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A Systematic Review of *Cassia alata* Extract as an Anticancer Agent in Human Breast Cancer Cell Lines from *In Vitro* Studies

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ABSTRACT

Introduction: As one of the leading causes of cancer-related deaths in women, breast cancer still poses major treatment challenges. Chemotherapy often causes toxicity and resistance, emphasizing the need for safer options. *Cassia alata*, rich in flavonoids like kaempferol shows potential anticancer effects through apoptosis induction. This review evaluated the in vitro evidence of *C. alata* against human breast cancer cell lines.

Methods: This review followed the PRISMA 2020 guidelines. A total of 1,183 records were retrieved from six databases (PubMed, Scopus, ScienceDirect, ProQuest, EBSCOhost, and Google Scholar). After screening records, eight studies met the eligibility criteria. Eligible studies were *in vitro* experiments evaluating *C. alata* crude extracts, fractions, or derived formulations on human breast cancer cell lines (MCF-7, T47D, MDA-MB-231, and others). Data on interventions, concentrations, cytotoxicity assays, and molecular mechanisms were extracted. Quality assessment was performed independently by two reviewers using the ToxRTool, with discrepancies resolved through consensus. Inter-rater reliability was not applied due to small sample size and consensus scoring.

Results: *C. alata* consistently reduced breast cancer cell viability, with reported IC_{50} values ranging from 0.013 to 456 µg/mL depending on extract and cell line. Most studies showed dose-dependent cytotoxicity and apoptosis via caspase activation, nuclear fragmentation, and PI3K/AKT inhibition. Variations in extraction methods and assay conditions explained inter-study heterogeneity.

Conclusion: Evidence from studies indicates that *C. alata* reduces breast cancer cell viability and promotes apoptosis. Further in vivo and clinical studies are required to validate efficacy and safety.

Keywords: apoptosis; breast cancer; *Cassia alata*; cytotoxicity; *in vitro*

INTRODUCTION

Breast cancer remains one of the most prevalent malignancies worldwide, with an increasing incidence and mortality rate each year. According to the World Health Organization (WHO), there were approximately 2 million new breast cancer cases and 700,000 related deaths in 2020[1]. In Indonesia, breast cancer remains the most common cancer among women, with approximately 66,271 new cases in 2022 and an age-standardized incidence rate of 42.1 per 100,000 women in 2020 as the highest incidence rate across all cancer types, highlighting the urgent need for effective and accessible treatment options in low- and middle-income countries[1]. Despite advances in detection and treatment, current chemotherapeutic regimens face significant limitations, including severe adverse effects, high toxicity to normal cells, increasing drug resistance, and a substantial financial burden on patients and healthcare

systems[2]. These limitations highlight the necessity for exploring alternative therapeutic approaches with improved selectivity and reduced toxicity.

In recent years, natural products have been widely investigated as potential anticancer agents. Their sustainability, structural bioactive compounds, and multi-targeted mechanisms make them valuable sources for drug discovery, often with lower toxicity profiles compared to conventional chemotherapeutics[3]. The exploration of plant-derived compounds as anticancer agents is grounded in both traditional medicine and modern pharmacology. Historically, natural products have served as the backbone of anticancer drug discovery, exemplified by agents such as paclitaxel, vincristine, and camptothecin, all of which originated from plants and later revolutionized chemotherapy[4]. Plants produce a vast repertoire of structurally diverse secondary metabolites as part of their evolutionary defense mechanisms, many of which exhibit bioactivities relevant to cancer treatment, including induction of apoptosis, inhibition of angiogenesis, modulation of oxidative stress, and interference with oncogenic signaling pathways[5]. Unlike single-target synthetic agents, phytochemicals frequently exert polypharmacological effects, allowing simultaneous modulation of multiple molecular pathways that drive tumor initiation, progression, and resistance[6]. This multi-targeted approach is particularly important in breast cancer, which is characterized by heterogeneity across molecular subtypes, such as estrogen receptor-positive luminal tumors and triple-negative breast cancers[7]. Moreover, plant-derived compounds are increasingly recognized for their ability to enhance chemosensitivity and overcome resistance mechanisms when used in combination with standard therapies[8]. Taken together, these properties underscore the scientific rationale for systematically evaluating *Cassia alata* as a potential anticancer candidate, building on both its ethnomedicinal use and emerging mechanistic evidence from *in vitro* studies[9].

Recent experimental studies have highlighted *Cassia alata*, which stands out among medicinal plants for its rich content of flavonoids and other secondary metabolites that play important roles in anticancer mechanisms[9]. Kaempferol, one of the major flavonoids in *Cassia alata*, has been known to suppress BCL-2 expression, an anti-apoptotic protein that contributes to tumor cell survival and resistance to chemotherapy in many cancers, making it a critical target for anticancer strategies. Kaempferol promotes mitochondrial outer membrane permeabilization and activation of pro-apoptotic proteins such as BAX and BAK, leading to mitochondrial dysfunction and apoptotic cell death[10,11]. In addition to kaempferol, various other phytochemicals in *Cassia alata* are also reported to play significant roles in mediating its anticancer effects. Aloe-emodin, an anthraquinone derivative, has been reported to induce apoptosis by activating caspase-3 and caspase-9, while also inhibiting anti-apoptotic proteins such as BCL-2[12]. Quercetin, another abundant flavonoid, exerts antiproliferative effects through cell cycle arrest and inhibition of the PI3K/AKT signaling pathway, a critical axis for cancer cell survival and growth[13]. Emodin and rhein, also present in *Cassia alata*, have been shown to modulate oxidative stress and mitochondrial function, contributing to apoptosis and reducing tumor cell proliferation[14]. These findings suggest that these metabolites act on multiple molecular targets, supporting the multi-mechanistic anticancer activity of *Cassia alata* extracts observed *in vitro*.

