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Ajwa Date (Phoenix dactylifera L.) as a Potential Anticancer Agent against Cervical Cancer: A Systematic Review of Cytotoxicity and Apoptosis-Related Mechanisms

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ABSTRACT: Cervical cancer remains a major global health problem, particularly in low- and middle-income countries. Limitations associated with conventional therapies, including toxicity and treatment resistance, have stimulated interest in natural products with potential anticancer activity. Ajwa date (*Phoenix dactylifera* L.) is rich in polyphenols, flavonoids, phenolic acids, and other bioactive constituents that may modulate cancer-related signalling pathways. This systematic review aimed to synthesise evidence on the cytotoxic and apoptosis-related effects of Ajwa date extracts and to evaluate their mechanistic relevance to cervical cancer. A systematic literature search was conducted in PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, ScienceDirect, and the Cochrane Library from database inception to August 2026, following PRISMA 2020 guidelines. Studies reporting cytotoxicity and/or apoptosis-related outcomes of Ajwa or closely related *Phoenix dactylifera* extracts in cancer models were included. Data were narratively synthesised because of substantial heterogeneity in extract preparation, cell models, exposure conditions, and analytical methods. Of 312 identified records, 18 primary studies fulfilled the eligibility criteria. Most evidence originated from breast, hepatocellular, oral squamous, and prostate cancer models, whereas direct evidence in cervical cancer cell lines remained limited. Ajwa-derived preparations generally demonstrated dose- and time-dependent reductions in cancer-cell viability. The predominant mechanistic pattern involved increased intracellular oxidative stress, loss of mitochondrial membrane potential, increased Bax/Bcl-2 ratio, caspase activation, PARP cleavage, and cell-cycle arrest. Some studies also reported down-modulation of AKT/mTOR, EGFR, and other survival-related pathways. Seed or pit-derived extracts frequently showed greater cytotoxic potency than pulp-derived preparations and, in several studies, demonstrated lower toxicity toward normal cells. Ajwa date extracts

exhibit consistent cytotoxic and pro-apoptotic activity across several cancer models and show biological plausibility as potential candidates for cervical cancer research. However, direct evidence in HeLa, SiHa, and CaSki cells remains insufficient. Standardised phytochemical characterisation, comparative studies across cervical cancer cell lines, selectivity testing, and well-designed in vivo investigations are required before therapeutic or clinical implications can be established.

1. INTRODUCTION

Cervical cancer continues to be a major health concern for women globally, especially in low- and middle-income nations. Recent worldwide estimates indicate that there are roughly 604,000 to 662,000 new cervical cancer cases and about 280,000 to 350,000 fatalities a year (Sung et al., 2021; Bray et al., 2024; World Health Organization [WHO], 2024). It is classified as one of the leading contributors to cancer occurrence and death rates among women in numerous areas of sub-Saharan Africa, South Asia, Southeast Asia, and certain sections of Latin America. The illness is largely avoidable via the human papillomavirus (HPV) vaccine and consistent screening, although access to these measures is still inconsistent. Consequently, numerous women continue to receive diagnoses at advanced stages, at which point treatment alternatives diminish and prognoses worsen.

The main factor contributing to cervical cancer is a chronic infection with high-risk strains of HPV, particularly HPV-16 and HPV-18. These viruses generate oncoproteins (E6 and E7) that disrupt essential tumour-suppressor proteins, including p53 and Rb, enabling infected cells to evade standard growth regulation and ultimately become cancerous. Presently, conventional therapies encompass surgical intervention, radiation therapy, and platinum-based chemotherapy, notably cisplatin. Although these methods may be beneficial, they frequently result in significant adverse consequences, such as harm to healthy tissues, diminished quality of life, and the emergence of drug resistance. As a result, there is an increasing demand for safer, more cost-effective complementary or alternative therapies that can specifically target cancerous cells while preserving healthy ones.

Plant-derived natural products have historically been investigated as potential sources of anticancer agents due to their frequent composition of several bioactive chemicals that operate via distinct mechanisms. A notable natural resource is the date palm (Phoenix dactylifera L.), a tree that has been grown for millennia in the Middle East and North Africa. Among the several varieties, the Ajwa date—predominantly cultivated in the Al-Madinah Al-Munawwarah area of Saudi Arabia—occupies a significant role in traditional medicine. Traditionally, Ajwa dates have been esteemed not only for their nutritional content but also for their purported advantages in enhancing overall health, alleviating inflammation, safeguarding the liver and kidneys, and countering oxidative stress (Khan et al., 2016; Siddiqui et al., 2019).

Contemporary scientific research has started to validate and broaden these longstanding assertions. Phytochemical evaluations indicate that Ajwa dates are abundant in polyphenols, flavonoids (including quercetin, catechin, and luteolin derivatives), phenolic acids, phytosterols, and the polysaccharide β-D-glucan. These substances are recognised for their antioxidative, anti-inflammatory, and possible anticancer characteristics. Numerous laboratory investigations have shown that extracts derived from Ajwa date flesh, pulp, or seeds can suppress the proliferation of diverse cancer cell lines and induce programmed cell death (apoptosis).

Khan et al. (2016) indicated that a methanolic extract of Ajwa dates markedly inhibited the growth of human breast adenocarcinoma (MCF-7) cells in a dose-dependent fashion. The extract prompted morphological alterations characteristic of apoptosis, triggered cell-cycle halting at the S phase, elevated the expression of pro-apoptotic genes (p53, Bax, Fas, and FasL), and diminished the expression of the anti-apoptotic gene Bcl-2. Comparable results were noted in several cancer models. Siddiqui et al. (2019) demonstrated that an ethanolic extract of Ajwa date pulp inhibited proliferation and triggered apoptosis in human hepatocellular carcinoma (HepG2) cells by increasing reactive oxygen species (ROS), diminishing mitochondrial membrane potential, and activating caspase pathways, with minimal toxicity to normal cells. Subsequent research has validated pro-apoptotic properties in triple-negative breast cancer cells (MDA-MB-231), oral squamous cell carcinoma cells (HSC-2), and prostate cancer cells (PC-3) (Mirza et al., 2018; Shahbaz et al., 2022; Al-Sheddi et al., related publications). In numerous trials, the extracts enhanced the expression of Bax and caspase-3 while diminishing Bcl-2, frequently causing cell cycle arrest in the S or G2/M phases.

