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REVIEW ARTICLE

Systematic Review of the Anti-Cancer Activity of Green Tea (*Camellia sinensis*)-Derived Compounds in Breast Cancer *In vitro*

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A B S T R A C T

Breast cancer is the most common type of cancer occurring in women with increasing prevalence in these past few years. Although many targeted therapies have been developed to increase the specificity of treatment, many patients still suffer from cancer resistance and relapse. Green tea, a common beverage derived from natural plants, has been shown to induce chemo-preventive effects and exhibit anti-cancer activity through its catechins and polyphenols content. The main well-known compound that induces these effects is epigallocatechin-3-gallate (EGCG). Green tea also contains other naturally occurring compounds such as catechin (C), epicatechin (EC), epigallocatechin (EGC), and epicatechin-3-gallate (ECG), to name a few. In this study, we assessed and compared the anti-cancer activity of these green tea-derived compounds toward different types of breast cancer cell lines. A total of 14 original research papers from PubMed, Google Scholar, and DOAJ databases were collected and evaluated for data extraction. The results showed that EGCG was the most potent compound in green tea that was able to reduce cell viability, promote wound closure, and induce apoptosis even in highly aggressive MDA-MB-231 and lower grade MCF-7 cell lines with ranging concentrations. The second potent compound was ECG, followed by EGC and EC which exhibited intermediate effects. Lastly, catechin was shown to have the lowest anti-cancer activity among all other compounds. Flavonols were also shown to exert cytotoxic effects on breast cancer cells. However, further study is needed to discover the exact mechanism of each compound and determine its relationship to different types of breast cancer cell lines.

K E Y W O R D S

breast cancer; green tea; catechins; epigallocatechin-3-gallate; epicatechin; catechin; epicatechin-3-gallate; epigallocatechin; anti-cancer; In vitro

H I G H L I G H T S

- Green tea naturally contains catechins and polyphenols that show anti-cancer effects.
- EGCG in green tea shows the most anti-cancer activity even from very low concentrations.
- ECG shows the second-most anti-cancer activity.
- EGC and EC shows only intermediate anti-cancer effects.
- Catechin has the lowest anti-cancer activity compared to other compounds.

INTRODUCTION

Breast cancer is one of the most common cancers found in women. Based on the data collected by GLOBOCAN, in 2021, there were approximately 1.7 million women diagnosed with breast cancer with 522,000 related mortalities. It was predicted that the number of cases would significantly increase by 18% from 2008. Thus, it is predicted that by 2050, the number of breast cancer cases will be elevated to 3.2 million new cases per year (Momenimovahed & Salehiniya, 2019). The very high number of breast cancer cases is commonly associated with several challenges arising in treating breast cancer, such as the heterogeneity of the cell followed by complex gene and epigenetic factors (Tao et al., 2014). Modern lifestyles with excessive alcohol and fat consumption can elevate the number of estrogen-related hormones and trigger cancer cell formation through the estrogen-receptor activation pathways. Several prevention methods have been employed, including improved screening methods for better early diagnosis. Several targeted therapies also have been developed to increase the specificity and the efficacy of the treatment in targeting breast cancer, such as Trastuzumab which specifically targets HER2 receptors (Sun et al., 2017). However, despite these advancements in the prevention and treatment of breast cancer, there remains an urgency to find a suitable therapy or treatment to cure and eradicate breast cancer, especially from natural compounds due to their accessibility and complementary effects in eliminating cancer cells (Seely et al., 2005).

Tea is a beverage that has a high level of polyphenols. Tea also exists in high demand only second to water. Aside from its affordable price, tea has been constantly consumed since ancient times due to its wellness benefits. One way to categorize tea is based on its processing or the state of harvested leaves; they are black tea, green tea, and oolong tea (Khan & Mukhtar, 2013). Among all, green tea is the most well-studied for its health-promoting effects, especially in its chemo-preventive effect (Surh, 2003). These biological roles of green tea are mainly mediated by the catechins and polyphenols contained inside, which include catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). Many naturally occurring polyphenols have been reported to exhibit different targeted anti-cancer activities, including cellular proliferation and apoptosis (Ramos, 2008), angiogenesis (Granci et al., 2010), and reversal of drug and radiation resistance (Garg et al., 2005). In a review written by Wu & Butler (2011), it was stated that green tea catechins possessed chemo-preventive properties, such as antioxidant, anti-inflammatory, anti-proliferative, and anti-angiogenic effects.

This narrative review aims to assess and compare the anti-cancer activity of green tea-derived compounds towards breast cancer cell lines *In vitro*, which include green tea catechins and other compounds, through quantitative and qualitative assessment of cell viability, cell migration, and apoptosis induction.

MATERIAL AND METHODS

The alternative hypothesis of this study is that green tea-derived compounds are potentially used for breast cancer therapy observed *In vitro*. The methodology of this study is based on the PRISMA protocol guidelines and using the PICO method for data collection. The collected data was then filtered according to the inclusion and exclusion criteria.

Search

Three databases namely DOAJ, PubMed, and Google Scholar were employed for data gathering. For data retrieval, the PICO analysis method was used, which stands for Population, Intervention, Comparisons, and Outcomes. For the PubMed database, the keywords were searched against the medical subject

headings (MeSH) term before inputting the keywords into the databases. In particular, the two keywords used include green tea and breast cancer or breast neoplasm.

Population (P). The population or the participant of this study is breast cancer cell lines, since the aim of this study was *in vitro* observation. All types of breast cancer cell lines including the metastatic cells were included for the data collection. Based on the results obtained from MeSH, the suitable keyword of the population to be input into PubMed was breast neoplasm, while the keyword for Google Scholar and DOAJ was breast cancer.

Intervention (I). The intervention of this study is the ability of several compounds derived from green tea to eliminate or reduce the growth of breast cancer cells. Since the focus of the study is to observe the effects of green tea, green tea was used as the intervention keyword in all databases.

Comparisons (C). The comparisons were broadly addressed related to the intervention. All types of controls were included in the study collection, including the use of other compounds as the positive control, the test on the untreated cells, or the use of healthy breast cells. All were selected to be collected and assessed for further analyses.

Outcomes (O). The main outcome of this study is to determine the anti-cancer activity of green tea-derived compounds towards breast cancer cells, such as the wound healing ability, cytotoxicity, and the apoptotic effects. However, due to the limited number of papers obtained upon the input of “anti-cancer” as the keyword for the outcome, the keyword was excluded from the search in the databases.

Study identification and selection

The flow of study identification and selection using the PRISMA method was shown in **Figure 1**. Study identification and selection processes were done using Rayyan web server (Ouzzani et al., 2016). In the identification process, the total number of studies collected from all selected databases was 722 studies, of which 110 of them are excluded due to the duplicates, which leads to the 612 papers being collected for further screening. In the further screening process, 289 from 612 studies were excluded due to irrelevant content or the absence of an abstract. Next, to select the eligible paper, full-text screening was done on 323 studies based on the inclusion and exclusion criteria (**Table 1**). In total, there were 14 papers included in the synthesis of this narrative review. Afterwards, the quality of these papers was assessed using a software-based tool named ToxRTool (Toxicological data Reliability Assessment Tool) to ensure the validity and reliability of the collected data (Schneider et al., 2009). In the end, a total of 14 papers were included for the data extraction process.