Several *in vitro* studies have reported the cytotoxic potential of *Cassia alata*, consistently demonstrating growth inhibition in human breast cancer cell lines such as MCF-7, T47D, and the triple-negative MDA-MB-231, although differences in extract types and experimental approaches have led to variability in the reported outcomes[15,16,17]. These cell lines represent distinct breast cancer subtypes, with MCF-7 and T47D being estrogen receptor-positive (luminal type) and MDA-MB-231 representing the aggressive triple-negative subtype, which enables assessment of the extract's therapeutic relevance across distinct subtypes of breast cancer[7]. Investigations using *in vitro* models provide an essential approach to evaluate these subtype-specific responses under controlled conditions, thereby offering early insight into the molecular mechanisms of potential anticancer agents[3].

METHOD

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[18]. A comprehensive literature search was conducted in PubMed, Scopus, ScienceDirect, ProQuest, EBSCOhost, and Google Scholar for studies published up to January 2025. The search strategy combined both Medical Subject Headings (MeSH) and free-text terms: (*Cassia alata* OR *Senna alata*) AND ("Breast Neoplasms" OR "Breast Cancer" OR "MCF-7" OR "T47D" OR "ZR-75-1" OR "BT-474" OR "SK-BR-3" OR "MDA-MB-231" OR "MDA-MB-468" OR "Hs578T") AND ("Cell Line, Tumor" OR *in vitro* OR "Cytotoxicity" OR "Apoptosis").

The inclusion criteria were as follows: (1) original *in vitro* studies on human breast cancer cell lines (e.g., MCF-7, T47D, MDA-MB-231, and other subtypes); (2) interventions involving *Cassia alata* extracts in any preparation form (crude ethanolic, methanolic, aqueous, or purified fractions); (3) studies employing negative controls (vehicle or untreated cells) and/or positive controls (e.g., doxorubicin, tamoxifen); (4) outcomes reporting anticancer activity, including cell viability (IC_{50} values), cytotoxicity, apoptosis, antiproliferative activity, cell cycle arrest, or molecular mechanisms; and (5) full-text articles published in English or Indonesian with no restriction on publication year or country.

The exclusion criteria were: (1) studies not involving *Cassia alata* or those using mixed herbal extracts where its individual effect could not be distinguished; (2) studies conducted outside the *in vitro* setting (e.g., *in vivo*, *in silico*, or clinical trials); (3) secondary articles (reviews, meta-analyses), conference abstracts, theses, editorials, or short communications without primary data; (4) publications without accessible full text or published in languages other than English or Indonesian; and (5) studies with outcomes unrelated to anticancer activity (e.g., antibacterial or anti-inflammatory properties).

All identified references were imported into Mendeley Reference Manager for organization and duplicate removal. Screening was conducted in Rayyan software to facilitate blinded review. The screening process was performed in three stages: (1) title screening, (2) abstract screening, and (3) full-text eligibility assessment. Two independent reviewers screened the studies, and any discrepancies were resolved through discussion or by consulting a third reviewer.

Data extraction was performed using a standardized Excel sheet, capturing the following categories: author and year of publication, country of study, cell lines used, extract type and preparation method, control type, assay methods (e.g., MTT assay, flow cytometry, cell cycle analysis), primary outcomes (IC_{50}, apoptosis induction, cell viability reduction, antiproliferative activity), and secondary findings such as molecular pathway modulation or phytochemical characterization. Limitations reported by the original studies were also extracted.

An adapted version of the ToxRTool (Toxicological data Reliability Assessment Tool) was used to evaluate the methodological quality and risk of bias of the included *in vitro* studies. The tool provides a structured framework consisting of 18 criteria divided into five reliability categories: (1) test substance identification, (2) test system characterization, (3) study design description, (4) study results documentation, and (5) plausibility of study design and results. Each criterion was scored as "yes" (1 point) or "no/unclear" (0 points), generating a total score between 0 and 18. In summary, studies were assigned to four categories according to their relevance and scored 0–18 points (reliable without restrictions, 15–18 points; reliable with restrictions, 11–14 points, not reliable, < 11 points; not assignable, if insufficient documentation to assess the study was provided). In this review, studies scoring ≥11 points were classified as sufficiently reliable (high methodological quality), while those below this threshold were considered at high risk of bias. Assessment was initially performed by one reviewer (FPA), and subsequently verified through discussion with a second reviewer (FA) to resolve any uncertainties. Discrepancies were discussed until a consensus was reached, and final scores were determined through agreement, if consensus was not achieved, a third reviewer was consulted to reach agreement. Because all studies were jointly reviewed and consensus-based, inter-rater reliability (e.g.,

Cohen's κ) was not calculated, in accordance with the ToxRTool guidelines, which consider consensus scoring acceptable for a small number of studies when disagreement is minimal.