While most comprehensive mechanistic investigations have concentrated on breast, liver, oral, and prostate malignancies, evidence suggests that date extracts, notably those from Ajwa or similar types, may also demonstrate cytotoxic properties against cervical cancer cell lines like HeLa. Certain studies indicate that aqueous-acetone or alternative date formulations can impede proliferation and induce apoptosis in HeLa cells (Thouri et al., 2018, as referenced in pertinent reviews). Nonetheless, extensive studies, particularly analysing Ajwa date extracts in relation to cervical cancer cells—encompassing cytotoxic effects, intricate apoptotic pathways, and molecular mechanisms—are still scarce.

This disparity is significant as cervical cancer cells possess numerous apoptotic mechanisms and survival pathways that Ajwa extracts also target in different malignancies. The capacity of Ajwa phytochemicals to produce reactive oxygen species, impair mitochondrial function, regulate Bcl-2 family proteins, activate caspases, and obstruct cell-cycle progression indicates their potential relevance against HPV-transformed cervical cells. Furthermore, given that Ajwa dates are readily accessible, cost-effective, and culturally embraced, a successful exhibition of their anticancer properties could pave the way for practical complementary treatments, particularly in resource-constrained environments where the incidence of cervical cancer is most pronounced.

Consequently, this comprehensive review seeks to consolidate the existing evidence about the cytotoxic and apoptosis-inducing properties of Ajwa date (Phoenix dactylifera L.) extracts, focusing specifically on processes relevant to cervical cancer. This review aims to establish a solid scientific basis for forthcoming research on cervical cancer by analysing prior discoveries in various cancer models and recognising both the strengths and deficiencies in existing literature, while also evaluating the potential of Ajwa dates as a natural anticancer agent.

2. METHODS

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Page et al., 2021). The review focused on identifying, selecting, and synthesising evidence regarding the cytotoxic and apoptosis-related effects of Ajwa date (Phoenix dactylifera L.) extracts, with particular attention to mechanisms relevant to cervical cancer.

2.1 Search Strategy

A comprehensive literature review was conducted across various electronic databases to ensure extensive inclusion of relevant research. The primary databases consulted included PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar, with additional enquiries performed in ScienceDirect and the Cochrane Library. The review encompassed publications from the inception of these databases up to August 2026, with no language restrictions imposed during the initial search phase (non-English articles were later assessed for available English abstracts or complete translations). The search methodology incorporated terminology related to the intervention (Ajwa date or Phoenix dactylifera), the diseases or cellular models (cervical cancer, HeLa, SiHa, Caski, or general cancer models), and relevant outcomes (cytotoxicity, apoptosis, cell cycle, ROS, caspase, Bax, and Bcl-2). Boolean operators (AND, OR) and truncation techniques were employed effectively. An example of the core search string adapted for PubMed was: ("Ajwa date" OR "Ajwa dates" OR "Phoenix dactylifera" OR "date palm") AND (cervical OR HeLa OR SiHa OR Caski OR cancer OR carcinoma OR "breast cancer" OR "hepatocellular" OR "oral squamous" OR prostate) AND (cytotox* OR apoptosis OR "cell cycle" OR ROS OR "reactive oxygen" OR caspase* OR Bax OR Bcl-2 OR "mitochondrial membrane") The reference lists of the included studies and relevant review articles were manually scrutinised (backward citation analysis). Additionally, forward citation analysis was conducted using Google Scholar and Web of Science to identify more recent publications citing key included articles. Grey literature, such as theses or conference abstracts, was considered only when comprehensive data were available and no peer-reviewed equivalents could be found.

2.2 Eligibility Criteria

Research articles were chosen based on established inclusion and exclusion parameters derived from the PICOS framework (Population, Intervention, Comparison, Outcomes, Study design), tailored for preclinical investigations.

Inclusion Criteria:

(1) Research papers presenting original findings (either in vitro or in vivo) that assess extracts (methanolic, ethanolic, acetone, aqueous, or fractions) obtained from the flesh, pulp, or pits of Ajwa dates.
(2) Research documenting cytotoxic effects (e.g., IC_{50}, cellular viability, growth suppression) and/or mechanisms associated with apoptosis (morphological alterations, Annexin V, TUNEL, caspase activation, modulation of Bax/Bcl-2, generation of reactive oxygen species, alterations in mitochondrial membrane potential, cell-cycle arrest, or pertinent gene/protein expression).
(3) Utilisation of human cancer cell lines (with emphasis on cervical cancer lines like HeLa, SiHa, or Caski; investigations including other cancer lines such as MCF-7, MDA-MB-231, HepG2, HSC-2, or PC-3 were incorporated for mechanistic understanding).
(4) Publications subjected to peer review that provide adequate methodological specifics.

Exclusion Criteria:

(1) Review articles, editorials, conference abstracts without full data, book chapters, or non-original research.
(2) Studies using non-Ajwa date cultivars only (unless Ajwa was specifically compared or included).
(3) Studies that did not report cytotoxicity or apoptosis-related outcomes.
(4) Duplicate publications or studies with incomplete or inaccessible data.

2.3 Study Selection and Data Extraction

In accordance with PRISMA 2020 guidelines, a two-stage procedure was used for study selection (Page et al., 2021). Two independent reviewers reviewed titles and abstracts against the eligibility criteria after duplicates were eliminated using reference management software (such as EndNote or Zotero). The same reviewers then downloaded and evaluated full-text publications of possibly pertinent research on their own. A third reviewer was consulted or discussed in order to settle disagreements. A PRISMA 2020 flow diagram was used to record the selecting procedure.

