is a process through which the cell cytoplasmic contents are engulfed in vesicles, bind with lysosomes and then undergo degradation resulting in protein and ATP production (Han et al., 2018; Jain et al., 2013; Karantza & White, 2007). On one hand, autophagy can promote cell survival while on the other, it can contribute to cell death. Therefore, it can either have a positive or negative effect on tumorigenesis (Han et al., 2018; Jain et al., 2013). It is mainly regulated by the mTOR pathway. Han et al reckon that the activated mTOR pathway enhances progression of tumour and as a result has a deleterious effect on the survival of patients with breast cancer. Thus, increased expression of mTOR in breast cancer is a poor prognostic factor (Han et al., 2018). Chen et al concluded that delphinidin augmented protective autophagy through repression of AKT/mTOR/eIF4e/p70s6k signalling cascade and stimulation of the LKB1/AMPK/ULK1/FOXO3a signalling cascade in HER-2 positive MDA-MB-453 nad BT 474 cells (Chen & Liu, 2018). Quercetin also suppressed glycolysis and cell mobility through Akt-mTOR pathway mediated autophagy (Jia et al., 2018).

Apoptosis

Aberrant control of apoptotic cell death eventually leads to inactivation of apoptosis and has been linked to the pathogenesis of several diseases including cancer (Abotaleb et al., 2019; Kikuchi et al., 2019). Cancer cells usually exhibit resistance to apoptosis through enhanced expression of pro-oncogenes (*c-Myc* which enhances proliferation and suppresses p53) and anti-apoptotic proteins such as Bcl-2, survivin and livin. In contrast, cancer cells also resist apoptosis by downregulating apoptotic proteins such as caspases, Bad and Bax in addition to enhancing the loss of tumor suppressor function of p53(Campbell & Tait, 2018). Natural tumor suppressor factors such as p53 gene will exert their action through induction of apoptosis and thus dysregulation of such factors is an important molecular mechanism for the development of malignancies such as breast cancer (Whibley et al., 2009).

In most of the papers reviewed, induction of apoptosis was showed to be a major mechanism by which breast cancer progression was attenuated by flavonoids. Flavonoids including calycopterin, apigenin, KARP, tangeretin, xanthomycrol, PMF, kaempferol, aviculin, genistin, PEC, hyperoside, nobiletin, quercetin, liquiritigenin, isoquercitrin, ampelopsin, MEQ induced apoptosis in different BC cell lines. The mechanisms involved included reduction in expression of antiapoptotic genes such as Bcl2, XIAP and increased expression of Bax, PARP 1, caspases 3, 7, 8, 9. Other mechanisms included increased mitochondrial oxidative stress, downregulation of mitochondrial membrane potential, inhibition of NF-κB, modulation of miR-200b/JAZF1 pathway and activation of PAX8-AS1-N/miR-17-5p/PTEN, CDKNIA,ZBTB4 regulation axis (Abbaszadeh et al., 2018; Anaya-Eugenio et al., 2021; Attari et al., 2021; Harrath et al., 2021; Hwang et al., 2020; Ko et al., 2020; Lee et al., 2020; Liang et al., 2021; Moradi et al., 2020; Pham et al., 2021; Qiu et al., 2019; Truong et al., 2021; Vrhovac Madunić et al., 2018; Wang et al., 2021; Xu et al., 2019b; Yu et al., 2018; Zhang et al., 2020; Zhu & Xue, 2019).

Limitations
We acknowledge that we restricted the review to articles only published in English. This might have eliminated some articles with useful information on the scope of the study. Another limitation is the articles reviewed used different methodologies and breast cancer cell lines and/or *in vivo* models hence making it difficult to compare the findings. The enlisted limitations notwithstanding, we believe that the findings of this study were not significantly affected,

Conclusions
Dietary flavonoids modulate anti-cancer effects through various mechanisms such as; cell cycle arrest, enhanced apoptosis, inhibited proliferation, invasion, angiogenesis and metastasis. Their safety, easy availability and anti-tumour effect may render them as effective alternative strategies for battling breast cancer. We recommend that further research be done to explore the possibility of using flavanoids as lead molecules for anti-cancer drug development.

Data availability
Underlying data
All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines
Harvard Dataverse. Murithi, Mary, 2022, "Replication Data for Molecular mechanisms of flavonoids and their modulatory effects against breast cancer: A scoping review", DOI: https://doi.org/10.7910/DVN/V5YGQP, Harvard Dataverse, DRAFT VERSION.

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Open Peer Review

Current Peer Review Status: ? ? ✗

Version 1

Reviewer Report 12 October 2023

https://doi.org/10.5256/f1000research.120351.r198139

✗ **Nupur Mukherjee**
Department of Molecular and Cellular Biology, ICMR-National Institute for Research in Reproductive and Child Health, Mumbai, 400012, India

In the present study, the authors have tried to enumerate and evaluate the outcomes of in-vitro and in-vivo studies focused on analyzing antitumor potential of different flavonoids in breast cancer. Following are the comments regarding the systematic review:

Major concern

1. The authors have mentioned in their manuscript title that they are exploring both "molecular mechanisms of flavonoids and their modulatory effects against breast cancer". It is not clear why they have only preclinical studies in the manuscript. Why have they excluded cohort, case-control or in silico based studies which better reflect the antitumor potential of phytochemicals in breast cancer. Also why have they not included "studies were co-chemotherapies (combined flavonoids with conventional anti-cancers)" in exclusion criterion? There are papers which have indicated a role of phytochemicals in improving chemotherapy response in breast cancer [eg: PMID: 33357918[1], PMID: 29635751[2], PMID: 31593704[3]].