A total of 8 eligible studies were included after screening[16,17,19,20,21,22,23,24]. Most studies originated from Asian countries (Indonesia, Malaysia, India, and Bangladesh), reflecting regions where *Cassia alata* is traditionally used[19,20,21]. The majority of studies employed ethanolic or methanolic extracts, with some using aqueous fractions[16,20]. Commonly used breast cancer cell lines included MCF-7 and T47D (luminal type), as well as MDA-MB-231 (triple-negative subtype)[16,21]. Most studies applied the MTT assay for cytotoxicity testing, while several incorporated apoptosis assays (Annexin V staining, caspase activity) and cell cycle analysis[12,16]. Positive controls such as doxorubicin were employed in several studies, while others used untreated cells as the baseline comparison[16,20]. Across these studies, *Cassia alata* consistently demonstrated cytotoxic and pro-apoptotic effects, although variations in extract preparation and methodological rigor contributed to differences in reported outcomes.

RESULT

Study selection

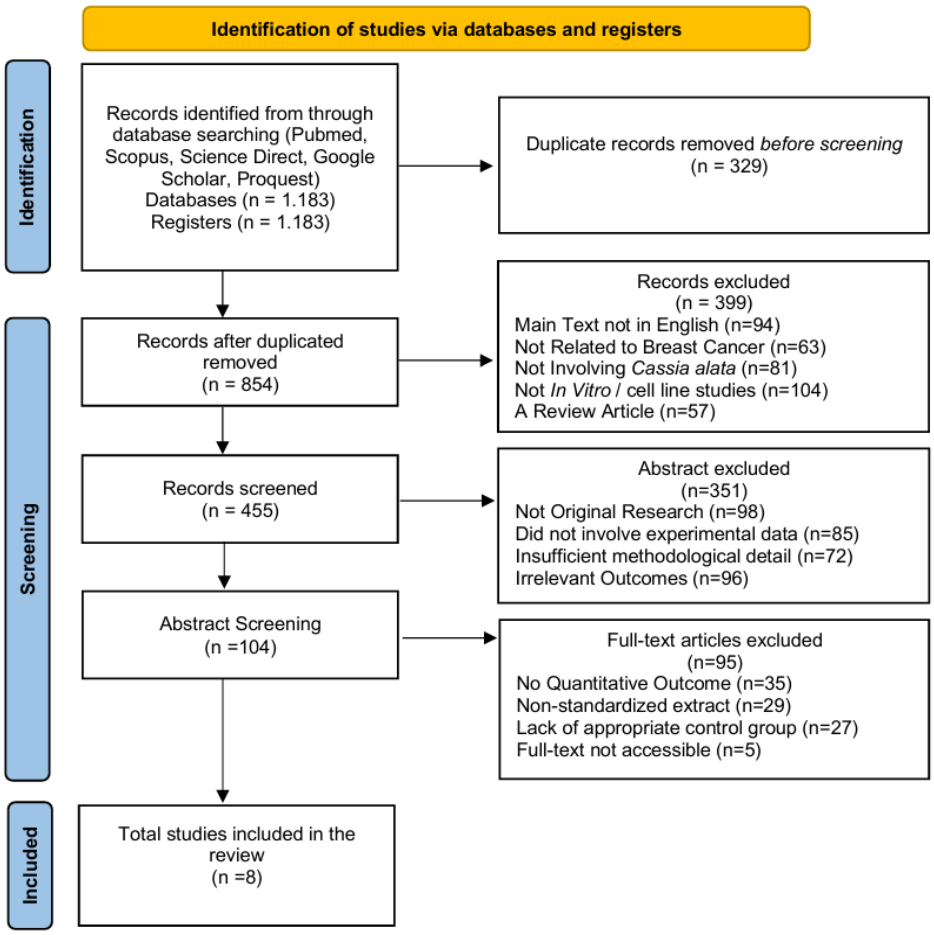

Figure 1. Flow Diagram of Systematic Review Search Procedures according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

A total of 1,183 records were identified from six electronic databases (PubMed, Scopus, ScienceDirect, ProQuest, EBSCOhost, and Google Scholar). After removing 329 duplicates, 854

records remained for screening. During the title and abstract screening, 399 records were excluded for reasons such as being non-English (n = 94), not related to breast cancer (n = 63), not involving *Cassia alata* (n = 81), not conducted in *in vitro* or cell line settings (n = 104), or being review articles (n = 57). Subsequently, 351 articles were excluded at the abstract level due to being non-original research (n = 98), lacking experimental data (n = 85), insufficient methodological detail (n = 72), or reporting irrelevant outcomes (n = 96). The remaining 104 articles were assessed in full text, of which 95 were excluded because they did not provide quantitative outcomes (n = 35), used non-standardized extracts (n = 29), lacked appropriate control groups (n = 27), or had inaccessible full text (n = 5). Ultimately, 8 studies fulfilled the eligibility criteria and were included in the qualitative synthesis (Figure 1).

Study Characteristics

A total of eight studies published between 2011 and 2025 were included in this review (Table 1). Most studies originated from Asian countries, particularly India (n = 3), Indonesia (n = 2), and the Philippines (n = 1), while others were conducted in Nigeria, Vietnam, and the United States. All studies employed *in vitro* models to assess the cytotoxic or antiproliferative activity of *Cassia alata* or *Senna alata* extracts against human cancer cell lines, with one study by Wiedadgo et al. (2025) extending the analysis to an in-silico and nanodelivery system approach.

Table 1. Identification of Studies Included.