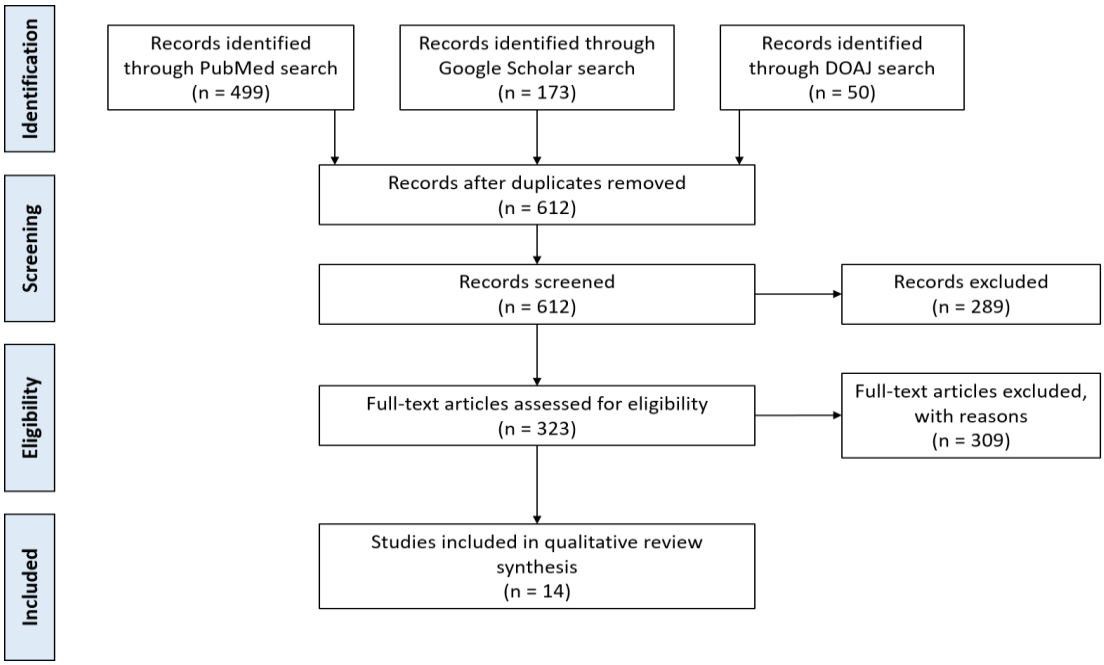

Figure 1. PRISMA Flow Diagram

Table 1. The inclusion and exclusion criteria for study selection

Inclusion Criteria	Exclusion Criteria
• Data range from 2011 to 2021 • Published as an original research paper • Reported to be *In vitro* study • Methods conducted are either cytotoxic assay, apoptotic assay, or wound healing assay	• Reported to reduce breast cancer risk • Study related to combinatorial effects with other compounds • Study written in a language other than English • Published as publications other than original research paper (systematic review, commentary, letter, poster presentations, etc.

The collected studies were screened and collected based on the inclusion and exclusion criteria shown in **Table 1.** The included studies were ranging from 2011 to 2021, while other studies published before 2011 were excluded from further analysis. The year 2011 was used as the year limit due to the application of the 10-year period of references. This was done to minimize the number of outdated papers to ensure the validity of the obtained data in the systematic review. Moreover, only the original research paper was included in the study for the relevant information collection. This study specifically discusses the *in vitro* experiments conducted on breast cancer cell lines. Therefore, other types of studies including *in silico, in vivo,* and clinical trials were excluded. Since this study aims to assess the ability of green tea-derived compounds in eliminating cancer, the included papers were related to the elimination or reduction of breast cancer by the potential compounds. Meanwhile, the papers with the aim of reducing cancer risks were excluded due to their irrelevance. The included papers must also be in the form of journal publications, while books and patents were excluded. Since the anti-cancer activity is relatively broad, this study specifically chose three methods to be assessed, which were related to the effects of the compounds towards cell growth, cell migration and invasion, and apoptosis capability, by using cytotoxic assay, apoptotic assay, or wound healing assay. There are other methods that are commonly used to observe the anti-cancer ability of a compound, including western blotting and real-time PCR. However, these methods

were excluded due to the high variability in results, which resulted in incomparable data. Hence, the three selected methods were chosen for ease of comparison. Aside from that, the selected methods are considered as basic procedures to conduct an anti-cancer study. Thus, it allows a higher number of studies to be compiled and included in the systematic review. Papers with no selected methods were excluded from further analyses. Lastly, the chosen papers also must be written in English, with publications using other languages were excluded.

Data extraction

A total of fourteen studies were selected for the data extraction. Further, these studies were categorized based on green tea-derived compounds for comparison purposes, which were divided into catechin (C), epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3 gallate (EGCG), and other green tea-derived compounds that differ from it. The acquired data from the studies include the author, the year of publication, the type of used cell lines, the selected concentrations of the used compound, and the outcome. The outcome of the data that was collected according to its methods, including the wound healing assay, apoptotic assay, and cytotoxic assay.

RESULTS

In total, there were fourteen papers collected for the study synthesis. **Table 2** displayed the characteristics of the included papers, which includes the assessed compounds, the cell lines used, the included methods according to the inclusion criteria, and the comparison or the control. To assess and compare the effectiveness of each compound, the collected papers are grouped according to each assessed compound.

Table 2. The characteristics of the included studies.

Reference (year)	Compound	Cell Types	Methods	Comparison
Braicu et al. (2013)	EGCG	Hs578T	Cytotoxic assay	Negative control (no treatment)
De Amicis et al. (2013)	EGCG	MCF-7, T47D, and SkBr3	Cytotoxic assay	Negative control (no treatment)
Deb et al. (2014)	EGCG	MCF-7 and MDA-MB-231	Wound healing assay	Negative control (no treatment) Other epigenetic modifying agents: 5-Aza-CdR, TSA, DZNep"
Farhan et al. (2016)	C EC EGC EGCG	MCF10A and MDA-MB-231	Cytotoxic assay	Negative control (no treatment + normal healthy cells)
Ho et al. (2013)	Green Tea Seeds (GTS) extract	MDA-MB231	Cytotoxic assay Wound healing assay	Negative control (no treatment)
Kuban-Jankowska et al. (2020)	EC ECG EGC	MCF-7	Cytotoxic assay	Negative control (no treatment)

	EGCG			
Meeran et al. (2011)	EGCG	MCF-7, MDA-MB-231, and MCF10A	Cell viability assay Apoptotic assay	Negative control (no treatment + normal healthy cells)
Mineva et al. (2013)	EGCG	SUM-149 and SUM-190.	Wound healing assay	Negative control (no treatment)
Moradzadeh et al. (2017)	EGCG	T47D and HFF	Cytotoxic assay Apoptotic assay	Positive control: tamoxifen (0, 2.5, 5,10 and 20 μM)
Rha et al. (2019)	Fraction Rich in Flavonol Glycosides (FLG) Fraction Rich in Flavonol Aglycones (FLA)	E0771	Cytotoxic assay	Positive control: paclitaxel
Sheng et al. (2019)	EC ECG EGC EGCG	MCF-7 and MDA-MB 231	Cytotoxic assay Wound healing assay	Negative control (no treatment)
Wei et al. (2018)	EGCG	4T1	Cytotoxic assay Wound healing assay	Negative control (no treatment)
Xu et al. (2020)	EGCG	4T1	Cytotoxic assay Apoptotic assay Wound healing assay	Negative control (no treatment)
Zeng et al. (2014)	EGCG	MDA-MB-231, MCF-7, T47D, MCF10A	Cytotoxic assay Apoptotic assay	Negative control (no treatment)

Catechin (C)

Our search only returned one result for papers that assess the ability of catechin towards the cancer cells activity, which was written by Farhan and colleagues in 2016 (**Table 3**). In the paper, the cell viability assay on the MDA-MB-231 cells proved that catechin contained in green tea has a very low anti-cancer activity in comparison to the other green tea-derived compounds. This was shown by a high cell viability percentage (far more than 50%) despite the usage of catechin maximum concentration in the paper. Thus, in this study, the IC50 was not even achieved by the catechin.