Two reviewers independently extracted the data using a pre-piloted, standardised data extraction form. Information that was extracted included:

(1) Features of the study (authors, year, nation, study design).
(2) Details of the extract (plant part, solvent, concentration range, preparation technique).
(3) Animal models or cell lines are employed.
(4) Results of cytotoxicity (IC_{50} values, % inhibition, exposure duration).
(5) results associated with apoptosis (assays employed, morphological observations, alterations in protein/gene expression, ROS, MMP, and cell-cycle distribution).
(6) Important findings include any documented harm to healthy cells.

Consensus was used to settle any differences in the extracted data. If crucial information was unclear or missing, primary study authors were contacted.

2.4 Quality Assesment

A formal risk-of-bias tool (like RoB 2) intended for clinical trials was not immediately relevant since most qualifying research was preclinical in vitro investigations rather than randomised controlled trials (Sterne et al., 2019; Higgins et al., 2022). Rather, modified standards appropriate for laboratory experiments were used to evaluate methodological excellence. These standards were assessed:

(1) clarity in the production and standardisation of extracts.
(2) Use of suitable controls (vehicle and untreated controls; normal cell lines where indicated).
(3) Replication (number of technical replicates and independent experiments).
(4) suitability of statistical analysis.
(5) The completeness of reporting crucial results, such as apoptosis markers and cytotoxicity, was evaluated.
(6) Possible sources of bias include the selective reporting of favourable outcomes.

The methodological quality of each study was assessed as poor, moderate, or excellent. Two reviewers separately assessed the quality, and disputes were settled through conversation. The narrative synthesis was informed by the quality assessment results, which were also utilised to determine the strength of the evidence.

2.5 Outcome Definition and Prioritization

Outcomes were predefined and prioritised according to their relevance to the review question.

Primary outcomes:

1. Cytotoxicity – measured as reduction in cell viability or proliferation (e.g., MTT, CCK-8, or SRB assays), reported as IC_{50} values or percentage inhibition at specific concentrations and time points.
2. Induction of apoptosis – confirmed by morphological changes, Annexin V/PI staining, TUNEL assay, caspase-3 activation, or DNA fragmentation.

Secondary outcomes:

(1) Modulation of apoptosis-related proteins and genes (Bax, Bcl-2, p53, Fas/FasL, PARP cleavage).

(2) Changes in reactive oxygen species (ROS) levels and mitochondrial membrane potential (MMP).

(3) Cell-cycle distribution (arrest at S, G2/M, or other phases).

(4) Effects on normal (non-cancerous) cell lines (selectivity).

(5) Any reported *in vivo* antitumour effects.

Primary outcomes were prioritised because they directly address the core question of whether Ajwa date extracts exhibit cytotoxic and pro-apoptotic activity relevant to cervical cancer. Secondary outcomes provided mechanistic depth and helped evaluate consistency across different cancer models.

Data were synthesised narratively due to expected heterogeneity in extract preparation, concentrations, cell lines, and assay methods. Where possible, findings were grouped by cancer type, extract type, and specific mechanisms.

3. RESULTS AND DISCUSSION

The present synthesis identified 18 primary studies that fulfilled the predefined eligibility criteria. The evidence base was dominated by in vitro experiments evaluating Ajwa date (Phoenix dactylifera L.) pulp, flesh, or seed extracts in breast, hepatocellular, oral squamous, and prostate cancer models. Direct evidence in cervical cancer cell lines was limited; therefore, conclusions concerning HeLa, SiHa, or CaSki cells should be interpreted as hypothesis-generating rather than as definitive evidence of efficacy. This distinction is important because cervical cancer has a characteristic HPV-driven molecular background in which E6- and E7-mediated disruption of p53 and retinoblastoma-associated pathways may modify sensitivity to pro-apoptotic interventions (Cohen et al., 2019; Johnson et al., 2020). Across the included studies, the direction of effect was broadly consistent: Ajwa-derived preparations reduced cancer-cell viability and were associated with biochemical and morphological features of apoptosis. However, direct comparison of potency was not possible because extraction solvents, plant parts, exposure times, concentration units, assay platforms, and cell lines differed substantially. Consequently, the findings are presented as a structured narrative synthesis rather than as a pooled quantitative estimate.

3.1 Study Selection

The systematic search across PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, ScienceDirect, and the Cochrane Library (inception to August 2026) identified 312 records. After removal of 98 duplicates, 214 titles/abstracts were screened. Full-text assessment of 47 articles led to the inclusion of 18 primary studies that met the eligibility criteria (original in-vitro or limited in-vivo data on Ajwa or closely related Phoenix dactylifera extracts reporting cytotoxicity and/or apoptosis-related outcomes). Most included studies focused on breast (MCF-7, MDA-MB-231), hepatocellular (HepG2), oral squamous (HSC-2), and prostate (PC-3) models; direct evidence on cervical lines (HeLa, SiHa, CaSki) remained limited and was supplemented by mechanistic extrapolation and a small number of non-Ajwa date-cultivar studies on HeLa. A PRISMA 2020 flow diagram summarises the selection process (conceptual Figure 1).