Author (Year)	Country	Title
Chahardeh et al. (2021)	India	Cytotoxic activity of fractions from *Senna alata* leaves on human breast cancer cell line (MCF-7)
Khoerunisah et al. (2021)	Indonesia	Anticancer activity of crude ethanol extract and isolated compounds from *Cassia alata* leaves against MCF-7 cell lines
Fernand et al. (2011)	United States	Rhein inhibits angiogenesis and the viability of hormone-dependent and -independent cancer cells under normoxic or hypoxic conditions in vitro
Onyegeme-Okerenta et al. (2018)	Nigeria	Evaluation of the anti-cancer potential of *Senna alata* leaf extract on human breast cancer cell line (MCF-7)
Saravanan et al. (2022)	India	Green synthesis of silver nanoparticles using *Senna alata* leaf extract and evaluation of their anticancer activity against MCF-7 cells
Bhaduhsha et al. (2019)	India	Evaluation of anticancer activity of hydroalcoholic extract of *Cassia alata* Linn. against MCF-7 and PC-3 cancer cell lines
Olarte et al. (2013)	Filipina	Isolation of polyunsaturated fatty acid esters from *Cassia alata* leaves with cytotoxic activity against MCF-7 cells
Wiedagdo et al. (2025)	Indonesia	Chitosan–folate-coated nanoliposomes loaded with *Cassia alata* extract enhance cytotoxicity in T47D breast cancer cells

The majority of studies focused on the MCF-7 cell line, representing hormone receptor-positive breast cancer, making it the most frequently used model across the dataset. Additional breast cancer cell lines included T47D (ER^+), MDA-MB-435s (hormone-independent), and MDA-MB-231 in related literature, reflecting efforts to test extracts against different molecular subtypes of breast cancer. Some studies also incorporated non-breast cancer models such as PC-3 (prostate), HT-29 (colon), Col2 (colorectal), and T24 (bladder), primarily for comparative cytotoxicity profiling.

Table 2. Characteristic on Studies Included.

Author (Year)	Study Type	Cell Line(s)
Chahardeh et al. (2021)	In-vitro cytotoxicity & antiproliferation	MCF-7 (breast cancer)
Khoerunisah et al. (2021)	In-vitro cytotoxicity study	MCF-7
Fernand et al. (2011)	In-vitro cytotoxicity & anti-angiogenesis	MCF-7 (hormone-dependent breast cancer) MDA-MB-435s (hormone-independent breast cancer)
Onyegeme-Okerenta et al. (2018)	In-vitro cytotoxicity screening (MTT, clonogenic, Trypan-Blue, methylene-blue)	MCF-7 (breast cancer); C4-2WT (prostate cancer), HT-29 & HCT-116 (colorectal cancer)
Saravanan et al. (2022)	In-vitro cytotoxicity & antimicrobial activity	MCF-7 (breast cancer)
Bhaduhsha et al. (2019)	In-vitro cytotoxicity (MTT assay)	MCF-7 (breast cancer); PC-3 (prostate cancer)
Olarte et al. (2013)	In-vitro sitotoksisitas & apoptosis	MCF-7 (breast cancer); T24 (bladder cancer); Col2 (colorectal cancer)
Wiedagdo et al. (2025)	In-silico & in-vitro evaluation of a nanodrug delivery system	T47D (ER^+ breast cancer)

Main Findings

In vitro experiments consistently show that leaf extracts of Senna alata and Cassia alata exert strong cytotoxic activity against breast cancer cells. Studies on Senna alata have revealed potent anti-cancer properties. An ethyl-acetate extract of S. alata achieved a notable GI_{50} of approximately 5.9 µg mL^{-1} on the MCF-7 cell line[20]. Similarly, a hydroalcoholic extract of Senna alata leaves demonstrated significant inhibition of MCF-7 cancer cell proliferation with an IC_{50} of approximately 46.7 µg mL^{-1} (and PC-3 cells with ~62.5 µg mL^{-1})[21]. These results were typically derived from MTT or clonogenic assays, confirming dose-dependent reductions in cell viability across the observed cancer lines. The ethyl-acetat e extract of S. alata achieved the lowest GI_{50} (≈ 5.9 µg mL^{-1}) on the MCF-7 line, while the hydro-alcoholic extract produced an IC_{50} of ≈ 46.7 µg mL^{-1} on the same cells. Fractionated ethanol extracts and isolated compounds (aloe-emodin, emodin, kaempferol) yielded IC_{50} values ranging from 12.7 ppm to 131.3 ppm, confirming the contribution of specific phytochemicals to the anti-breast-cancer effect.

Further investigation into Senna alata also included sequential solvent extracts. The n-hexane fraction of Senna alata leaves proved to be the most potent, exhibiting an IC_{50} of 0.013 µg mL^{-1} on MCF-7 cells in a 72-hour SRB assay, with the DCM fraction yielding an IC_{50} of 57.61 µg mL^{-1}. Interestingly, brine shrimp lethality tests showed no toxicity at concentrations up to 5,000 µg mL^{-1}, suggesting a selective cytotoxicity towards cancer cells[22]. Despite this impressive potency, specific molecular targets were not reported, and only dose-response data was provided [22]. The potential of Cassia alata extracts has also been explored through advanced delivery systems. Chitosan-folate-coated nanoliposomes loaded with C. alata extract showed high encapsulation efficiency (96.7%) and sustained release (approximately 90% within 72 hours). This formulation resulted in an IC_{50} of approximately 53.46 µg mL^{-1} against the T47D breast cancer cell line, indicating moderate activity and better efficacy compared to the free extract [17]. The authors attributed this enhanced cytotoxic effect to improved cellular uptake via folate receptors, though specific mechanistic pathways were not explored [17].

