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ORIGINAL PAPER

Anti-cancer activity of Ajwa dates extract (Phoenix Dactylifera L.) through analysis of MCL-1 levels, EGFR, and p53 expressions on apoptosis in human prostate cancer cell lines PC3: An in vitro study

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Summary *Introduction & Objectives: Prostate Cancer is recognized as a global burden disease related to malignancy in men. Several fruit and plant-based supplementation have been studied to evaluate their utility for the management of prostate cancer. Ajwa dates (Phoenix Dactylifera L.) have been known to contain various beneficial compounds, which makes them a potential anti-cancer therapy. The aim of this study was to assess the effect of Ajwa dates on prostate cancer cell lines PC3 through analysis of MCL-1 levels, EGFR, and p53 expressions on apoptosis.*

Materials & Methods. This study was an experimental in vitro study with post-test-only control design. Groups were divided into four: control group, abiraterone group, Ajwa dates group, and combination group (abiraterone and Ajwa dates). Viability test was conducted using the CCK-8 method to determine the inhibitory concentration (IC50) of Ajwa dates after a 72-hour incubation period of PC3 cells. The MCL-1 levels were rated using ELISA, while EGFR and p53 expressions were analyzed using immunofluorescence microscopic staining. Apoptosis was measured using Fluorescence-activated cell sorting (FACS). SPSS version 25 and R-studio were used for statistical analysis.

Results: This study found the IC50 for Ajwa dates was 913.3 µg/ml. Our data indicated that Ajwa dates decrease the MCL-1 levels, EGFR and p53 expression and also induced apoptosis compared to control. Furthermore, these effects became more evident when combined with abiraterone.

Conclusions: This study demonstrates the potential of Ajwa dates as a complementary therapy for prostate cancer, but further research is still needed before clinical testing is carried out.

KEY WORDS: *Prostate cancer; Ajwa dates; MCL-1; EGFR; p53.*

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INTRODUCTION

Prostate cancer is the fourth most common cancer worldwide and the second most frequently diagnosed in men, according to global cancer statistics (GLOBOCAN), which accounted for 7.3% of all new cancer cases in 2022 (1). Drug toxicity and resistance often limit current treatments, keeping prostate cancer as a major cause of cancer-related mortality worldwide (1, 2). This has led more research on natural chemicals found in fruits and vegetables for prostate cancer treatment (2).

Date palm (*Phoenix dactylifera L.*) is an essential fruit in Arabian countries, which showed several anticancer effects in many studies through antioxidant, pro-apoptotic, and cell cycle regulating mechanisms (2-4). The Ajwa date is preferred among other dates because of its great nutritional value, which is important for human diet and health (5, 6). Many studies showed that by disrupting membrane potential, raising oxidative stress, and generating DNA fragmentation, Ajwa date extracts cause mitochondrial-mediated death in PC3 cells and Oral Squamous Cell Carcinoma (HSC-2) cell culture (2, 7). However, the precise molecular pathways of key regulators and oncogens still needs further study (8).

Evidence supports the role of MCL-1 in prostate cancer cell survival, as its reduction causes rapid apoptosis by interacting with the dephosphorylation of the *Bcl2-Associated Death* (BAD) promoter (9). As demonstrated in a study where phytochemicals such as phenethyl isothiocyanate restored mutant p53 function to induce cell cycle arrest, p53 mutational status concurrently influences therapeutic responses (10). Moreover, the progression of prostate cancer is linked to EGFR signaling and EGFR-based therapy has the potential to make prostate cancer more responsive to treatment (11). Abiraterone acetate is a potent inhibitor of androgen biosynthesis, which has demonstrated significant cytotoxic and anti-proliferative effects in human prostate cancer PC3 cells of a model for *castration-resistant prostate cancer* (CRPC) (12). This study aims to investigate whether Ajwa date extract can modu-

late MCL-1 levels, EGFR, and p53 expression to induce apoptosis in PC3 cells. The findings could provide information on Ajwa dates as a complementary therapeutic agent with abiraterone acetate to target multiple oncogenic pathways simultaneously.

Methods

Study design and setting

The in vitro experimental study was performed at the *Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia*. Experimental design applied a laboratory-based approach with a post-test-only control group design. PC3 is a human mCRPC cell line, which in this study was categorized into four groups consisting of: negative control, abiraterone, Ajwa date extract, and combination of abiraterone and Ajwa dates extract. After 72 hours exposure, the MCL-1 levels were measured using ELISA method, while EGFR and p53 expressions were analyzed using immunofluorescence microscopic staining.