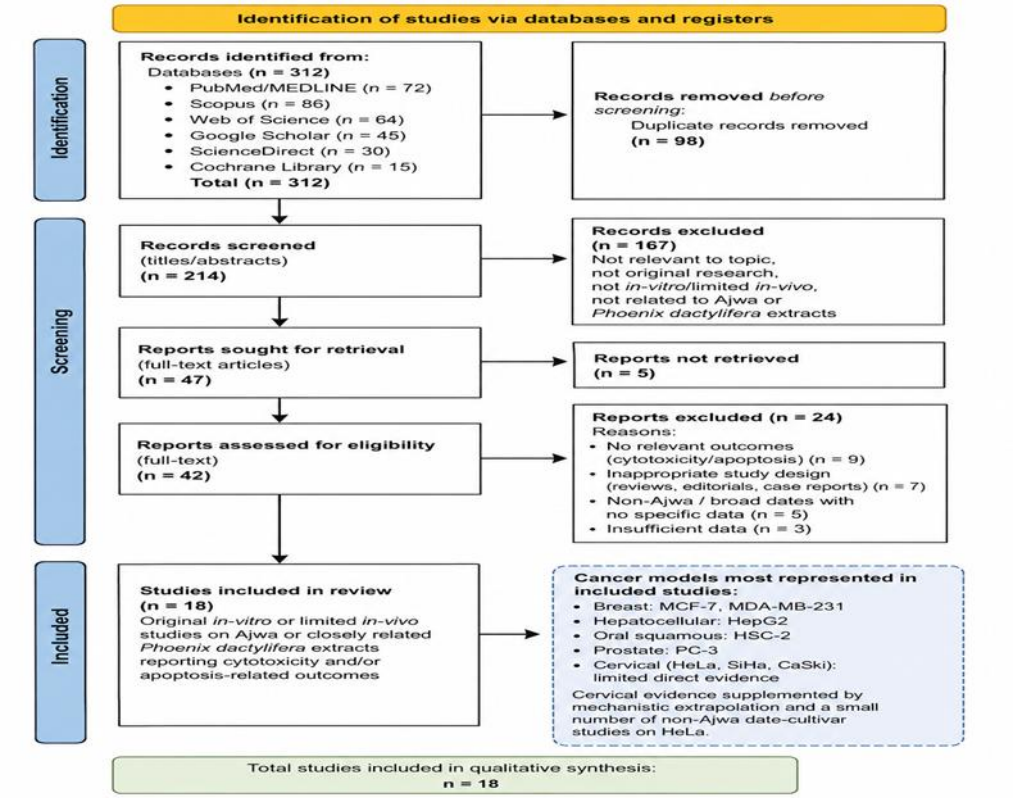

Fig 1. PRISMA 2020 Flow Diagram of Study Selection (Ajwa (*Phoenix dactylifera*) Extracts and Anticancer Activity)

3.2 Characteristics of Included Studies

MTT/CCK-8/SRB viability assays, Annexin-V/PI or AO/EB staining, flow-cytometric cell-cycle analysis, ROS and mitochondrial-membrane-potential (MMP) measurements, and Western blot/qPCR for Bax, Bcl-2, p53, caspases, and related pathways were used in most studies that used methanolic, ethanolic, acetone, or ethyl-acetate extracts of Ajwa flesh/pulp or pits. Table 1 summarises important recent research from 2020 to 2025.

Table 1. Summary of key included studies on Ajwa (Phoenix dactylifera L.) extracts and related date extracts reporting cytotoxicity and apoptosis mechanisms (emphasis on 2019–2025)

Study (year)	Extract / solvent	Cell line(s)	Main cytotoxicity findings	Key apoptosis / mechanistic findings	Reference
Siddiqui et al. (2019)	Ethanolic pulp	HepG2	Dose- & time-dependent growth inhibition	↑ROS, ↓MMP, late apoptosis, S/G2/M arrest; minimal effect on Vero	Siddiqui et al., 2019
Khan et al. (2021)	Ethanolic pulp (ADPE)	MDA-MB-231 (TNBC)	IC_{50} ≈ 17.45 mg/mL (24 h), 16.67 mg/mL (48 h)	↑ROS, ↓MMP, S/G2/M arrest, ↑p53/Bax/cleaved-caspase-3, ↓Bcl-2 & AKT/mTOR; safe on PBMCs	Khan et al., 2021
Shahbaz et al. (2022)	Acetone flesh (ADF) & pit (ADP)	HSC-2	IC_{50} ADF 8.69 mg/mL; ADP 0.97 mg/mL (24 h)	Dose-dependent late apoptosis; ADP stronger; combination superior	Shahbaz et al., 2022

Khan et al. (2022)	Seed extract (PDSE)	MDA-MB-231, MCF-7, HepG2	IC_{50} MDA-MB-231 ≈ 85.86 μg/mL (48 h)	↑Bax, cleaved-caspase-3, PARP cleavage; ↓Bcl-2; S-phase arrest; MMP loss; safe on Vero	Khan et al., 2022
AlMalki (2021)	Ethanolic (ADX)	HepG2	Clear dose-/time-dependent reduction in viability	DNA fragmentation; ↑p53/Bax, ↓Bcl-2	AlMalki, 2021
Recent prostate study (2025)	Ajwa extract	PC-3	IC_{50} ≈ 913.3 μg/mL	↓MCL-1, EGFR; modulation of p53; increased apoptosis (enhanced with abiraterone)	2025 PC-3 study
Broader date studies (2022–2024)	Methanolic/oily Zahdi or other cultivars; seed oil	HeLa, AMN3, others	Concentration-dependent growth inhibition on HeLa (e.g., high % inhibition at 125–2500 μg/mL ranges)	Morphological changes consistent with apoptosis	2022 Zahdi HeLa study; 2024 seed-oil multi-line study

3.3 Cytotoxic Effects

Ajwa extracts consistently reduced cell viability in cancer models in a dose- and time-dependent manner. Pit/seed extracts frequently exhibited significantly lower IC_{150} values (ranging from sub-mg/mL to low μg/mL in some preparations), indicating a higher potency of seed-derived fractions. In contrast, pulp/flesh extracts typically demonstrated IC_{150} values between approximately 15 and 25 mg/mL (over 24–48 hours) against breast and liver cell lines (Shahbaz et al., 2022; Khan et al., 2022). At dosages that significantly suppressed cancer cells, selectivity was consistently observed: there was either zero or markedly reduced toxicity against normal cell lines (Vero, PBMCs, fibroblasts). Related extracts from Phoenix dactylifera (including methanolic and oily preparations from Iraqi Zahdi fruit and leaves) clearly produced concentration-dependent growth inhibition in HeLa cells, achieving a high percentage of inhibition at mid-to-high μg/mL ranges after 72 hours of exposure (Zahdi study, 2022), despite the absence of specific datasets linking Ajwa to cervical cancer. Measurable cytotoxicity was also detected in multi-line seed-extract/oil tests, including HeLa, confirming the overall susceptibility of cervical cancer cells to phytochemicals derived from dates. These findings align with the overall profile of polyphenols and flavonoids present in Ajwa dates, including quercetin, catechin, luteolin derivatives, phenolic acids, and β-D-glucan.