Furthermore, individual compounds found in related species demonstrate significant anti-cancer potential. Rhein, a pure anthraquinone, effectively inhibited angiogenesis and reduced the viability of hormone-dependent (MCF-7) and -independent (MDA-MB-435s) breast cancer cells under both normoxic and hypoxic conditions. Rhein achieved an IC_{50} of approximately 81 µM for MCF-7

(normoxia) and 52 µM for MDA-MB-435s (normoxia)[19]. Its anti-angiogenic effects were attributed to the down-regulation of PI3K/AKT, ERK pathways, Hsp90α, and VEGF signaling, linking reduced angiogenic activities under both normoxic and hypoxic conditions[19].

Table 3. Summarization on Studies Included.

Author (Year)	Intervention	Comparison	Assay/ Method	Outcome (IC_{50})
Chahardeh et al. (2021)	Sequential solvent extracts of Senna alata leaves (n-hexane, DCM, $CHCl_3$, water)	Vehicle (≤0.1 % DMSO)	SRB assay; Brine-shrimp lethality test; GC-MS profiling	IC_{50} (72 h): n-hexane = 0.013 µg mL^{-1}; DCM = 47.11 µg mL^{-1}; $CHCl_3$ = 57.61 µg mL^{-1}
Khoerunisah et al. (2021)	Crude ethanol extract of *Cassia alata* leaves, its solvent fractions (n-hexane, ethyl-acetate, water) and isolated compounds (aloe-emodin, emodin, kaempferol)	Vehicle (≤0.1 % DMSO)	MTT assay	IC_{50} (72 h): aloe-emodin = 12.7 ppm; emodin = 18.1 ppm; kaempferol = 131.3 ppm; n-hexane fraction also active
Fernand et al. (2011)	Rhein (pure compound, 0-200 µM)	Vehicle (≤0.1 % DMSO)	MTT cell-viability assay; HUVEC tube-formation; proliferation; migration assays	MCF-7 IC_{50} ≈ 81 µM (normoxia) / 71 µM (hypoxia) MDA-MB-435s IC_{50} ≈ 52 µM (normoxia) / 127 µM (hypoxia)
Onyegeme-Okerenta et al. (2018)	Ethyl-acetate leaf extract of Senna alata (0.1 – 100 µg mL^{-1})	Vehicle (≤0.1 % DMSO)	MTT (viability); clonogenic survival; Trypan-Blue exclusion; methylene-Blue; proliferation	GI_{50} (MTT, 72 h): • MCF-7 = 5.90 µg mL^{-1} • HT-29 = 4.97 µg mL^{-1} • HCT-116 = 11.86 µg mL^{-1} • C4-2WT = 9.48 µg mL^{-1}
Saravanan et al. (2022)	Bio-active Ag-nanoparticles (Ag-NPs) synthesized using Senna alata leaf extract (various concentrations)	Implicit vehicle control (≤0.1 % DMSO); exact control not detailed	MTT assay (cell viability); Disc-diffusion assay (antibacterial)	• Cytotoxicity: dose-dependent decrease in MCF-7 viability; IC_{50} values reported in the paper • Antimicrobial: inhibition zones measured against six pathogenic strains; highest activity against B. subtilis and V. cholerae

Author (Year)	Intervention	Comparison	Assay/ Method	Outcome (IC_{50})
Bhaduhsha et al. (2019)	Hydroalcoholic extract of Senna alata leaves (0.1 – 100 µg mL^{-1})	Vehicle (≤0.1 % DMSO)	MTT assay (72 h incubation)	• MCF-7 IC_{50} ≈ 46.7 µg mL^{-1} • PC-3 IC_{50} ≈ 62.5 µg mL^{-1}
Olarte et al. (2013)	Pure isolate TLC f6l from *Cassia alata* leaves (polyunsaturated fatty acid esters)	Vehicle (≤0.1 % DMSO)	MTT; colorization DAPI; assay TUNEL; caspase-3 activity	• MCF-7 IC_{50} ≈ 16 µg mL^{-1} • T24 IC_{50} ≈ 17 µg mL^{-1} • Col2 IC_{50} ≈ 17 µg mL^{-1}
Wiedagdo et al. (2025)	Chitosan-folate coated nanoliposomes loaded with Cassia alata leaf extract (Lip-CA@Chi-FA)	Non-coated liposome (Lip-CA) and free extract (CA) as comparators	ATR-FTIR & TEM (particle characterization) ; in-vitro release (cumulative % over 72 h); MTT cytotoxicity	• Encapsulation efficiency ↑ from 94.4 % (Lip-CA) to 96.7 % (Lip-CA@Chi-FA) • Cumulative release ≈ 90.6 % at 72 h (targeted conditions) • IC_{50} ≈ 53.46 µg mL^{-1} against T47D (moderate activity, better than free extract)

Key Result and Study Limitation

Across the eight included studies, all reported significant dose-dependent cytotoxicity of *Cassia alata* (syn. *Senna alata*) extracts or isolated compounds against breast cancer cell lines. The majority employed MCF-7 cells, with some extending analysis to T47D, MDA-MB-231, and other cancer models, allowing cross-subtype evaluation.

Several studies emphasized the potency of solvent fractions. For example, the *n-hexane* fraction was consistently reported as the most active, achieving very low IC_{50} values, while brine shrimp lethality assays demonstrated minimal toxicity to non-cancerous systems, suggesting a degree of selectivity. Isolated phytochemicals, including aloe-emodin, emodin, and kaempferol, also exhibited strong cytotoxicity, with aloe-emodin being the most potent.