Preparation of Ajwa dates extract

Fresh Ajwa dates (1 kilogram) were prepared and separated from the seeds. The Ajwa date flesh (600 grams) was then cut into small pieces and dried at 600C. The results were refined with a blender to make a "*simplisia*" (or symplicia) that was stored in a closed container in the refrigerator. The "*simplisia*" was then extracted using the maceration method in 90% ethanol to extract more compounds. We collected the maceration content and re-macerated it by adding half the volume of ethanol from the first maceration. The maceration result was then concentrated using a rotary evaporator. The final product of the Ajwa date extract had a paste-like consistency. The extract results were weighed at 100 mg/ml working stock and stored in a refrigerator at a temperature of 40C for further use on the PC3 cells intervention.

PC3 cell culture

The PC3 cells (mCRPC PC3 cell lines), F-12K medium, *Dimethyl Sulfoxide* (DMSO), penicillin/streptomycin solution, *Phosphate Buffered Saline* (PBS), and *Fetal Bovine Serum* (FBS) were obtained from *American Type Culture Collection* (ATCC, Manassas, VA, USA). PC3 cells were cultured in F-12K with 10% FBS at 37°C in a humidified environment containing 5% CO2 until they attained 80% confluency. All cell lines were thereafter grown in full media for a minimum of 24 hours prior to experimental treatment.

Cell viability analysis and IC50 measurement

PC3 cells were seeded in 96-well plates at a density of 3.000-4.000 cells per well and let to adhere overnight. The cells were subsequently treated with Ajwa dates extract for 72 hours in full medium (0-20.000 µg/mL). The cells were subsequently treated at 37°C for 1-2 hours with the *Cell Counting Kit-8* (CCK-8) test (50µl CCK-8 per ml of culture media). The relative absorbance of the cell culture was subsequently measured at an excitation wavelength of 485 nm. The IC50 value of abiraterone was 66.9 µg/ml, as determined in a prior work utilizing the identical PC-3 cells (13).

MCL-1 levels, EGFR, and p53 expressions analysis

PC3 cells were seeded in a 24-well culture plate at a density of around 500.000-1.000.000 cells/mL and incubated for 24 hours. The cells received treatment with IC50 abiraterone acetate, IC50 Ajwa date extract, and a combination of IC50 Ajwa date extract and IC50 abiraterone acetate. Subsequently, the cells were incubated for 72 hours and further examination was carried out to assess MCL-1 levels, EGFR, and p53 expressions.

MCL-1 levels were examined using the MCL-1 monoclonal antibody reagent of Invitrogen® (*Thermofisher, USA*) with Enzyme-linked Immunosorbent Assay (ELISA). The ELISA procedure includes the antigen binding step, addition of biotin conjugate, Streptavidin-HRP, TMB substrate, and addition of stop solution, followed by absorbance reading at 450 nm wavelength to calculate MCL-1 levels based on the standard curve of *Optical Density* (OD). EGFR and p53 expressions were examined using staining and immunofluorescence examination. The preparation was performed by washing the cells using PBS and with 0.1% Triton-X 100. Following this, the cells were incubated with 1% Bovine Serum Albumin (BSA) at room temperature and then incubated with primary antibodies overnight at 4°C. Subsequently, the cells were incubated with secondary antibodies for 30 minutes at room temperature. Further, the cells were incubated with 4',6-diamidino-2-phenylindole (DAPI) 1:1000. Finally, the cells were covered with mounting media and cover glass, later the cells were observed with a fluorescence microscope.

Cell apoptosis analysis

PC-3 cells were washed twice with BioLegend Cell Stain Buffer (*Elabsciences, Houston, TX, USA*) and subsequently suspended in Annexin V Binding Buffer (*Elabsciences, Houston, TX, USA*) at a concentration of 1.0×107 cells/mL Subsequently, 100 µL of the cell suspension was transferred into a 5 mL reaction tube, followed by the addition of 5 µL FITC Annexin V (*Elabsciences, Houston, TX, USA*) and 10 µL propidium iodide solution (*Elabsciences, Houston, TX, USA*). The cells were then incubated at room temperature of 25°C. Subsequently, 400 µL of Annexin V Binding Buffer was introduced into each tube. This study analyzed 5.000 cells in each cycle. The flowcytometry results were analyzed by *Fluorescence-activated cell sorting* (FACS), which employed data bars of gating quadrants The total mean in early and late apoptosis was counted in this study. The fluorescence emitted by cells was quantified using the *Becton Dickinson* (BD) FACSCalibur (*BD Bioscience, Franklin Lakes, NJ, USA*).