Table 3. The characteristics and results of catechin assessment show the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (Year)	Cell Types	Concentration	Wound Healing Results	Cytotoxic Results	Apoptotic Results
Farhan et al. (2016)	MDA-MB-231	25 µM 50 µM 75 µM 100 µM	-	**MTT Assay** C possesses low anti-cancer activity, which by the concentration 100 µM, the cell viability is observed approximately around 72%. *IC_{50}: 173.33 µM.*	-

* Sentences in bold denote the conducted assay.

Epicatechin (EC)

Four out of fourteen selected studies assess the anti-cancer activity of EC towards breast cancer cell lines, which include MCF10A-NB, MCF-7 and MDA-MB-231 (**Table 4)**. EC possesses an intermediate inhibitory effect towards the cell viability. Although its effects on the cell viability were slightly higher compared to the previous compound (C), it also failed to achieve IC50. Moreover, the variance in cell viability depends on the type of breast cancer cell line used as a subject. Although EC was shown to have some effects on the overall viability of breast cancer cells, MDA-MB-231 was shown to have a better resistance toward the inhibitory effect of EC compared to MCF-7 despite the varying results.

Table 4. The characteristics and results of EC assessment showing the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (year)	Cell Types	Concentration	Wound Healing Results	Cytotoxic Results	Apoptotic Results
Farhan et al. (2016)	MDA-MB-231	25 µM 50 µM 75 µM 100 µM	-	**MTT Assay** <u>MDA-MB-231</u> EC possesses quite low inhibition in the cell viability in which 100 µM EC resulted in approximately 65% cancer cell viability. *IC_{50}: 156.05 µM.*	-
Kuban-Jankowska et al. (2020)	MCF-7	0.98 µM 1.95 µM 3.90 µM 7.81 µM 15.625 µM 31.25 µM 62.5 µM 125 µM	-	**MTT Assay** Dose 0.98-62.5 µM has no significant effect towards the cell viability. 125 µM reduces the cell viability to 41% upon 24 h treatment. *IC_{50}: 112.22 µM.*	-

Sheng et al. (2019)	MCF-7 MDA-MB-231	5 µM 10 µM 20 µM 50 µM 100 µM	-	**MTT Assay** MCF-7: % Cell viability 80% (100 µM) *IC_{50}: 250 µM.* MDA-MB-231: % Cell viability 85% (50 µM) 70% (100 µM) *IC_{50}: 166.67 µM.* Other concentrations of EC had no significant effect on the cell viability. EC exerts low viability inhibition effects toward MCF-7 and intermediate inhibition to MDA-MB-231.	-

* Sentences in bold denote the conducted assay; sentences in underline denote the tested cell lines.

Epicatechin-3-Gallate (ECG)

Another assessed green tea-derived compound is the ECG. In total, there were three papers out of fourteen that discuss and assess the anti-cancer properties of ECG, whose characteristics and results are displayed in **Table 5**. Based on these studies, ECG was found to have sufficient anti-cancer effects based on the evaluation of its apoptotic activity and inhibition towards the migratory and proliferation capability. Different from the results of EC, the different cell types tested with ECG, which in this study were the MCF-7 and MDA-MB-231 cell lines, did not affect the outcome on the anti-cancer effects of ECG. Unlike the two previous compounds, ECG successfully achieved its IC50 at around 50-100 µM for both MCF-7 and MDA-MB-231 cells according to the study done by Sheng et al. (2019). This shows the higher ability of ECG compounds to induce anti-cancer activity to the breast cancer cells.

Table 5. The characteristics and results of ECG assessment showing the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (Year)	Cell Types	Concentration	Wound Healing Results	Cell Viability Results	Apoptotic Results
Kuban-Jankowska et al. (2020)	MCF-7	0.98 µM 1.95 µM 3.90 µM 7.81 µM 15.625 µM 31.25 µM 62.5 µM 125 µM	-	**MTT Assay** Treatment with concentrations 7.81, 15.625, and 125 µM lowered the viability of MCF-7 to 81%, 74%, and 65%, respectively, while other concentrations had no significant impact on cell	-

				viability. *IC_{50}: 307.29 µM.*	
Sheng et al. (2019)	MCF-7 MDA-MB-231	MTT Assay 5 µM 10 µM 20 µM 50 µM 100 µM	-	**MTT Assay** <u>MCF-7:</u> % Cell viability N/A (5 µM) N/A (10 µM) 70% (20 µM) 52% (50 µM) 48% (100 µM) *IC_{50}: 75 µM.* <u>MDA-MB-231:</u> % Cell viability N/A (5 µM) N/A (10 µM) 80% (20 µM) 50% (50 µM) 50% (100 µM) *IC_{50}: 50-100 µM.* ECG has similar viability inhibition effects toward MCF-7 and MDA-MB-231.	-

*Sentences in bold denote the conducted assay; sentences in underline denote the tested cell lines.

Epigallocatechin (EGC)

There are four studies that assessed the ability of EGC in eliminating and reducing breast cancer progression (**Table 6**). Overall, the effect of high EGC concentration towards cell viability showed intermediate inhibitory results. However, the inhibitory effects on cell viability were different between MD-MB-231 and MCF-7 cell lines. Different studies on MDA-MB-231 cells showed similar results on the effect of EGC, while different studies on MCF-7 provided varying results of EGC efficacy. For instance, the study conducted by Kuban-Jankowska et al. (2020) resulted in 50% reduction of MCF-7 cell viability (IC50) at 35.9±10.6 µM, while Sheng et al., (2019) found that even a higher concentration (50 µM) was only able to reduce the cell viability to 75%. Despite this, EGC effects on breast cancer cells proved to be more potent compared to the previous compounds so far. In addition, low concentration of EGC (10 µg/ml) resulted in intermediate inhibition of wound closure, and EGC was not able to induce apoptosis in both MCF-7 and MCF10A-NB, while high EGC concentration (40 µg/ml and 100 µg/ml) was able to induce MCF-7 apoptosis.

Table 6. The characteristics and results of EGC assessment show the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (Year)	Cell Types	Concentration	Wound Healing Results	Cell Viability Results	Apoptotic Results
Farhan et al. (2016)	MDA-MB-231	25 µM 50 µM 75 µM 100 µM	-	**MTT Assay** 100 µM EGC possesses intermediate anti-cancer activity towards the cell viability (65%). **IC50: 50.52 µM**	-
Kuban-Jankowska et al. (2020)	MCF-7	0.98 µM 1.95 µM 3.90 µM 7.81 µM 15.625 µM 31.25 µM 62.5 µM 125 µM	-	**MTT Assay** % Cell viability 57% (15.625 µM) 49% (31.25 µM) 37% (62.5–125 µM) Other concentrations had no effect. **IC50: 35.9±10.6 µM**	-
Sheng et al. (2019)	MCF-7 MDA-MB-231	5 µM 10 µM 20 µM 50 µM 100 µM	-	**MTT Assay** <u>MCF-7:</u> % Cell viability N/A (5 µM) N/A (10 µM) 85% (20 µM) 75% (50 µM) 70% (100 µM) IC50: 206.67 µM <u>MDA-MB-231:</u> % Cell viability N/A (5 µM) N/A (10 µM) 85% (20 µM) 70% (50 µM) 65% (100 µM) EGC has similar viability inhibition effects toward MCF-7 and MDA-MB-231. IC50: 160 µM	-

*Sentences in bold denote the conducted assay; sentences in underline denote the tested cell lines.