Other research highlighted mechanistic insights, though unevenly. Fernand et al. (2011) provided the most detailed analysis, linking rhein's effects to inhibition of angiogenic signaling pathways such as PI3K/AKT, ERK, NF-κB, and VEGF under both normoxic and hypoxic conditions. Similarly, Olarte et al. (2013) demonstrated apoptosis induction through caspase-3 activation, while more recent studies explored nanotechnology-based delivery systems. Wiedagdo et al. (2025) developed folate-modified nanoliposomes, achieving enhanced cellular uptake and improved cytotoxicity compared to free extracts, underscoring the therapeutic promise of advanced formulations.

However, notable limitations were identified across studies. Many failed to explore underlying molecular mechanisms, reporting only cell viability data without pathway analysis. Others lacked standardized extraction methods or sufficient methodological detail, limiting comparability. Only a minority tested extracts against normal cell lines or incorporated advanced mechanistic assays, making it difficult to fully establish selectivity and safety profiles.

Taken together, the studies collectively support the anticancer potential of *Cassia alata* through multiple bioactive fractions and compounds, while highlighting important gaps in mechanistic elucidation, standardization, and translational relevance that should be addressed in future research.

Table 4. Key Result and Limitation of Studies Included.

Author (Year)	Key Result	Study Limitation
Chahardeh et al. (2021)	n-Hexane extract most potent; brine-shrimp assay showed no toxicity up to 5 000 µg mL^{-1}	No specific molecular targets reported; only dose-response data
Khoerunisah et al. (2021)	Aloe-emodin most cytotoxic among isolated compounds; n-hexane fraction showed strong activity	No signalling pathway evaluated – only cell-viability data
Fernand et al. (2011)	Dose-dependent inhibition of cancer-cell viability; significant suppression of VEGF-stimulated HUVEC angiogenic activities under both normoxic and hypoxic conditions	Rhein ↓PI3K, phospho-AKT, phospho-ERK (total unchanged); ↓Hsp90α, NF-κB, COX-2, HER-2 – linking reduced angiogenic signalling
Onyegeme-Okerenta et al. (2018)	Dose-dependent reduction of viability in all four cancer lines; clonogenic assay showed significant colony loss at GI_{50} and 2× GI_{50}; increased non-viable cells (Trypan-Blue); decreased growth (methylene-blue) with higher extract concentration	No molecular pathways investigated; only phenotypic cytotoxicity reported
Saravanan et al. (2022)	Ag-NPs significantly reduced MCF-7 cell viability and displayed strong antibacterial activity; authors propose potential use against cholera and as a novel anticancer agent	No molecular mechanism (e.g., apoptosis markers, ROS generation) was investigated or reported
Bhaduhsha et al. (2019)	Significant dose-dependent cytotoxicity observed in both cancer cell lines; extract inhibited cell proliferation without affecting normal fibroblast cells in preliminary tests	Not reported (no mechanistic study conducted in this paper)
Olarte et al. (2013)	Isolation F6L shows dose-dependent cytotoxicity; changes in cell morphology and apoptosis markers (DAPI, TUNEL, caspase-3 in Col2 cells).	The mechanism is thought to occur through induction of apoptosis (increased caspase-3).
Wiedagdo et al. (2025)	Folate-mediated coating improves targeting and release kinetics; nanoliposome formulation enhances cytotoxic effect compared with free extract	No detailed intracellular signaling studied; authors attribute improved effect to better cellular uptake via folate receptor but mechanistic pathways not explored

Assessment of Quality

Based on the Toxicological data Reliability Assessment Tool (ToxRTool), which comprises 18 criteria for evaluating the reliability of in vitro studies, most of the included articles demonstrated high methodological quality (Reliability category 1), with total scores ranging from 15 to 18. Studies by

Chahardeh et al. (2021), Saravanan et al. (2022), and Wiedagdo et al. (2025) achieved the maximum score (18/18), reflecting comprehensive reporting of test substance identification and purity, detailed cell culture conditions, appropriate use of positive and negative controls, clear replication strategies, and transparent statistical analyses. Fernand et al. (2011) and Bhadusha et al. (2019) also scored highly (17/18), though they provided limited information on the physicochemical characterization or purity of the tested extracts.

Table 5. Assessment of Quality using ToxRTool.

Criteria and Reference	Chaharde h et al. (20 21)	Khoerunisah et al. (2021)	Fernand e t al. (2011)	Onyegeme-Ok erenta et al. (2018)	Saravanan et al. (2022)	Bhaduhsha et al. (2019)	Olarte et al. (2013)	Wiedagdo et al. (2025)
Substance identification	1	1	1	1	1	1	1	1
Purity of substance	1	1	1	0	1	1	1	1
Source/origin of substance	1	1	1	1	1	1	1	1
Nature/physico -chemical properties of test substance	1	1	0	1	1	1	1	1
Stability of the test substance	1	0	1	0	1	1	0	1
Species/strain of the cell line	1	1	1	1	1	1	1	1
Source/origin of the cell line	1	1	1	1	1	1	1	1
Authentication of the cell line	1	0	1	0	1	1	1	1
Culture conditions described	1	1	1	1	1	0	1	1
Passage number or population	1	1	1	1	1	1	1	1
Test concentrations	1	1	1	1	1	1	0	1
Exposure duration specified	1	1	1	1	1	1	1	1
negative (vehicle) and positive controls	1	1	1	1	1	1	1	1
Number of replicates	1	1	1	1	1	1	1	1
Methods of measurement	1	1	1	1	1	1	1	1
Statistical method reported	1	1	1	1	1	0	1	1
Quantitative results reliability	1	1	1	1	1	1	1	1
Adequate study design	1	1	1	1	1	1	1	1
Total Score	18	16	17	15	18	17	16	18
Reliability	1	1	1	1	1	1	1	1

In contrast, Khoerunisah et al. (2021) and Olarte et al. (2013) scored 16/18, primarily due to insufficient reporting on cell line authentication and test substance stability. Onyegeme-Okerenta et al. (2018) obtained the lowest score (15/18), mainly because of incomplete information regarding the test material's purity and stability as well as the lack of clear cell line authentication. Nevertheless, all

studies adequately described experimental design, test concentrations, exposure duration, control groups, and quantitative outcomes such as IC_{50} values.