Statistical analysis

MCL-1 expressions obtained from ELISA were analyzed alongside the expression percentage values of EGFR and p53 derived from immunofluorescence staining. A normality test was performed to determine the data distribution. The one-way *analysis of variance* (ANOVA) test was utilized for data with a normal distribution, while the Kruskal-Wallis non-parametric test was applied for data exhibiting non-normal distribution. A subsequent multiple comparison test, post hoc test, and regression analysis were conducted. The statistical analysis utilized SPSS version 25 (*IBM, New York, US*) and R Studio.

Results

Effect of Ajwa dates on cell viability and IC50 values in PC3 cells

Viability CCK8 assay was performed to determine the IC50 of Ajwa dates. Absorbance results were collected 72 hours later. The administration of Ajwa dates resulted in a viability reduction of PC3 cells, as indicated by an IC50 value of 913.3 µg/ml.

Effect of Ajwa dates, abiraterone acetate, and combinations on MCL-1 levels

The PC3 cells underwent interventions for 72 hours, with dosages determined by the IC50 value obtained. MCL-1 levels were subsequently measured using sandwich ELISA. The results demonstrated that MCL-1 levels were statistically different across groups ($p < 0.001$). The bar chart illustrates the variations in MCL-1 levels among the groups presented in Figure 1. A post-hoc analysis utilizing Tukey's HSD was performed to compare MCL-1 levels across groups. The analysis indicated the combination of abiraterone acetate and Ajwa dates resulted in significantly lower MCL-1 levels compared to the abiraterone acetate group ($p = 0.002$), the Ajwa dates group ($p < 0.001$), and the control group ($p < 0.001$), as shown in Table 1. The findings show that using a combination of abiraterone acetate with Ajwa date led to a significant decrease in MCL-1 levels when compared to the control and individual treatment groups.

Figure 1.
The effect of abiraterone, ajwa, and combination of abiraterone with ajwa on MCL-1 levels. The bars in the graph represent standard deviation.

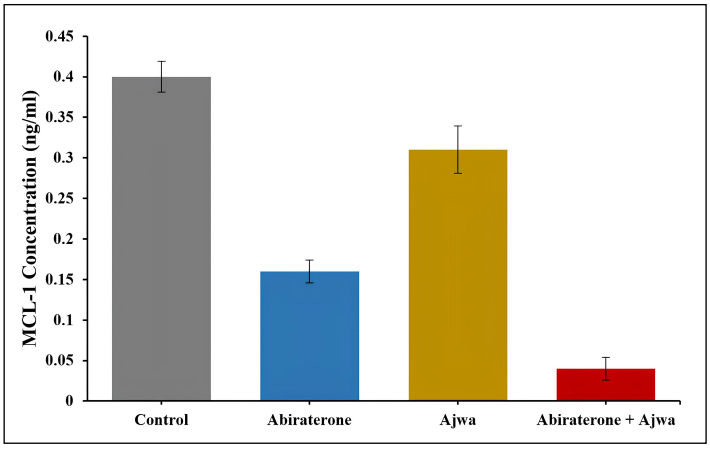

Table 1.
Post hoc analysis comparison of MCL-1 levels between groups.

Groups	Mean Difference (95% CI)	P-value
Abiraterone *vs* Control	-0.24 [-0.3 - (-0.18)]	< 0.001*
Ajwa *vs* Control	-0.08 [-0.14 - (-0.02)]	0.007*
Abiraterone + Ajwa *vs* Control	-0.35 [-0.41 - (-0.29)]	< 0.001*
Abiraterone *vs* Ajwa	-0.15 [-0.21 - (-0.09)]	< 0.001*
Abiraterone + Ajwa *vs* Abiraterone	-0.11 [-0.17 - (-0.05)]	0.002*
Abiraterone + Ajwa *vs* Ajwa	-0.26 [-0.32 - (-0.2)]	< 0.001*

*Tukey's HSD test. *Statistically significant at p < 0.05.*
CI: Confidence Interval.