Epigallocatechin-3-Gallate (EGCG)

EGCG was the most studied compared to other green tea-derived compounds, as indicated by thirteen out of fourteen studies being found to assess EGCG towards breast cancer activity (**Table 7**). Overall, it was found that EGCG has a potent effect in suppressing the viability of various cancer cell lines. Various methods were used to assess the cell viability, such as MTT assay, trypan blue exclusion assay, and

CCK8 assay. Most studies used EGCG at concentrations ranging from 10 µg/ml - 300 µg/ml and 1 µM - 320 µM and various types of cell lines with MCF-7, T47D, and MDA-MB-231 as the predominant ones. Different cell types possess different susceptibilities toward EGCG treatment. MDA-MB-231 cell line viability was found to be very susceptible to EGCG, both at low and high EGCG concentrations. Significant inhibition by low EGCG concentration was also found in the studies on MCF-7 cell lines. According to the studies conducted by Kuban-Jankowska et al. (2020) and Sheng et al. (2019), EGCG was able to reduce 50% of the MCF-7 cell viability (IC50) at around 13.9 and 54.25 µM in 24 hours upon the incubation. Based on these studies, EGCG possesses the strongest cytotoxic effect on MCF-7's cell viability among the other green tea-derived compounds that were mentioned before. Other uncommonly used cell lines like Hs578T and 4T1 also provided similar results of EGCG efficacy either using MTT or CCK8 assay, in which high EGCG concentration was able to induce inhibitory effects towards cell viability (Braicu et al., 2013; Xu et al., 2020). However, T47D was found to have a rather different response toward EGCG treatment in each related study, with Moradzadeh et al. (2017) and De Amicis et al. (2013) proving T47D as the most susceptible cell line, while Zeng, Holly, & Perks (2014) discovered the T47D resistance towards EGCG with only 8% growth inhibition upon treatment with 1 µM EGCG. On top of that, most papers showed that EGCG was likely to exhibit a dose-dependent effect. In contrast, the effect of EGCG was not potent in normal breast epithelial cells MCF-10A.

The wound healing assay was done using EGCG treatment at a concentration ranging from 10 µM - 20 µM. Most studies showed a visible result in the anti-migratory activity of EGCG starting from 20 µM with minimal variation in the result depending on the cell line used and the incubation time. The migratory capability of MDA-MB-231 (highly migratory and invasive phenotype) is also shown to be highly susceptible towards EGCG treatment. Similar efficacy was also achieved by MCF-7, SUM-190, and 4T1.

The apoptotic activity of EGCG was also assessed with various methods such as PI/Annexin V-FITC flow cytometry, and comet assay. PI/Annexin V-FITC flow cytometry detected a dose-dependent apoptotic cell death with effective concentration starting from 40 µg/ml. The early and late apoptosis of 4T1 cells was reported to be affected by different concentrations of EGCG. Overall, all cell lines were found to undergo apoptosis upon high EGCG concentration treatment.

Table 7. The characteristics and results of EGCG assessment showing the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (Year)	Cell Types	Concentration	Wound Healing Results	Cell Viability Results	Apoptotic Results
De Amicis et al. (2013)	MCF-7 T47D	10 µM 20 µM 40 µM	-	**MTT Assay** <u>MCF-7:</u> % Cell Inhibition 20% (10 µM) 25% (20 µM) 50% (40 µM) *IC_{50}: 40 µM.* <u>T47D:</u> % Cell Inhibition 50% (10 µM) 52% (20 µM) 67% (40 µM) *IC_{50}: 10 µM.* EGCG has more potent	-

				inhibition effects toward T47D cells compared to the MCF-7 cell lines.	
Braicu, et al. (2013)	Hs578T	10 µM	-	**MTT Assay** Cell proliferation of the cells treated at 72 hours was significantly lower than control. *IC_{50}: N/A*	-
Deb et al. (2014)	MCF-7 MDA-MB-231	20 µM	**Scratch Assay** MCF-7 20 µM of EGCG resulted in the 33% wound closure after 48 hours of treatment (control:95%) MDA-MB-231 **(**highly invasive and migratory) 20 µM of EGCG resulted in the 18% wound closure after 24 hours of treatment. 20 µM of EGCG was sufficient in reducing the cell motility in both MCF-7 and MDA-MB-231 cells.	-	-
Farhan et al. (2016)	MDA-MB-231 MCF-10A	25 µM 50 µM 75 µM 100 µM	-	**MTT Assay** MDA-MB-231 EGCG is effective in inhibiting cell viability, which is shown by approximately 20% cell viability at 100 µM concentration. *IC_{50}: 62.5 µM.* MCF-10A The cytotoxic effect of EGCG is not potent to normal breast epithelial cells. 50 µM results in 90% of cell viability. *IC_{50}: 250 µM.*	-

Kuban-Jankowska et al. (2020)	MCF-7	0.98 µM 1.95 µM 3.90 µM 7.81 µM 15.625 µM 31.25 µM 62.5 µM 125 µM	-	**MTT Assay** EGCG induce effective inhibitory effect on MCF-7 cells, especially on concentration 15.625 µM and 62.5 µM. % Cell Viability 0.98 µM (78%) 1.95 µM (85%) 3.90 µM (83%) 7.81 µM (71%) 15.625 µM (31%) 31.25 µM (29%) 62.5 µM (30%) 125 µM (42%) *IC_{50}:* 13.9±3.1 *µM.*	-
Meeran et al. (2011)	MCF-7 MDA-MB-231	**Cell Counting** 20 µmol/L 40 µmol/L 60 µmol/L **Apoptotic Assay** EGCG (40 µmol/L) pEGCG (20 µmol/L)	-	**Trypan blue exclusion assay** Doses of up to 60 µmol/L of EGCG and 40 µmol/L of pEGCG can inhibit the proliferation activity of MCF-7 and MDA-MB-231. Upon 12 hours EGCG incubation: <u>MCF-7</u> *IC_{50}: 36.27 µmol/L* <u>*MDA-MB-231*</u> *IC_{50}: 36.24 µmol/L.*	**Annexin-V** EGCG (40 µmol/L) and pro-EGCG (20 µmol/L) induces apoptosis in both MCF-7 and MDA-MB-231 cancer cells in time-dependent manner. **Immunofluorescence staining** Same concentration of EGCG and pEGCG as above were able to induce apoptotic cells in MCF-7 and MDA-MB-231 but pEGCG is more potent than EGCG as it requires a lower dose to induce the same % apoptosis.
Mineva et al. (2013)	SUM-149 SUM-190	40 mg/ml	EGCG can inhibit the stimulation of lymphatic endothelial cell migration by SUM-190 cells' secretion factors	-	-
Moradzadeh et al.	T47D	10 µM 20 µM	-	**MTT Assay** EGCG decreased cell	-