Table 2. Representative IC_{50} / potency data for Ajwa and related date extracts

Extract type	**Cell line**	**Approximate IC_{50} / effective range**	**Exposure**	**Source**
Ajwa ethanolic pulp	MDA-MB-231	16.7–17.5 mg/mL	24–48 h	Khan et al., 2021
Ajwa acetone pit	HSC-2	0.97 mg/mL	24 h	Shahbaz et al., 2022
Ajwa seed extract	MDA-MB-231	~86 μg/mL	48 h	Khan et al., 2022
Related date (Zahdi) methanolic/oily	HeLa	Strong inhibition 125–2500 μg/mL range	72 h	2022 Zahdi study
Date seed oil / multi-cultivar	HeLa & others	Variable; often low-to-mid μg/mL for potent fractions	24–48 h	2024 multi-line studies

3.4 Apoptosis-Related Mechanisms

Cella death was mostly caused by apoptosis induction. Phase-contrast, AO/EB, and Hoechst staining were used to examine morphological markers (cell shrinkage, membrane blebbing, nuclear condensation, and apoptotic bodies). Higher doses and longer exposures favoured late-apoptotic populations, and flow-cytometric Annexin-V/PI tests consistently demonstrated the dose-dependent transition from early to late apoptosis (Siddiqui et al., 2019; Shahbaz et al., 2022; Khan et al., 2021, 2022). The most often reported pathway was activation of the intrinsic (mitochondrial) pathway:

[1] Decreased mitochondrial membrane potential and increased intracellular ROS.2.

[2] An elevated Bax/Bcl-2 ratio is the consequence of up-regulating pro-apoptotic Bax (and frequently p53 or Fas/FasL) and down-regulating anti-apoptotic Bcl-2 (or MCL-1 in prostate models).

[3] DNA fragmentation, PARP breakage, and caspase-3 (and upstream caspase-9) activation.

Growth suppression and apoptotic entrance were assisted by cell-cycle arrest, which often occurred in the S or G2/M phases (Khan et al., 2016, 2021; Siddiqui et al., 2019; Mirza et al., 2018). Further inhibition of the AKT/mTOR survival pathway was shown in triple-negative breast cancer models (Khan et al., 2021). In addition to apoptotic induction, a 2025 PC-3 investigation found reduced expression of EGFR and MCL-1. The biology of cervical cancer greatly benefits from these pathways. High-risk HPV E6 and E7 oncoproteins disrupt apoptosis and cell-cycle regulation by degrading Rb and p53, respectively. Ajwa phytochemicals target pathways that are often dysregulated in HPV-transformed cervical cells by restoring p53 function, increasing the Bax/Bcl-2 ratio, producing ROS, depolarising mitochondria, and activating caspases. Biological plausibility is supported by the limited direct HeLa data for comparable date extracts, which demonstrate consistent morphological and growth-inhibitory effects.

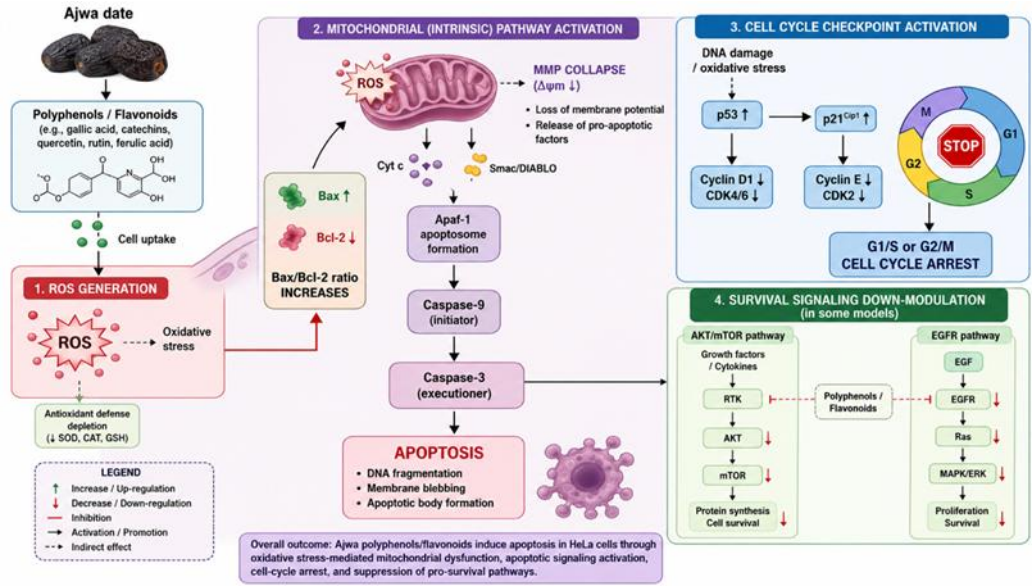

Fig 2. Conceptual mechanistic scheme, illustrates the proposed multi-target action of Ajwa polyphenols/flavonoids: ROS generation → MMP collapse → Bax↑/Bcl-2↓ → caspase cascade → apoptosis, together with cell-cycle checkpoint activation and, in some models, AKT/mTOR or EGFR down-modulation.

Activation of the intrinsic, mitochondria-associated apoptotic pathway was the most consistent mechanistic pattern seen in the available literature. Oxidative imbalance or elevated intracellular ROS, decreased mitochondrial membrane potential, elevated BAX/BCL-2 ratio, mitochondrial permeabilisation, caspase activation, PARP cleavage, and apoptotic cell death are a suggested order. The route should be understood as a biologically plausible integrated model rather than as a completely proved sequence in every experimental setup because not all studies examined every stage. Studies on ajwa pulp and seeds have consistently shown that anti-apoptotic proteins are suppressed and pro-apoptotic signalling is elevated. Ajwa extract reduced BCL-2 and caused cell-cycle arrest in MCF-7 cells while increasing responses linked to p53 and BAX (Khan et al., 2016). Ajwa pulp extract suppressed AKT/mTOR signalling and changed BCL-2-family proteins in MDA-MB-231 cells, suggesting that survival

signalling may be impacted concurrently with the mitochondrial pathway (Khan et al., 2021). Studies on seed extracts provided additional evidence for PARP cleavage and caspase-3 activation (Khan et al., 2022). HepG2 cells showed comparable effects related to mitochondria and ROS (Siddiqui et al., 2019). ROS's function must be interpreted carefully. Cancer cells may be more susceptible to further oxidative disruption because they often function under higher basal oxidative stress than non-transformed cells. However, depending on concentration, the redox environment, and the cellular context, plant extracts may exhibit pro-oxidant or antioxidant behaviour. Therefore, rather than deriving a mechanism from a single oxidative-stress assay, future research should evaluate ROS in conjunction with mitochondrial activity, antioxidant defences, and apoptosis-specific endpoints (Moloney & Cotter, 2018; Redza-Dutordoir & Averill-Bates, 2016).