Figure 2.
The impact of abiraterone, ajwa, and combination of abiraterone with ajwa on EGFR expressions. The bars in the graph represent standard deviation.

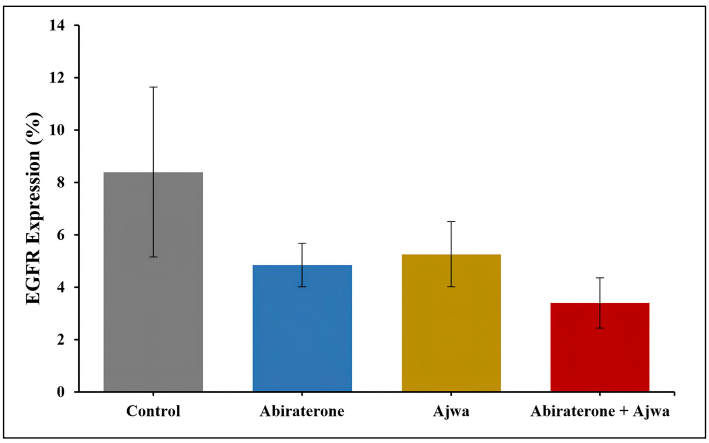

Table 2.
Post hoc analysis comparison of EGFR expression between groups.

Groups	Mean difference (95% CI)	P-value
Abiraterone *vs* Control	-3.55 (-8.38 - 1.28)	0.165
Ajwa *vs* Control	-3.14 [-7.97 - (1.68)]	0.237
Abiraterone + Ajwa *vs* Control	-5 [-9.83 - (-0.16)]	0.043*
Abiraterone *vs* Ajwa	-0.4 (-5.23 - 4.42)	0.993
Abiraterone + Ajwa *vs* Abiraterone	-1.45 (-6.28 - 3.38)	0.774
Abiraterone + Ajwa *vs* Ajwa	-1.85 (-6.69 - 2.97)	0.626

*Tukey's HSD test. *Statistically significant at p < 0.05.*
CI: Confidence Interval.

Effect of Ajwa dates extract, abiraterone acetate, and combinations on EGFR expressions

Following exposure of PC3 cells to the specified interventions, EGFR expressions were quantified as the percentage of cells exhibiting EGFR with a microscopic immunofluorescence analysis. The results indicated that the expressions of EGFR were statistically different among groups ($p = 0.03$). The bar chart illustrates the variations in EGFR expressions among the groups presented in Figure 2. Post hoc analysis indicated that the combination of abiraterone acetate and Ajwa dates resulted in significantly lower EGFR expressions compared to the control group ($p = 0.43$), as shown in Table 2. The findings show that using a combination of abiraterone acetate with Ajwa date led to a significant decrease in EGFR expressions when compared to the control group.

Effect of Ajwa dates extract, abiraterone acetate, and combinations on p53 expressions

Following exposure of PC3 cells to the specified interventions, p53 expressions were quantified as the percentage of cells exhibiting p53 with a microscopic immunofluorescence analysis. The result indicated that the expressions of p53 were statically different among groups ($p = 0.006$). The bar chart shows the differences in p53

Figure 3.
The impact of abiraterone, ajwa, and combination of abiraterone with ajwa on p53 expressions. The bars in the graph represent standard deviation

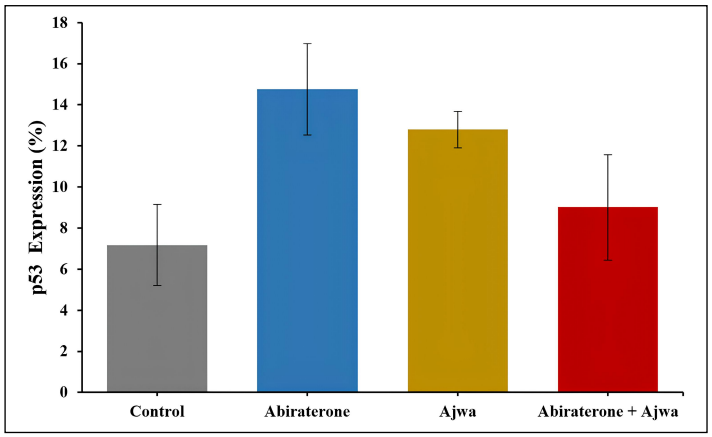

Table 3.
Post hoc analysis comparison of p53 expression between groups.