(2017)		40 µM 80 µM		viability as concentration- and time- dependent. *IC_{50}: 14.17 µM.* The toxicity of EGCG was significantly high towards T47D cells.	
Sheng et al. (2019)	MCF-7 MDA-MB-231	**MTT Assay** 5 µM 10 µM 20 µM 50 µM 100 µM **Wound healing assay** 20 µM	MCF-7 Cells significantly have higher width ratio of cell-free areas compared to the control group. MDA-MB 231 Cells still showed 42.2% cell-free area after 72h scratch and treatment.	**MTT Assay** MCF-7 IC50: 54.25 ± 7.15 µM (24h) 40.35 ± 5.54 µM (48h) 27.53 ± 1.02 µM (72h) MDA-MB-231 IC50: 54.07 ± 9.52 µM (24h) 44.03 ± 3.61 µM (48h) 27.12 ± 0.41 µM (72h)	-
Wei et al. (2018)	4T1	10 µM 20 µM 40 µM 80 µM 160 µM 240 µM 320 µM	-	**CCK8 Assay** An escalating dose of EGCG in combination with longer incubation time enhances the effect on lowering the viable 4T1 cells significantly. 80 µL of EGCG significantly reduced 4T1 cell growth by 41% but had minimal effect on HC11 (17%). *IC_{50}: 97.56 µM*	**Annexin V** Escalating dose of EGCG increases the overall rate of apoptosis Effective induction at dose 20-240 µg/mL, in which the rates of both early and late apoptosis were increased.
Xu et al. (2020)	4T1	50 µg/mL 100 µg/mL 150 µg/mL 200 µg/mL 250 µg/mL 300 µg/mL	**Scratch &Transwell Assay** EGCG significantly inhibit migration and invasion in a dose-dependent manner. Effective inhibition at dose 50-250 µg/mL. Relative invasion became 20% at dose 250ug/mL, compared to control: 100%.	**CCK8 Assay** EGCG significantly suppressed the cell viability in a dose-dependent manner. Effective suppression at dose 150-350 µg/mL. Cell viability became 20% at dose 350 µg/mL. *IC_{50}: 218.75 µg/mL*	**Annexin V** EGCG significantly induces apoptosis in a dose-dependent manner. Effective induction at dose 50-250 ug/mL. Apoptosis rate was 12% at dose 250 µg/mL.
Zeng et al. (2014)	MDA-MB-231 MCF-7 T47D	0.1 µM 1 µM	-	**Trypan blue exclusion assay** MDA-MB-231 25% growth inhibition with 1 µM EGCG *IC_{50}: 2 µM*	-

					<u>MCF-7</u> 28% growth inhibition with 1 µM EGCG *IC_{50}: 1.785 µM* <u>T47D</u> 8% growth inhibition with 1 µM EGCG. T47D was found to be resistant towards the viability inhibitory effect of EGCG *IC_{50}: 6.25 µM*	

*Sentences in bold denote the conducted assay; sentences in underline denote the tested cell lines.

Compounds other than catechins

Other than catechins, there were compounds like flavonols and green tea seeds components that were assessed for their anti-cancer activity, which can be seen in **Table 8**. The flavonol fraction in green tea was also proven to reduce cell viability of EO771 (luminal B breast cancer cell line) when used at 100 µg/mL (Rha et al., 2019). Studies on green tea seeds (GTS) by Ho et al. (2013) also proved its ability in suppressing the migration activity of MDA-MB-231 (high migratory phenotype) in a dose-dependent manner and reduce cell viability even at the lower concentration.

Table 8. The characteristics and results of other green tea-derived compound assessments showing the cited paper, the breast cancer cell line that was used, the concentration, and its corresponding result.

Reference (year)	Cell types	Compound	Concentration	Wound healing results	Cell viability results	Apoptotic results
Ho et al. (2013)	MDA-MB231	Green Tea Seeds (GTS)	**MTT Assay:** 1 µg/ml 5 µg/ml 10 µg/ml 25 µg/ml 50 µg/ml 100 µg/ml **Migration assay:** 1 µg/ml 5 µg/ml 10 µg/ml	**Migration assay** GTS significantly repressed cell migration after 48h in a dose-dependent manner. At 10 µg/mL, GTS-treated cells showed 42.7% wound closure compared to untreated cells (66.1%).	**Cell viability** GTS treatment with dose 25,50, and 100 µg/mL significantly inhibited cancer cell growth by 28.5%, 87.9%, and 85.7%, respectively. **IC50= 72.74 µg/mL**	-
Rha et al. (2019)	EO771	Fraction Rich in Flavonol Glycosides (FLG)	1 µg/ml 10 µg/ml 100 µg/ml	-	**MTT assay** FLG or FLA at 100 µg/mL concentration decreased the viability of the breast cancer cell line EO771.	-

Fraction Rich in Flavonol Aglycones (FLA)	FLG can exert cytotoxic activity when used as a monotherapy in EO771. IC50 of FLG: 97.88µg/ml IC50 of FLA: 100µg/ml

*Sentences in bold denote the conducted assay; sentences in underline denote the tested cell lines

DISCUSSION

Green tea was found to have various positive effects on health. One of the effects is an anti-cancer activity towards various types of cancers. However, despite its potential effects as an anti-cancer agent, the efficacy of each compound from green tea is not well known. Aside from that, there are limited studies related to green tea efficacy specifically for breast cancer therapy. This review was synthesized to compile and summarize the efficacy of the anti-cancer activity of several green tea-derived compounds, including C, EC, EGC, ECG, EGCG, and others towards several breast cancer cells. The collected studies ranging from 2011 to 2021 to obtain the newest and reliable information related to green tea-derived compounds treatment towards breast cancer cells. A similar parameter was applied by observing the effects of the compounds towards three main cellular activity, which were migration and invasion through wound healing assay, proliferation capability and viability through cell viability assay, and apoptotic activity by apoptotic assay.

In total, there were fourteen collected studies that are in line with the inclusion criteria, most of which were assessing the anti-cancer activity of EGCG. From all of the collected data, by comparing each compound's efficacy, EGCG is found to be the most potent for breast cancer cell activity. EGCG was able to induce very high inhibitory cellular viability and migration in most of the cell lines compared to other compounds. EGCG was very effective in inhibiting cell proliferation of highly aggressive MDA-MB-231 and lower grade MCF-7 cell lines with sufficient migratory inhibition and apoptosis induction. Other breast cancer cell lines including Hs578T and 4TI also proved the efficacy of EGCG. So far, these results proved that EGCG has the most potent anti-cancer activity in green tea, which indicates that EGCG has the potential to be used and studied further as a natural therapeutic agent for breast cancer.

EGCG is known to be the most abundant catechin in green tea and is widely researched to have anti-cancer properties toward many cancers including breast cancer (Asensi et al., 2011). Based on the review written by Min & Kwon (2014), EGCG was mentioned to be able to modulate the signaling molecules related to cancer activity. EGCG is found to be able to modulate the ROS level. EGCG is known as a potent antioxidant, which leads to its ability in ROS scavenging. EGCG can indirectly reduce the amount of ROS by the antioxidant enzyme induction. Aside from that, several studies showed that EGCG is also able to exert pro-oxidant function, which is very important in EGCG cytotoxicity towards the cancer cells. Aside from ROS modulation, EGCG also affects the activity of nuclear factor-κB or NF-κB. NF-κB is known to be involved in various cancer progressions due to its ability to regulate gene expression. In normal conditions, NF-κB will reside in the cytosol and be inactivated. However, in the cancer progression, the activation of NF-κB leads to progressive cell proliferation, apoptosis inhibition, and cellular migration and invasion. EGCG is able to downregulate the NF-κB activation by repressing IKK activation, which has been shown in various cancers, such as bladder cancer (Qin et al., 2012), lung cancer (Yang et al., 2005), prostate cancer (Hastak et al., 2003), head and neck cancer, and breast cancer (Masuda et al., 2002). The reduction of NF-κB activation leads to the decrease of cancer progression, which is shown by the decrease of migratory effect, proliferative ability, and increase of apoptotic capability. EGCG also modulates cell invasion and cell death

through the activity of MAPK. MAPK deregulation is commonly associated with tumorigenesis. EGCG is proven to be able to decrease MMP production through ERK phosphorylation suppression in various cancers, such as fibrosarcoma (Maeda-Yamamoto et al., 2003) and gastric carcinoma (Kim et al., 2004). EGCG also leads to cell death through the release of cytochrome c through JNK pathways, which are observed in colorectal carcinoma cells (Kim et al., 2005) and thyroid carcinoma cells (Lim & Cha, 2011). Lastly, EGCG is found to be involved in epigenetic regulation, such as suppressing the activity of DNA methyltransferase and increasing histone acetylation, which leads to the elevation of tumor suppressor genes activity (Min & Kwon, 2014). In the study done by Choudhary et al. (2012), non-cytotoxic dose of EGCG was shown to cause DNA damage upon the addition of carcinogen (PhIP) onto MCF-10A cell line, but not when carcinogen was not introduced. Additionally, (Farhan et al., 2016) showed an impotent effect of EGCG toward normal MCF-10A, even at a moderate concentration. This signifies that EGCG might have a selective effect on more cancerous cells, since it was tested that the effect of EGCG is insignificant towards MCF10A, which is a normal epithelial breast cell line. However, despite being the most extensively studied green tea-derived compound, several studies also showed that other green tea-derived compounds elicit anti-cancer effects, which indicates that EGCG is not the only compound that is able to eliminate breast cancer in green tea.