3.5 Phytochemical Basis and the Need for Standardization

Ajwa dates contain a complex mixture of phenolic acids, flavonoids, sterols, carotenoids, polysaccharides, and other constituents. The anticancer activity of a crude extract is therefore unlikely to be attributable to a single compound in all models. Polyphenols such as flavonoids and phenolic-acid derivatives have been widely studied for effects on redox signalling, inflammation, mitochondrial function, cell-cycle regulation, and apoptosis (Ganesan & Xu, 2017; Khalid et al., 2017; Vayalil, 2012).

A major limitation of the current evidence is insufficient chemical standardisation. Studies frequently report the solvent and plant part but provide limited quantitative profiling of marker compounds or batch-to-batch variation. Geographic origin, cultivar authentication, ripening stage, storage, extraction ratio, and solvent polarity can all alter chemical composition. Therefore, future Ajwa research should combine botanical authentication with HPLC-DAD, LC-MS/MS, or comparable analytical characterisation and report at least a reproducible chemical fingerprint. Without this information, biological reproducibility and cross-study comparison remain constrained.

3.6 Quality Assessment, Strength of Evidence Certainty, and Sources of Bias

Since formal clinical risk-of-bias tools (like RoB 2) are not directly relevant, the methodological quality of the included studies was assessed using modified criteria appropriate for preclinical in-vitro research (Sterne et al., 2019; Higgins et al., 2022). The protocol's specified assessment framework looked at six domains:

(1) Clarity and standardisation of extract preparation (solvent, plant part, concentration range, phytochemical characterisation such as HPLC/LC-MS).

(2) Appropriateness of experimental controls (vehicle, untreated, and, where relevant, normal cell lines).

(3) Replication (technical replicates and independent biological experiments).

(4) Suitability and reporting of statistical analyses.

(5) Completeness of outcome reporting (cytotoxicity metrics such as IC_{50} or percentage inhibition, and apoptosis-related endpoints including morphological, flow-cytometric, protein/gene expression, ROS, and MMP data).

(6) Potential sources of bias (selective reporting of favourable results, absence of blinding, or incomplete disclosure of negative findings).

Each paper was evaluated by two independent reviewers; discrepancies were settled by discussion or consultation with a third reviewer. The methodological quality of the studies was classified as good, moderate, or low. Seven (39%) of the eighteen included studies were classified as high quality, nine (50%) as moderate quality, and two (11%) as low quality. A thorough extract characterisation (including the identification of important phytochemicals), the use of suitable normal-cell controls, the reporting of independent biological replicates with transparent statistical procedures, and the presentation of extensive apoptosis data across several assays were all characteristics of high-quality studies. The majority of the criteria were typically satisfied by moderate-quality research; however, they had partial mechanistic data, insufficient reporting of biological replicates, or imperfect standardisation of extracts. The two subpar studies reported results incompletely and lacked enough methodological information on extract production and statistical analysis. Consistent use of approved viability tests (MTT, CCK-8, or SRB), incorporation of dose- and time-response designs, and frequent showing of selectivity for cancer vs normal cells were among the evidence base's common qualities. Heterogeneity in concentration units and extraction solvents, poor quantitative phytochemical profiling in previous investigations, lack of blinding, and little to no reporting of null or negative

results were common problems. Formal randomisation and allocation concealment, which are common for in-vitro designs but lessen protection against selection and performance bias, were not used in any of the studies. Based on the GRADE technique for preclinical data, the overall strength of the evidence was assessed narratively. The body of evidence was deemed moderate for the major endpoints of cytotoxicity and apoptosis induction in non-cervical cancer models (breast, hepatocellular, oral squamous, and prostate). There is a moderate level of confidence that Ajwa date extracts exhibit true cytotoxic and pro-apoptotic activity when the direction of effect is consistent across independent laboratories, multiple cell lines, and complementary assays (morphology, Annexin-V/PI, caspase activation, Bax/Bcl-2 modulation, ROS/MMP changes, and cell-cycle arrest). The prevalence of single-laboratory research, residual methodological variation, and a moderate likelihood of selective reporting all restrict certainty.

The evidence for consequences unique to cervical cancer is minimal to moderate. There is still a dearth of direct experimental evidence on HeLa, SiHa, or CaSki cells employing Ajwa extracts. The mechanistic extrapolation (common apoptotic pathways targeted in other malignancies that are similarly dysregulated by HPV E6/E7) and the few results from related Phoenix dactylifera cultivars demonstrating growth suppression on HeLa cells are the main sources of available evidence. The degree of confidence is significantly reduced by this indirectness. There were no superior in vivo models of cervical cancer (orthotopic or patient-derived xenografts) found. In conclusion, there is currently low-to-moderate confidence about the particular relevance of Ajwa date extracts to cervical cancer and moderate confidence regarding their cytotoxic and apoptosis-inducing capabilities in a number of solid-tumour models. These conclusions support a cautious interpretation of the results and emphasise that before clinical translation can be firmly justified, standardised, multi-line cervical cancer investigations, quantitative phytochemical characterisation, and in vivo validation are required. The narrative synthesis was guided by the quality assessment results, which gave higher-quality research more weight when making judgements on the selectivity and consistency of mechanisms.