Groups	Mean difference (95% CI)	P-value
Abiraterone *vs* Control	7.56 (2.3 - 12.82)	0.008*
Ajwa *vs* Control	5.6 (0.34 - 10.86)	0.037*
Abiraterone + Ajwa *vs* Control	1.8 (-0.43 - 7.08)	0.693
Abiraterone *vs* Ajwa	1.96 (-3.29 - 7.22)	0.646
Abiraterone + Ajwa *vs* Abiraterone	-5.74 [-11 - (-0.48)]	0.033*
Abiraterone + Ajwa *vs* Ajwa	-0.377 (-9.03 - 1.48)	0.177

*Tukey's HSD test. *Statistically significant at p < 0.05.*
CI: Confidence Interval.

expressions between the groups presented in Figure 3. Post-hoc analysis indicated that the abiraterone acetate group and Ajwa dates group had significantly higher p53 expressions than the control group (p = 0.008; p = 0.037; respectively), as shown in Table 3. This result highlighted that the abiraterone acetate or Ajwa dates administration led to an increase in p53 expressions in comparison to the control group.

Effect of Ajwa dates extract, abiraterone acetate, and combinations on apoptosis in PC3 cells

This study employed FACS to evaluate the proportion of cells undergoing apoptosis following treatment. PC3 cells were categorized into four quadrants according to the cell emission signals illustrated in Figure 4. Early apoptosis was observed in the lower right quadrant, while late apoptosis was noted in the upper right quadrant. The data in this study exhibited a normal distribution, and one-way ANOVA analysis revealed a significant difference in total mean apoptosis among the groups (p = 0.009). The bar chart illustrates the variations in total mean apoptosis among the groups presented in Figure 5. Post hoc analysis indicated that all groups demonstrated increased total apoptosis with respect to the control group with the combination of abiraterone acetate and Ajwa dates demonstrating significantly higher total mean apoptosis compared to other groups (p = 0.012), as

Figure 4.
Fluorescence-activated cell sorting (FACS) analysis showing gating and quadrant distribution of apoptosis. (A-B) control group; (C-D) abiraterone; (E-F) ajwa; (G-H) combination of abiraterone and ajwa.