ECG is the third most abundant catechin in green tea, which also elicits sufficient anti-cancer properties towards the breast cancer cell lines although not as potent as EGCG (Lim et al., 2006). Based on the results in **Table 5**, it was shown that the anti-cancer activity of ECG was found to be effective for all of the predominant cell lines. Reduction in cell viability combined with the inhibition of migratory ability and increase of apoptosis induction effectively proved the potential of ECG as an anti-cancer agent obtained from green tea. However, despite its potent anti-cancer activity, the exact molecular mechanisms are not well studied. A study showed that ECG induced potent inhibitory effects toward prostate and ovarian cancer cells derived from patients (Ravindranath et al., 2006). Importantly, ECG is the only green tea catechins that were shown to significantly induce NAG-1 expression leading to cell apoptosis and growth inhibition (Baek et al., 2004). A study done by Kürbitz et al. (2011) also mentioned the strong ability of ECG in diminishing the aggressive phenotype in pancreatic cancer by almost completely inhibiting TNFα-induced NF-κB activity, which completely reduced the secretion of IL-8 and uPA that contributes to the aggressiveness of pancreatic cancer. A study by Lim et al. (2006) also showed that ECG in head and neck squamous cell carcinoma is more potent in downregulating the expression of cyclin D1, which is a protein that is strongly involved in the cell cycle progression. The reduction of cyclin D1 expression resulted in the inhibition of cell proliferation and growth, which is the desired effect in eliminating cancer cells. However, despite all findings, the clear mechanism is not well established, especially in breast cancer.

Other catechin-derived compounds, including EGC and EC, are shown to have similar intermediate effects on breast cancer activity. It was found that EGC and EC have resulted in 65% to 80% of cellular viability in various cell lines. However, these results vary across different studies, which indicates the need for further assessment to establish the relationship between EGC and EC towards different breast cancer cell lines. Although EGCG has higher potency, EGC is still found to be able to induce cellular apoptosis. In a study conducted by Das et al. (2006), EGC treatment to malignant neuroblastoma cells induces apoptosis process through both of the intrinsic and extrinsic apoptosis pathways, which are mitochondrial and death receptor-mediated apoptosis, respectively. However, the number of studies of anti-cancer effects induced by EGC is still very little, which leads to unclear mechanisms on how EGC could induce apoptosis. EC anti-cancer effects towards breast cancer are also poorly studied. In other cancers, such as acute myeloid leukemia, Papież et al. (2010) discovered that EC can induce DNA damage and apoptosis in rats. Another study by Mackenzie & Oteiza (2006) showed that EC was able to inhibit the proliferation of Hodgkin Lymphoma cells and Jurkat T cells by the inhibition of NF-κB binding to DNA. It is suspected that the Na^+/H^+ exchanger of NF-κB is strongly inhibited by EC. EC was also found to be able to modulate the epigenetic

patterns in prostate cancer through the inhibition of histone acetyltransferase activity (Siddique et al., 2012). Interestingly, the study done by Delgado et al. (2014) discovered that the interaction of DNA methyltransferase with EC was effectively inhibiting the cellular proliferation of MCF-7. EC was found to be involved in various mechanisms of cancer activity, which potentiates EC to be used as a therapeutic agent for breast cancer (Shay et al., 2015).

Based on this review finding, the lowest efficacy is achieved by catechin. There was only one study collected related to the catechin assessment of breast cancer cell lines (**Table 3**). This study also proved that catechin elicits a low inhibitory effect towards MDA-MB-231 cell lines in which 100 µM of catechin induces 80% cell viability.

Very little study was conducted towards catechin potency as an anti-cancer agent, this might suggest that the full potential of anti-cancer properties of catechin has not been discovered yet. Sun et al. (2020) stated that catechin is the most basic catechin compound without glycosylation, which leads to higher absorption efficiency. They found that catechin can increase the activity of cyclin inhibitor p21 in lung cancer cell lines. Catechin is also able to decrease the activity of cyclin E1 through the inhibition of P-AKT activity in lung cancer. Besides that, catechin is found to possess a comparable inhibitory activity towards cell viability compared to the EGCG. However, further study still needs to be done to validate the efficacy of catechin in reducing the progression of breast cancer.

Other than green tea catechins, several compounds are also determined to have anti-cancer activity obtained from green tea leaves and seeds (**Table 8**). Most of the results showed that other extracts and compounds also exhibit intermediate anti-cancer activity towards breast cancer cell lines. Therefore, it can be said that all green tea-derived compounds are able to be used as anti-cancer agents with varying efficacy towards breast cancer. The most potent anti-cancer effects are possessed by the most abundant catechins in green tea, which is EGCG. ECG also has comparable efficacy in eliminating cancer to EGCG. Other than that, EGC and EC have intermediate efficacy towards the inhibition of cell viability, cell migration, and induction of apoptosis. Although in this review it is shown that catechin possesses low anti-cancer activity towards breast cancer, further studies are needed to validate the potency of catechin in eliminating breast cancer. In addition, many green tea-derived compounds, such as catechin and flavonols have not been elaborated and studied in detail, especially in relation to breast cancer. Thus, further study is needed to promote the evidence of the compounds' anti-cancer activity. Further analysis on the side effects and toxicity of the compounds can be done to assess the ability of green tea-derived compounds as an alternative for breast cancer therapy. *In vivo* studies are also recommended to assess the accuracy of the compound's effectiveness in reducing the tumor size and progression.

Even though the summarized papers provided adequate information to compare the effectiveness of each compound, it was rather difficult to precisely develop a definite comparison due to the different concentration and breast cancer cell type used in each study. More specific criteria can be proposed to narrow down the variables that can influence the comparability.

CONCLUSION

In conclusion, most green tea-derived compounds were shown to have anti-cancer activity in terms of reducing cell viability, inducing apoptosis, and inhibiting the migration capability of breast cancer cell lines. Despite that, not all compounds showed similar anti-cancer effects. *In vitro* studies showed that EGCG was proven to be the most potent compound in green tea to be effective even against malignant breast cancer cell lines starting from a very low concentration. Various mechanisms were thought to underlie the anti-cancer activity of EGCG such as ROS modulation, downregulation of NF-κB signaling, and regulation of MAPK pathway via inhibition of ERK phosphorylation. Second to EGCG was ECG, although the exact mechanism of ECG anti-cancer properties is still a grey area. As for EGC and EC, they were able to induce intermediate effects toward the breast cancer cell lines. However, further assessment should be done to

assess the exact mechanism and their relation toward different types of breast cancer cell lines. Catechin was found to have the lowest anti-cancer activity among all, but further research is needed to assess the potency of catechin's anti-cancer properties. To sum up, green tea extract contains many different active compounds which allow green tea extract to be used as a potent anti-cancer agent.