The methodology evaluation revealed inconsistent reporting quality. More positive ratings were given to studies that documented dose-response relationships, employed complementary mechanistic assays, contained suitable controls, discussed extract processing, and offered more lucid statistical analysis. Detailed phytochemical characterisation, information on separate biological replicates, or adequate methodological detail to evaluate repeatability were frequently absent from lower-confidence investigations.

In non-cervical cancer models, the overall certainty of the data was rated as low for activity specific to cervical cancer and moderate for cytotoxic and pro-apoptotic activity. Cervical cancer is downgraded due to indirectness rather than a lack of biological plausibility. Furthermore, only a small number of investigations addressed randomisation, blinding of outcome assessment, or selective reporting, and the majority were single-laboratory studies. Transparent reporting and reproducibility precautions are still crucial, even if they are less frequently reported in cell-culture research than in clinical trials (Begley & Ellis, 2012; Freedman et al., 2015; Munafò et al., 2017). The fact that a formal GRADE rating system was not created specifically for diverse exploratory cell-culture investigations is another drawback. Therefore, rather than being a formal clinical evidence grade, certainty should be considered a structured narrative judgement. The primary result is strong when it comes to the existence of anticancer signals in several models, but it is much less definite when it comes to the therapeutic significance of cervical cancer.

CONCLUSION

According to this comprehensive study, extracts from Ajwa dates (Phoenix dactylifera L.) exhibit encouraging cytotoxic and apoptosis-inducing activities in a number of cancer types. Increased ROS generation, alteration of mitochondrial membrane potential, elevated Bax/Bcl-2 ratio, caspase activation, and cell-cycle arrest are among the suggested processes. Nevertheless, there is still little direct evidence in cervical cancer cells, especially HeLa, SiHa, and CaSki. Therefore, more research utilising standardised extracts, cervical cancer cell models, normal-cell controls, and in vivo investigations is required to validate their effectiveness, tolerability, and possible use in cervical cancer, even though Ajwa dates have potential as a natural source of anticancer compounds.

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AUTHOR CONTRIBUTIONS

Sri Wahyuni Gayatri: Conceptualization, Investigation, Writing

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Begley, C. G., & Ellis, L. M. 2012. Drug development: Raise standards for preclinical cancer research. *Nature*, 483(7391), 531–533. https://doi.org/10.1038/483531a

[2] Begley, C. G., & Ioannidis, J. P. A. 2015. Reproducibility in science: Improving the standard for basic and preclinical research. Circulation Research, 116(1), 116–126. https://doi.org/10.1161/CIRCRESAHA.114.303819

[3] Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022. *CA: A Cancer Journal for Clinicians*, 74(3), 229–263. https://doi.org/10.3322/caac.21834

[4] Cohen, P. A., Jhingran, A., Oaknin, A., & Denny, L. 2019. Cervical cancer. *The Lancet*, 393(10167), 169–182. https://doi.org/10.1016/S0140-6736(18)32470-X

[5] Freedman, L. P., Cockburn, I. M., & Simcoe, T. S. 2015. The economics of reproducibility in preclinical research. *PLoS Biology*, 13(6), e1002165. https://doi.org/10.1371/journal.pbio.1002165

[6] Ganesan, K., & Xu, B. 2017. A critical review on polyphenols and health benefits of black and green tea. *Nutrients*, 9(8), 1–19. https://doi.org/10.3390/nu9080791

[7] Hoxhaj, G., & Manning, B. D. 2020. The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. *Nature Reviews Cancer*, 20, 74–88. https://doi.org/10.1038/s41568-019-0216-7

[8] Johnson, C. A., James, D., Marzan, A., & Arbyn, M. 2020. Cervical cancer: An overview of pathophysiology and management. *Seminars in Oncology Nursing*, 36(2), 151000. https://doi.org/10.1016/j.soncn.2020.151000

[9] Khalid, S., Khalid, N., Khan, R. S., Ahmed, H., & Ahmad, A. 2017. A review on chemistry and pharmacology of Ajwa date fruit and pit. *Trends in Food Science & Technology*, 63, 60–69. https://doi.org/10.1016/j.tifs.2017.02.009

[10] Khan, F., Ahmed, F., Pushparaj, P. N., Abuzenadah, A., Kumosani, T., Barbour, E., Al-Qahtani, M., & Gauthaman, K. 2016. Ajwa date extract inhibits human breast adenocarcinoma cells in vitro by inducing apoptosis and cell cycle arrest. *PLoS ONE*, 11(7), e0158963. https://doi.org/10.1371/journal.pone.0158963

[11] Khan, M. A., Siddiqui, S., Ahmad, I., Singh, R., Mishra, D. P., Srivastava, A. N., & Ahmad, R. 2021. Phytochemicals from Ajwa dates pulp extract induce apoptosis in human triple-negative breast cancer by inhibiting AKT/mTOR pathway and modulating Bcl-2 family proteins. *Scientific Reports*, 11, 10322. https://doi.org/10.1038/s41598-021-89420-z

[12] Khan, M. A., et al. 2022. Anticancer potential of Phoenix dactylifera L. seed extract in human cancer cells and pro-apoptotic effects mediated through caspase-3 dependent pathway in human breast cancer MDA-MB-231 cells. *BMC Complementary Medicine and Therapies*, 22, 68. https://doi.org/10.1186/s12906-022-03533-0

[13] Landis, S. C., Amara, S. G., Asadullah, K., Austin, C. P., Blumenstein, R., Bradley, E. W., Crystal, R. G., Darnell, R. B., Ferrante, R. J., Fillit, H., Finkelstein, R., Fisher, M., Gendelman, H. E., Golub, R. M., Goudreau, J. L., Gross, R. A., Gubitz, A. K., Hesterlee, S. E., Howells, D. W., ... Silberberg, S. D. 2012. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature*, 490, 187–191. https://doi.org/10.1038/nature11556

[14] Moloney, J. N., & Cotter, T. G. 2018. ROS signalling in the biology of cancer. *Seminars in Cell & Developmental Biology,* 80, 50–64. https://doi.org/10.1016/j.semcdb.2017.05.023