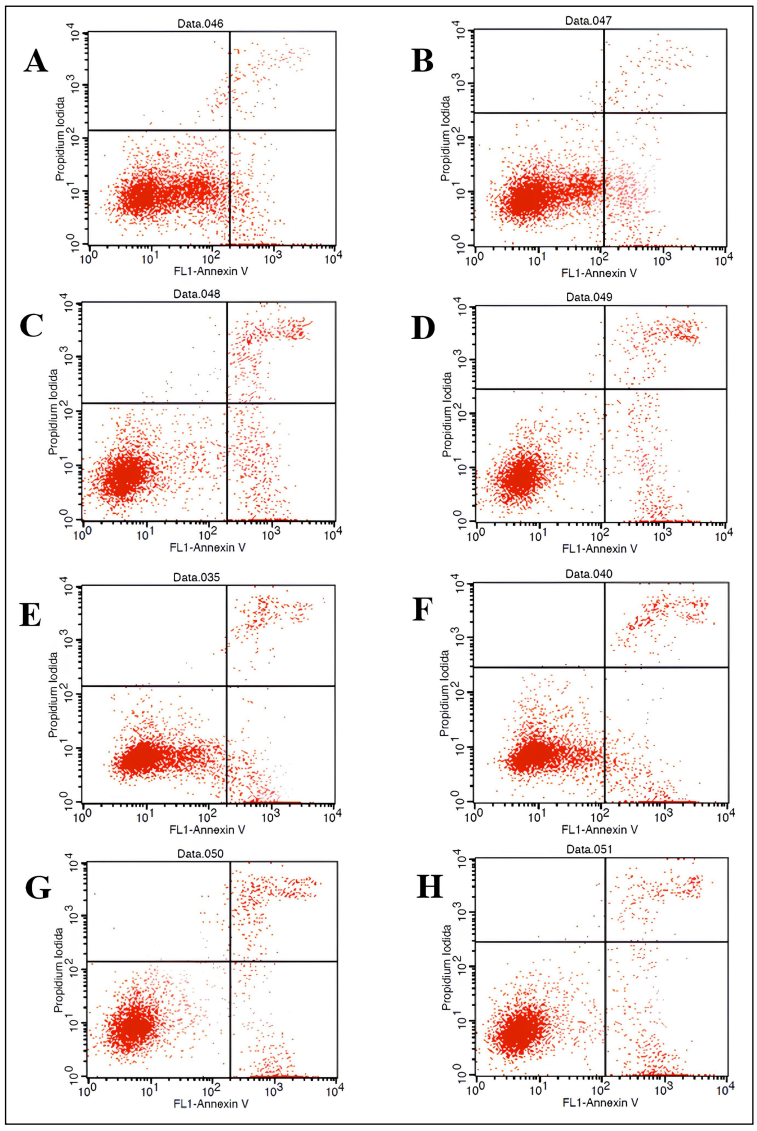

Figure 5.
The impact of abiraterone, ajwa, and combination of abiraterone with ajwa on mean percentage of total apoptosis. The bars in the graph represent standard deviation.

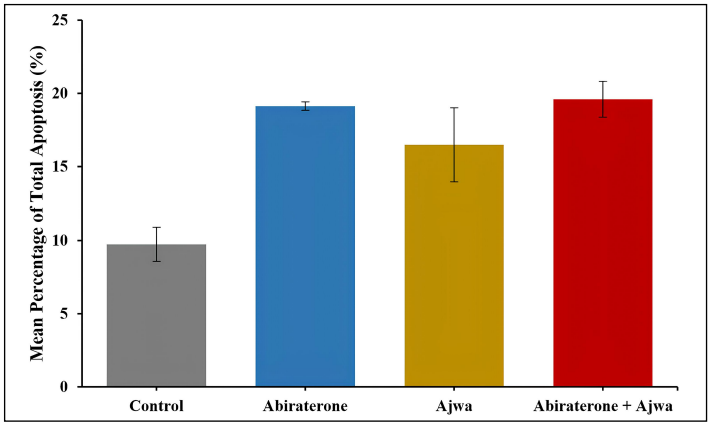

Table 4.
Post hoc analysis comparison of mean percentage of total apoptosis between groups.

Groups	Mean difference (95% CI)	P-value
Abiraterone *vs* Control	9.4 (3.2 - 15.59)	0.012*
Ajwa *vs* Control	6.75 (0.56 - 12.94)	0.038*
Abiraterone + Ajwa *vs* Control	9.86 (3.67 - 16.05)	0.010*
Abiraterone *vs* Ajwa	2.64 (-3.54 - 8.83)	0.413
Abiraterone + Ajwa *vs* Abiraterone	0.46 (-5.72 - 6.65)	0.989
Abiraterone + Ajwa *vs* Ajwa	3.11 (-3.08 - 9.3)	0.309

*Tukey's HSD test. *Statistically significant at p < 0.05.*
CI: Confidence Interval.

shown in Table 4. The findings highlight a significant mean percentage of total apoptosis among the treatment groups, with the combination of abiraterone and Ajwa dates consistently exhibiting the strongest effect.

Multiple linear regression analyses of MCL-1, EGFR, and p53 to predict apoptosis in PC3 cells

Indeed, multiple linear regression analyses of MCL-1 levels, EGFR and p53 expressions to predict apoptosis revealed that MCL-1 (beta = -0.434; p = 0.006), EGFR (beta = -0.417; p = 0.009), and p53 (beta = 0.334; p = 0.016) contributed to apoptosis in PC3 cells (r = 0.835; r2 = 0.698) as presented in Table 5. Concurrently, multivariate ridge regression analysis of MCL-1 levels, EGFR, and p53 expressions in each intervention group to predict apoptosis showed that MCL-1 (beta = -0.16; p = 0.006), EGFR (beta = -0.096; p = 0.004), and p53 (beta = 0.019; p = 0.002) in the Ajwa dates group and MCL-1 (beta = -0.091; p = 0.002), EGFR (beta = -0.085; p = 0.002), and p53 (beta = 0.005; p = 0.002) in the combination group analysis as presented in Table 6. These results highlighted that the changes in MCL-1, EGFR, and p53 sequentially contribute to apoptosis that occurs in PC3 cells in this study.

Table 5.
Multiple linier regression analysis with MCL-1, EGFR and p53 as independent and apoptosis as dependent variable.