REFERENCES

Asensi, M., Ortega, A., Mena, S., Feddi, F., & Estrela, J. (2011). Natural polyphenols in cancer therapy. *Critical Reviews In Clinical Laboratory Sciences, 48*(5-6), 197-216. https://doi.org/10.3109/10408363.2011.631268

Baek, S., Kim J., Jackson F., Eling T., McEntee M., Lee S. (2004). Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. *Carcinogenesis, 25*(12), 2425-2432. https://doi.org/10.1093/carcin/bgh255

Braicu, C., Gherman, C., Irimie, A., & Berindan-Neagoe, I. (2013). Epigallocatechin-3-Gallate (EGCG) Inhibits Cell Proliferation and Migratory Behaviour of Triple Negative Breast Cancer Cells. *Journal Of Nanoscience And Nanotechnology, 13*(1), 632-637. https://doi.org/10.1166/jnn.2013.6882

Choudhary, S., Sood, S., Donnell, R., & Wang, H. (2012). Intervention of human breast cell carcinogenesis chronically induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Carcinogenesis, 33*(4), 876-885. https://doi.org/10.1093/carcin/bgs097

Das, A., Banik, N., & Ray, S. (2006). Mechanism of apoptosis with the involvement of calpain and caspase cascades in human malignant neuroblastoma SH-SY5Y cells exposed to flavonoids. *International Journal Of Cancer, 119*(11), 2575-2585. https://doi.org/10.1002/ijc.22228

De Amicis, F., Russo, A., Avena, P., Santoro, M., Vivacqua, A., & Bonofiglio, D. et al. (2013). In vitro mechanism for downregulation of ER-α expression by epigallocatechin gallate in ER+/PR+ human breast cancer cells. *Molecular Nutrition & Food Research, 57*(5), 840-853. https://doi.org/10.1002/mnfr.201200560

Deb, G., Thakur, V., Limaye, A., & Gupta, S. (2014). Epigenetic induction of tissue inhibitor of matrix metalloproteinase-3 by green tea polyphenols in breast cancer cells. *Molecular Carcinogenesis, 54*(6), 485-499. https://doi.org/10.1002/mc.22121

Delgado, L., Fernandes, I., González-Manzano, S., de Freitas, V., Mateus, N., & Santos-Buelga, C. (2014). Anti-proliferative effects of quercetin and catechin metabolites. *Food & function, 5*(4), 797-803. https://doi.org/10.1039/C3FO60441A

Farhan, M., Khan, H., Oves, M., Al-Harrasi, A., Rehmani, N., & Arif, H. et al. (2016). Cancer Therapy by Catechins Involves Redox Cycling of Copper Ions and Generation of Reactive Oxygen Species. *Toxins, 8*(2), 37. https://doi.org/10.3390/toxins8020037

Garg, A., Buchholz, T., & Aggarwal, B. (2005). Chemosensitization and Radiosensitization of Tumors by Plant Polyphenols. *Antioxidants & Redox Signaling, 7*(11-12), 1630-1647. https://doi.org/10.1089/ars.2005.7.1630

Granci, V., Dupertuis, Y., & Pichard, C. (2010). Angiogenesis as a potential target of pharmaconutrients in cancer therapy. *Current Opinion In Clinical Nutrition And Metabolic Care, 13*(4), 417-422. https://doi.org/10.1097/mco.0b013e3283392656

Hastak, K., Gupta, S., Ahmad, N., Agarwal, M., Agarwal, M., & Mukhtar, H. (2003). Role of p53 and NF-κB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. *Oncogene, 22*(31), 4851-4859. https://doi.org/10.1038/sj.onc.1206708

Ho, J., Choue, R., & Lee, J. (2013). Green tea seed extract inhibits cell migration by suppressing the epithelial-to-mesenchymal transition (EMT) process in breast cancer cells. *Food Science And Biotechnology, 22*(4), 1125-1129. https://doi.org/10.1007/s10068-013-0193-7

Khan, N., & Mukhtar, H. (2013). Tea and Health: Studies in Humans. *Current Pharmaceutical Design, 19*(34), 6141-6147. https://doi.org/10.2174/1381612811319340008

Kim, H. S., Kim, M. H., Jeong, M., Hwang, Y. S., Lim, S. H., Shin, B. A., Ahn, B. W., & Jung, Y. D. (2004). EGCG blocks tumor promoter-induced MMP-9 expression via suppression of MAPK and AP-1 activation in human gastric AGS cells. *Anticancer research, 24*(2B), 747–753.

Kim, M., Murakami, A., Kawabata, K., &Ohigashi, H. (2005). (−)-Epigallocatechin-3-gallate promotes pro-matrix metalloproteinase-7 production via activation of the JNK1/2 pathway in HT-29 human colorectal cancer cells. *Carcinogenesis, 26*(9), 1553-1562. https://doi.org/10.1093/carcin/bgi104

Kuban-Jankowska, A., Kostrzewa, T., Musial, C., Barone, G., Lo-Bosco, G., Lo-Celso, F., & Gorska-Ponikowska, M. (2020). Green Tea Catechins Induce Inhibition of PTP1B Phosphatase in Breast Cancer Cells with Potent Anti-Cancer Properties: In Vitro Assay, Molecular Docking, and Dynamics Studies. *Antioxidants, 9*(12), 1208. https://doi.org/10.3390/antiox9121208

Kürbitz, C., Heise, D., Redmer, T., Goumas, F., Arlt, A., &Lemke, J. et al. (2011). Epicatechin gallate and catechin gallate are superior to epigallocatechin gallate in growth suppression and anti-inflammatory activities in pancreatic tumor cells. *Cancer Science, 102*(4), 728-734. https://doi.org/10.1111/j.1349-7006.2011.01870.x

Lim, Y., & Cha, Y. (2011). Epigallocatechin-3-gallate induces growth inhibition and apoptosis of human anaplastic thyroid carcinoma cells through suppression of EGFR/ERK pathway and cyclin B1/CDK1 complex. *Journal Of Surgical Oncology, 104*(7), 776-780. https://doi.org/10.1002/jso.21999

Lim, Y., Lee, S., Song, M., Yamaguchi, K., Yoon, J., Choi, E., & Baek, S. (2006). Growth inhibition and apoptosis by (−)-epicatechin gallate are mediated by cyclin D1 suppression in head and neck squamous carcinoma cells. *European Journal Of Cancer, 42*(18), 3260-3266. https://doi.org/10.1016/j.ejca.2006.07.014

Mackenzie, G., & Oteiza, P. (2006). Modulation of transcription factor NF-κB in Hodgkin's lymphoma cell lines: Effect of (−)-epicatechin. *Free Radical Research, 40*(10), 1086-1094. https://doi.org/10.1080/10715760600788396

Maeda-Yamamoto, M., Suzuki, N., Sawai, Y., Miyase, T., Sano, M., Hashimoto-Ohta, A., & Isemura, M. (2003). Association of Suppression of Extracellular Signal-Regulated Kinase Phosphorylation by Epigallocatechin Gallate with the Reduction of Matrix Metalloproteinase Activities in Human Fibrosarcoma HT1080 Cells. *Journal Of Agricultural And Food Chemistry, 51*(7), 1858-1863. https://doi.org/10.1021/jf021039l

Masuda, M., Suzui, M., Lim, J., Deguchi, A., Soh, J., & Weinstein, I. (2002). Epigallocatechin-3-gallate decreases VEGF production in head and neck and breast carcinoma cells by inhibiting EGFR-related pathways of signal transduction. *Journal Of Experimental Therapeutics And Oncology, 2*(6), 350-359. https://doi.org/10.1046/j.1359-4117.2002.01062.x