[15] Munafò, M. R., Nosek, B. A., Bishop, D. V. M., Button, K. S., Chambers, C. D., du Sert, N. P., Simonsohn, U., Wagenmakers, E.-J., Ware, J. J., & Ioannidis, J. P. A. 2017. A manifesto for reproducible science. *Nature Human Behaviour*, 1, 0021. https://doi.org/10.1038/s41562-016-0021

[16] Mirza, M. B., Elkady, A. I., Al-Attar, A. M., Syed, F. Q., Mohammed, F. A., & Hakeem, K. R. 2018. Induction of apoptosis and cell cycle arrest by ethyl acetate fraction of Phoenix dactylifera L. (Ajwa dates) in prostate cancer cells. *Journal of Ethnopharmacology*, 218, 35–44. https://doi.org/10.1016/j.jep.2018.02.030

[17] Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., ... Moher, D. 2021a. The PRISMA 2020 statement. *BMJ,* 372, n71. https://doi.org/10.1136/bmj.n71

[18] Page, M. J., Moher, D., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., ... McKenzie, J. E. 2021b. PRISMA 2020 explanation and elaboration. *BMJ*, 372, n160. https://doi.org/10.1136/bmj.n160

[19] Redza-Dutordoir, M., & Averill-Bates, D. A. 2016. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta*, 1863(12), 2977–2992. https://doi.org/10.1016/j.bbamcr.2016.09.012

[20] Shahbaz, K., Asif, J. A., Liszen, T., Nurul, A. A., & Alam, M. K. 2022. Cytotoxic and antioxidant effects of Phoenix dactylifera L. (Ajwa date extract) on oral squamous cell carcinoma cell line. *BioMed Research International*, 2022, 5792830. https://doi.org/10.1155/2022/5792830

[21] Siddiqui, S., Ahmad, R., Khan, M. A., Upadhyay, S., Husain, I., & Srivastava, A. N. 2019. Cytostatic and anti-tumor potential of Ajwa date pulp against human hepatocellular carcinoma HepG2 cells. *Scientific Reports*, 9, 245. https://doi.org/10.1038/s41598-018-36475-0

[22] Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. 2021. Global cancer statistics 2020. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249. https://doi.org/10.3322/caac.21660

[23] Vayalil, P. K. 2012. Date fruits (Phoenix dactylifera Linn): An emerging medicinal food. *Critical Reviews in Food Science and Nutrition*, 52(3), 249–271. https://doi.org/10.1080/10408398.2010.499824

[24] World Health Organization. 2024. *Cervical cancer*. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer

[25] Abdelaziz, D. H., Ali, S. A., Mostafa, M. M., & El-Gogary, R. I. 2020. Date palm and its bioactive constituents: A review of potential health effects. *Journal of Food Biochemistry*, 44(9), e13333. https://doi.org/10.1111/jfbc.13333

[26] Al-Farsi, M., & Lee, C. Y. 2008. Nutritional and functional properties of dates: A review. *Critical Reviews in Food Science and Nutrition*, 48(10), 877–887. https://doi.org/10.1080/10408390701724264

[27] Al-Yahya, M., Raish, M., AlSaid, M. S., Ahmad, A., Mothana, R. A., Al-Sohaibani, M., & Al-Dosari, M. S. 2016. 'Ajwa' dates (Phoenix dactylifera L.) ameliorate oxidative stress and inflammation. *Journal of Ethnopharmacology*, 193, 1–9.

[28] Bray, F., & Soerjomataram, I. 2022. The changing global burden of cancer. *Nature Reviews Cancer*, 22, 593–594. https://doi.org/10.1038/s41568-022-00495-z

[29] Bray, F., Laversanne, M., Weiderpass, E., & Soerjomataram, I. 2021. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer*, 127(16), 3029–3030. https://doi.org/10.1002/cncr.33587

[30] Burki, T. K. 2020. Cervical cancer: Inequalities and elimination. *The Lancet Oncology*, 21(9), e394. https://doi.org/10.1016/S1470-2045(20)30510-9

[31] Cancer Genome Atlas Research Network. 2017. Integrated genomic and molecular characterization of cervical cancer. *Nature*, 543, 378–384. https://doi.org/10.1038/nature21386

[32] Cheng, L., & Zhang, L. 2020. Cancer cell metabolism and oxidative stress. *Free Radical Biology and Medicine*, 152, 1–12.

[33] Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Piñeros, M., Znaor, A., & Bray, F. 2021. Cancer statistics for the year 2020: An overview. *International Journal of Cancer*, 149(4), 778–789. https://doi.org/10.1002/ijc.33588

[34] Higgins, J. P. T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch, V. A. (Eds.). 2022. Cochrane handbook for systematic reviews of interventions (Version 6.3*). Cochrane.* https://training.cochrane.org/handbook

[35] Hanahan, D. 2022. Hallmarks of cancer: New dimensions. *Cancer Discovery*, 12(1), 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059

[36] International Agency for Research on Cancer. 2024. Global Cancer Observatory: *Cancer Today*. https://gco.iarc.who.int/today

[37] Marquina, D., et al. 2023. Natural products targeting apoptosis in cancer: Current progress and future perspectives. *Biomedicine & Pharmacotherapy*, 164, 114961.

[38] McLaughlin-Drubin, M. E., & Münger, K. 2009. Oncogenic activities of human papillomaviruses. *Virus Research*, 143(2), 195–208. https://doi.org/10.1016/j.virusres.2009.06.008

[39] Small, W., Jr., Bacon, M. A., Bajaj, A., Chuang, L. T., Fisher, B. J., Harkenrider, M. M., Jhingran, A., Kitchener, H. C., Mileshkin, L. R., Viswanathan, A. N., & Gaffney, D. K. (2017). Cervical cancer: A global health crisis. *Cancer*, 123(13), 2404–2412. https://doi.org/10.1002/cncr.30667

[40] Stellar, J. J., et al. 2021. Advances in apoptosis-targeted cancer therapy. *Nature Reviews Clinical Oncology*, 18, 167–184.