Overall, these eight studies can be considered reliable (Reliability category 1), as the majority of ToxRTool criteria were fulfilled. Minor reporting gaps were observed, particularly concerning test substance characterization and cell line authentication, but the core methodological features were generally well described. This high overall reliability supports the inclusion of these studies in the present systematic review and strengthens confidence in their cytotoxicity findings regarding *Cassia alata* extracts in breast cancer cell models.

DISCUSSION

Cytotoxicity of *Cassia alata* Extracts Against Breast Cancer Cells

Several in vitro studies (n = 8) have reported cytotoxic effects of Cassia/Senna alata leaf preparations against breast cancer cell lines (predominantly MCF-7), although potency varies markedly with extraction solvent, fractionation, and assay method. Reported potencies include an exceptionally potent n-hexane fraction (IC50 ≈ 0.013 µg/mL, SRB), ethyl-acetate fractions with GI50 ≈ 5.9 µg/mL, and crude hydro-alcoholic extracts with IC50 values in the tens of µg/mL (e.g., ≈46.7 µg/mL on MCF-7)[16,21]. Isolated constituents such as aloe-emodin, emodin, and kaempferol generally display stronger activity than crude extracts (reported ranges ~12.7–131.3 ppm), suggesting specific phytochemicals drive much of the observed cytotoxicity. Nanoformulations improved in-vitro delivery: chitosan-folate coated nanoliposomes achieved high encapsulation efficiency (≈96.7%), sustained release (~90% over 72 h), and increased potency (IC50 ≈ 53 µg/mL on T47D versus the corresponding free extract). Mechanistic data indicate apoptosis induction (caspase-3/9 activation, DNA fragmentation), mitochondrial dysfunction, inhibition of PI3K/AKT and ERK signaling, downregulation of Hsp90α and VEGF (noted for anthraquinones), and modulation of oxidative stress. However, lack of standardization (assay type, exposure time, concentration units), limited reporting of selectivity against non-tumor cells, and sparse in vivo validation limit translational interpretation. We recommend standardized extraction/assay protocols, rigorous constituent identification, comprehensive selectivity testing, and in vivo efficacy/safety studies.

Phytochemical Basis and Potent Active Constituents

The anticancer activity of *Cassia alata* extracts is intrinsically linked to its complex phytochemical composition, predominantly characterized by anthraquinones (e.g., emodin, aloe-emodin, rhein) and flavonoids (e.g., kaempferol). Research indicates that isolated compounds often exhibit enhanced cytotoxic effects when compared to the crude extracts, affirming their role as primary active constituents. For example, specific studies on MCF-7 cells highlighted that emodin, aloe-emodin, and kaempferol demonstrate significant anti-proliferative activity, with emodin showing an IC50 of 28.69 µg/mL and kaempferol at 24.57 µg/mL in specific fractionated experiments[16].

Furthermore, rhein, a prominent anthraquinone, has been shown to inhibit angiogenesis and reduce the viability of both hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells under both normoxic and hypoxic conditions, with IC50 values ranging from 14.2 µM to over 100 µM depending on the cell line and conditions[19]. This robust activity across varying cellular microenvironments underscores the therapeutic potential of individual compounds within *Cassia alata*. The consistent presence and activity of these compounds across different extracts and fractions provide a strong foundation for future research focusing on their specific mechanisms and potential for drug development.

Molecular Mechanisms of Action in Breast Cancer Cells Subtypes and Clinical Implications

The available literature elucidates several key molecular mechanisms through which *Cassia alata* and its constituents exert their anticancer effects, primarily focusing on apoptosis induction and modulation of critical signaling pathways. Studies consistently demonstrate the induction of apoptosis in breast cancer cells treated with *Cassia alata* extracts and purified compounds. This is evidenced by apoptotic markers such as caspase-3/9 activation, DNA fragmentation (via TUNEL assay), and alterations in mitochondrial membrane potential. These findings confirm that the extracts engage programmed cell death pathways, a hallmark of effective anticancer agents.

Beyond apoptosis, the extracts have been shown to modulate vital cell signaling cascades. Rhein, for instance, significantly suppresses VEGF-stimulated angiogenesis and downregulates key pro-survival pathways, including PI3K/AKT and ERK signaling[19]. This compound also inhibits Hsp90α activity, a chaperone protein crucial for maintaining the stability of numerous oncogenic proteins. Another study with an isolate from *Cassia alata* leaves on MDA-MB-231 cells showed inhibition of cell growth and cell cycle arrest at the G1 phase along with an increase in apoptotic cells detected by DAPI staining[23]. These multi-targeted effects on angiogenic, proliferative, and survival pathways underscore the complex interplay of *Cassia alata* components in disrupting cancer cell functions.