2. The in-vivo studies should be discussed more elaborately as they more closely reflect the impact of flavonoid treatment on physiology and process of breast tumor progression

3. Also the impact on tumor immune responses should have been discussed

4. Since the article is systematic review, the methodologies should have been same in each paper, or the cell lines/animal model, patient cohort description should have been same for each study selected for review.

Minor comment

1. In table 1, the class/ subtype of flavonoid studied should have been included

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Are the rationale for, and objectives of, the Systematic Review clearly stated?
No

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Breast cancer biology and immunology, molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 22 September 2023

https://doi.org/10.5256/f1000research.120351.r198136

?

Sandip Patel
Department of Pharmacology, L.M. College of Pharmacy, Ahmedabad, India

The authors elaborated on the role of flavonoids in breast cancer. However, certain information is still required to be incorporated.

1. The source of flavonoids needs to be clarified as many of the flavonoids are derived from plants or by synthesis and for many flavonoids the sources are not mentioned.

2. Many flavonoids work through multiple molecular mechanisms. The author illustrates the molecular mechanisms of flavonoids through the schematic presentation or by graphics which make it very easy to understand the role of flavonoids in breast cancer and ultimately support the conclusion.

3. Authors need to discuss the current status of flavonoids in clinical practice. What are the potential problems that may hinder the usefulness of flavonoids in breast cancer?

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Partly

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Pharmacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 24 August 2023

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Muthi Ikawati
Laboratory of Macromolecular Engineering, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Special Region of Yogyakarta, Indonesia

The article is a scoping review focused on curating molecular mechanisms of flavonoids against breast cancer, aiming to develop flavonoids as potential chemotherapy agent for breast cancer. A sufficient detail of the methods and analysis has been incorporated. However, there are several points that need to be addressed to improve the quality of this work.

1. **The importance** of this scoping review should be highlighted more. The authors also lack in explaining the gap between the previous reviews, (i.e. Raffa et al., 2017 [1]; Kopustinskiene et al., 2020 [2]) with this scoping review.

2. The studies that were analyzed ranging from 2017-2021's publications. While constructing a scope review does take time, the peer review is taking place in the second half of 2023, thus there is a missing year (2022). The particular reason for this **publication's date criteria** should be explained.

3. **"The flavonoids were laboratory modified or synthetic" is one of the exclusion criteria**. By reading this criteria, readers may assume that all the flavonoids that were included in this scoping review were isolated. By checking 36 articles that were included, at least 9 of them were clearly isolated, 18 were purchased, 1 was derivative synthesized, 5 were unclear since it is not describe in the Materials and Methods in the respective articles (the other 3 cannot be confirmed due to my lack access of the full text). For those 18 flavonoids that were purchased, it is not clear whether they are isolates of synthetics. See the following lits for the details. Thus, **the authors should describe the identity/source of each flavonoid in more detail.**

Article no. based on Table 1:

1: isolated

2: Sigma

3: isolated

4: isolated

5: APExBIO Technology LLC (https://www.extrasynthese.com/flavone/887-rhoifolin.html)

6: isolated

7: Not confirmed (NC)

8: Unclear, since it is not described in the Materials and Methods in the respective articles (UC)

9: isolated

10: UC

11: UC

However, the publication referring previous publications as follows:

- BRA-90 anthocyanins (BRACs) extract was purchased from New Star (Jilin, China) (Chen et al., 2015 [3]).
- BRA-90 anthocyanins (BRACs) were purchased from New Star natural plant development company (Jilin, China) (Luo et al., 2014 [4]).
- Dp was purchased from Mansite Bio-technology Co (Chengdu, China) (Yang et al., 2016 [5]).

Still, it is unclear whether it is an isolate or synthetic one.

12: Langze Pharmaceutical

13: isolated

14: Wako Chemicals

15: Phytomarker

16: Sigma Chemical

17: isolated

18: UC

19: Sigma

20: Weikeqi Biological Technology

21: Sigma

22: Sigma-Aldrich

23: Weikeqi Biological Technology Co., Ltd.

24: National Institute for the Control of Pharmaceutical and Biological Products

25: NC

26: Sigma-Aldrich

27: isolated

28: Despite Biotech

29: Sigma-Aldrich

30: UC

31: Sigma

32: Sigma-Aldrich

33: Chengdu Must Biotechnology

34: UC

35: NC

36: isoflavone derivative synthesized by the method reported by Rajput et al. (2014) [6]

4. The discussion can be improved by incorporating different types of breast cancer cells and the molecular mechanisms involved, and also connecting the structure of flavonoids and their molecular mechanisms. A graphical summary can be provided, illustrating the structure of flavonoids and their molecular targets (i.e. protein kinase).

5. The conclusions are pretty much well-known. A more specific and in-depth conclusion regarding the molecular mechanism is expected.

6. Mistype, i.e. “flavanaids” in Conclusion is found. A thorough recheck is needed.

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Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Partly

Is the statistical analysis and its interpretation appropriate?

Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Chemoprevention, Molecular Biology, Immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.