Meeran, S., Patel, S., Chan, T., & Tollefsbol, T. (2011). A Novel Prodrug of Epigallocatechin-3-gallate: Differential Epigenetic hTERT Repression in Human Breast Cancer Cells. *Cancer Prevention Research, 4*(8), 1243-1254. https://doi.org/10.1158/1940-6207.capr-11-0009

Min, K., & Kwon, T. (2014). Anticancer effects and molecular mechanisms of epigallocatechin-3-gallate. *Integrative Medicine Research, 3*(1), 16-24. https://doi.org/10.1016/j.imr.2013.12.001

Mineva, N., Paulson, K., Naber, S., Yee, A., & Sonenshein, G. (2013). Epigallocatechin-3-Gallate Inhibits Stem-Like Inflammatory Breast Cancer Cells. *Plos ONE, 8*(9), e73464. https://doi.org/10.1371/journal.pone.0073464

Momenimovahed, Z., & Salehiniya, H. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer: Targets and Therapy*, *11*, 151–164. https://doi.org/10.2147/bctt.s176070

Moradzadeh, M., Hosseini, A., Erfanian, S., & Rezaei, H. (2017). Epigallocatechin-3-gallate promotes apoptosis in human breast cancer T47D cells through down-regulation of PI3K/AKT and Telomerase. *Pharmacological Reports, 69*(5), 924-928. https://doi.org/10.1016/j.pharep.2017.04.008

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews, 5*(1). https://doi.org/10.1186/s13643-016-0384-4

Papież, M., Baran, J., Bukowska-Straková, K., & Wiczkowski, W. (2010). Antileukemic action of (−)-epicatechin in the spleen of rats with acute myeloid leukemia. *Food And Chemical Toxicology, 48*(12), 3391-3397. https://doi.org/10.1016/j.fct.2010.09.010

Qin, J., Wang, Y., Bai, Y., Yang, K., Mao, Q., & Lin, Y., … & Xie, L. (2012). Epigallocatechin-3-gallate inhibits bladder cancer cell invasion via suppression of NF-κB-mediated matrix metalloproteinase-9 expression. *Molecular Medicine Reports, 6*(5), 1040-1044. https://doi.org/10.3892/mmr.2012.1054

Ramos, S. (2008). Cancer chemoprevention and chemotherapy: Dietary polyphenols and signalling pathways. *Molecular Nutrition & Food Research, 52*(5), 507-526. https://doi.org/10.1002/mnfr.200700326

Ravindranath, M., Saravanan, T., Monteclaro, C., Presser, N., Ye, X., Selvan, S., & Brosman, S. (2006). Epicatechins Purified from Green Tea (Camellia sinensis) Differentially Suppress Growth of Gender-Dependent Human Cancer Cell Lines. *Evidence-Based Complementary And Alternative Medicine, 3*(2), 237-247. https://doi.org/10.1093/ecam/nel003

Rha, C., Jeong, H., Park, S., Lee, S., Jung, Y., & Kim, D. (2019). Antioxidative, Anti-Inflammatory, and Anticancer Effects of Purified Flavonol Glycosides and Aglycones in Green Tea. *Antioxidants, 8*(8), 278. https://doi.org/10.3390/antiox8080278

Schneider, K., Schwarz, M., Burkholder, I., Kopp-Schneider, A., Edler, L.,Kinsner-Ovaskainen, A., ... & Hoffmann, S. (2009). "ToxRTool", a new tool to assess the reliability of toxicological data. *Toxicology Letters, 189*(2), 138-144. https://doi.org/10.1016/j.toxlet.2009.05.013

Seely, D., Mills, E., Wu, P., Verma, S., &Guyatt, G. (2005). The Effects of Green Tea Consumption on Incidence of Breast Cancer and Recurrence of Breast Cancer: A Systematic Review and Meta-analysis. *Integrative Cancer Therapies, 4*(2), 144-155. https://doi.org/10.1177/1534735405276420

Shay, J., Elbaz, H., Lee, I., Zielske, S., Malek, M., &Hüttemann, M. (2015). Molecular Mechanisms and Therapeutic Effects of (−)-Epicatechin and Other Polyphenols in Cancer, Inflammation, Diabetes, and Neurodegeneration. *Oxidative Medicine And Cellular Longevity, 2015*, 1-13. https://doi.org/10.1155/2015/181260

Sheng, J., Shi, W., Guo, H., Long, W., Wang, Y., Qi, J., … & Xu, Y. (2019). The Inhibitory Effect of (−)-Epigallocatechin-3-Gallate on Breast Cancer Progression via Reducing SCUBE2 Methylation and DNMT Activity. *Molecules, 24*(16), 2899. https://doi.org/10/molecules241628990.339

Siddique, H., Liao, D., Mishra, S., Schuster, T., Wang, L., Matter, B., … & Saleem, M. (2012). Epicatechin-rich cocoa polyphenol inhibits Kras-activated pancreatic ductal carcinoma cell growth in vitro and in a mouse model. *International Journal Of Cancer, 131*(7), 1720-1731. https://doi.org/10.1002/ijc.27409

Sun, H., Yin, M., Hao, D., & Shen, Y. (2020). Anti-Cancer Activity of Catechin against A549 Lung Carcinoma Cells by Induction of Cyclin Kinase Inhibitor p21 and Suppression of Cyclin E1 and P–AKT. *Applied Sciences, 10*(6), 2065. https://doi.org/10.3390/app10062065

Sun, Y., Zhao, Z., Yang, Z., Xu, F., Lu, H., & Zhu, Z. et al. (2017). Risk Factors and Preventions of Breast Cancer. *International Journal Of Biological Sciences, 13*(11), 1387-1397. https://doi.org/10.7150/ijbs.21635

Surh, Y. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer, 3*(10), 768-780. https://doi.org/10.1038/nrc1189

Tao, Z., Shi, A., Lu, C., Song, T., Zhang, Z., & Zhao, J. (2014). Breast Cancer: Epidemiology and Etiology. *Cell Biochemistry And Biophysics, 72*(2), 333-338. https://doi.org/0.1007/s12013-014-0459-6

Wei, R., Mao, L., Xu, P., Zheng, X., Hackman, R., Mackenzie, G., & Wang, Y. (2018). Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models. *Food & Function, 9*(11), 5682-5696. https://doi.org/10.1039/c8fo01397g

Wu, A., & Butler, L. (2011). Green tea and breast cancer. *Molecular Nutrition & Food Research, 55*(6), 921-930. https://doi.org/10.1002/mnfr.201100006

Xu, P., Yan, F., Zhao, Y., Chen, X., Sun, S., Wang, Y., & Ying, L. (2020). Green Tea Polyphenol EGCG Attenuates MDSCs-mediated Immunosuppression through Canonical and Non-Canonical Pathways in a 4T1 Murine Breast Cancer Model. *Nutrients, 12*(4), 1042. https://doi.org/10.3390/nu12041042

Yang, J., Wei, D., & Liu, J. (2005). Repressions of MMP-9 expression and NF-κB localization are involved in inhibition of lung carcinoma 95-D cell invasion by (–)-epigallocatechin-3-gallate. *Biomedicine & Pharmacotherapy, 59*(3), 98-103. https://doi.org/10.1016/j.biopha.2005.01.004

Zeng, L., Holly, J., & Perks, C. (2014). Effects of Physiological Levels of the Green Tea Extract Epigallocatechin-3-Gallate on Breast Cancer Cells. *Frontiers In Endocrinology*, 5. https://doi.org/10.3389/fendo.2014.00061