The consistent demonstration of cytotoxicity in luminal-type cell lines (MCF-7, T47D) as well as the aggressive triple-negative subtype (MDA-MB-231) is particularly important. Triple-negative breast cancer (TNBC) is clinically and biologically distinct, it lacks estrogen and progesterone receptors and HER2 amplification, is refractory to endocrine and HER2-targeted therapies, and is typically managed with cytotoxic chemotherapy, factors that contribute to poorer prognosis and higher recurrence rates compared with receptor-positive subtypes. The biology of TNBC (greater genomic instability, reliance on alternative survival pathways, and frequent activation of PI3K/AKT, MAPK/ERK and EMT-associated programs) creates an urgent need for novel agents that act on non-hormonal targets. The observed sensitivity of MDA-MB-231 and other TNBC models to *Cassia alata* extracts therefore flags the plant's phytochemicals as candidate leads for addressing a major unmet clinical need in breast oncology[1,25].

According to the mechanism, the phytochemicals identified in *Cassia alata* (anthraquinones such as emodin, aloe-emodin and rhein, plus flavonoids such as kaempferol and quercetin) target pathways that are relevant across breast cancer subtypes. These include induction of the intrinsic apoptotic cascade (mitochondrial dysfunction, caspase-3/9 activation), downregulation of anti-apoptotic BCL-2 family proteins, inhibition of PI3K/AKT and ERK signaling, and anti-angiogenic effects via VEGF/Hsp90α modulation[19,25]. Because these mechanisms do not depend exclusively on hormone receptor signaling, they provide a plausible explanation for activity in both luminal and triple-negative models[25,26]. At the same time, subtype-specific differences in basal pathway activity and drug-metabolizing enzymes may influence potency and should be explicitly tested during preclinical development[26].

Translational implications are multifold but must be tempered by realism. On the positive side, *Cassia alata*-derived compounds combine multi-target action (which may reduce resistance emergence) with chemical scaffolds that have precedents in pharmacology[3,25]. Nanoformulation approaches (e.g., chitosan-folate coated nanoliposomes) improved cellular delivery and potency in vitro, illustrating one route to enhance tumor targeting and bioavailability while potentially reducing systemic exposure[24]. Green-synthesized nanoparticles and other delivery platforms likewise show promise for improving pharmacokinetics and tumor uptake[8].

However, several substantial translational gaps remain. First, *in vitro* 2-D cell line, work while informative for mechanism, inadequately models drug pharmacokinetics, tumor microenvironment (stroma, immune cells, hypoxia), and three-dimensional tumor architecture. Moving forward, *Cassia*

alata leads should be evaluated in more predictive preclinical models, such as 3-D spheroids, co-culture systems, patient-derived organoids, and orthotopic or patient-derived xenograft (PDX) models that preserve tumor heterogeneity[25,26]. Second, comprehensive ADME/toxicity profiling is essential because several anthraquinones have known toxicities and complex metabolism[24]. Systemic safety, off-target effects, and potential herb–drug interactions (especially if combined with standard chemotherapy) must be defined before clinical translation[26].

Third, selectivity is critical. Only a minority of the included studies tested effects on non-tumorigenic mammary epithelial cells, those that did generally showed lower toxicity against normal cells, but data are sparse and inconsistent. Prioritizing selectivity assays (MCF10A or primary mammary epithelial cells, hepatocytes for metabolism, and relevant cardiac/hematologic safety panels) is mandatory to assess therapeutic windows[16,23].

Fourth, rational combination strategies deserve focused exploration. Because *Cassia alata* phytochemicals modulate apoptosis and survival signaling, combination with agents that induce DNA damage (e.g., doxorubicin) or with PARP inhibitors in HR-deficient TNBC could be synergistic; preclinical combination screens (dose-matrix and isobologram analysis) should be used to identify additive or synergistic regimens and to guide dose-reduction strategies that may lower toxicity[3,26].

Fifth, standardization and quality control are practical hurdles. Natural extracts vary by plant chemotype, harvest conditions, extraction solvent and fractionation; these variables change potency and composition. Developing standardized extraction procedures, quantifying marker compounds (e.g., kaempferol, emodin) and producing GMP-grade extracts/isolates will be required for reproducible preclinical and clinical work[3,22].

CONCLUSION

Cassia alata stands out as a profoundly promising natural agent, consistently exhibiting a broad and potent spectrum of anticancer activity against breast cancer cells, particularly MCF-7. Analysis of the results consistently highlights that the primary strength of *Cassia alata* lies in its intricate phytochemical composition. Isolated compounds such as emodin, aloe-emodin, rhein, and kaempferol repeatedly demonstrate significantly superior cytotoxic potential compared to crude extracts, achieving lower IC_{50} values across multiple studies, indicating enhanced efficacy at lower concentrations.

These powerful constituents critically orchestrate robust molecular mechanisms crucial for cancer inhibition. This includes the potent induction of apoptosis, leading to programmed cell death, and significant mitochondrial dysfunction, weakening cellular energy production and integrity. Furthermore, these compounds precisely modulate vital oncogenic signaling pathways like PI3K/AKT and ERK, which are often hyperactive in cancer and drive uncontrolled growth and survival. Concurrently, they effectively inhibit angiogenesis, blocking the formation of new blood vessels essential for tumor sustenance and metastasis. This comprehensive, multi-targeted action firmly positions *Cassia alata* as a compelling natural blueprint for developing innovative cancer therapies, offering a multifaceted and impactful approach to combating disease progression by attacking cancerous cells through diverse yet synergistic pathways.

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CONFLICT OF INTEREST

There is no conflict of interest.

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