Variable	Coefficients value			R	R^2
	Unstandardized Cofficients Beta	Standardized Cofficients Beta	p-value		
MCL-1	-510.739	-0.434	0.006*		
EGFR	-32.626	-0.417	0.009*	0.835	0.698
p53	15.041	0.334	0.016*		

**Statistically significant at p < 0.05.*

Table 6.
Multiple regression analysis of each intervention using ridge regression with MCL-1, EGFR and p53 as independent and apoptosis as dependent variable.

Group	Parameter	Standardized Coefficients Beta (95% CI)	p-value	MAPE (%)
Control	MCL-1	-0.068 [-0.187 - (-0.055)]	0.003*	39.868
	EGFR	-0.204 [-0.232 - (-0.089)]	0.003*	
	p53	0.015 (-0.097 - 0.056)	0.006*	
Abiraterone	MCL-1	-0.157 [-0.181 - (-0.126)]	0.002*	5.158
	EGFR	-0.101 [-0.161 - (-0.097)]	0.002*	
	p53	0.081 (0.077 - 0.085)	0.002*	
Ajwa	MCL-1	-0.16 [-0.228 - (-0.066)]	0.006*	10.645
	EGFR	-0.096 [-0.117 - (-0.052)]	0.004*	
	p53	0.019 (-0.077 - 0.041)	0.002*	
Abiraterone + Ajwa	MCL-1	-0.091 [-0.131 - (-0.079)]	0.002*	3.963
	EGFR	-0.085 [-0.127 - (-0.071)]	0.002*	
	p53	0.005 (0.003 - 0.008)	0.002*	

**Statistically significant at p < 0.05. Mean Absolute Percentage Error.*

Discussion

Our study demonstrated that Ajwa dates have potential as a promising natural anticancer agent. Ajwa dates contain various bioactive phytochemicals, such as flavonoids, phenolic acids, glycosides, and terpenoids, which together enhance their antioxidant, anti-inflammatory, and anticancer effects (14, 15). These compounds act via multiple mechanisms, including the induction of apoptosis, cell cycle arrest, and the inhibition of survival signals, thereby enhancing their efficacy and minimizing the risk of resistance compared to single-target chemotherapeutics (16). The favorable safety profile of Ajwa date extract, evidenced by its higher IC50 in normal cell lines, indicates a selective cytotoxic effect on cancer cells (15). This low-toxicity strategy exemplifies the characteristics of natural compounds that are gaining recognition for their capacity to modulate essential signaling pathways implicated in cancer progression, such as PI3K/AKT/mTOR, NF-κB, and MAPK/ERK cascades. The integration of these phytochemicals in cancer therapy may yield synergistic benefits, potentially improving outcomes and minimizing adverse effects (16).

The IC50 value of Ajwa dates extract determined in our study was 913.3 µg/mL. This result provides important context when compared to other published IC50 values for Ajwa date extracts against cancer cell lines. Notably, *Mirza et al.* reported a much lower IC50 for the *ethyl acetate fraction of Ajwa dates* (EAFAD) against the same PC3 cells, with values of 0.3887 mg/mL (388.7 µg/mL) at 24 hours and 0.4753 mg/mL (475.3 µg/mL) at 48 hours (2). This difference may be attributed to variations in extract preparation, the specific fraction used (e.g., ethyl acetate versus crude or other solvent extracts), or experimental conditions such as cell seeding density and assay protocols. In contrast, studies on other cancer cell lines, such as HSC-2, have reported higher IC50 values for Ajwa date flesh extract of 8690 µg/mL and pit extract of 970 µg/mL at 24 hours2 (7). These findings suggest that the cytotoxic potency of Ajwa date extracts can vary widely depending on both the cell type and the extraction method. Given the promising apoptotic and cytotoxic effects demonstrated across these studies (2, 7), further optimization of extraction strategies may enhance the anticancer potential of Ajwa date extracts for prostate cancer therapy.

In our study, we analysed MCL-1 levels, EGFR, and p53 expression parameters, which demonstrated the intervention of Ajwa dates extract in PC3 cells has the potential to target multiple interconnected signalling pathways that are essential for cancer cell survival and apoptosis. MCL-1 is a crucial anti-apoptotic protein within the Bcl-2 family that is often overexpressed in solid tumors. MCL-1 enhances cell survival through the inhibition of mitochondrial apoptosis and plays a role in chemoresistance (17). Targeting MCL-1 represents a promising therapeutic approach, as its downregulation may enhance the sensitivity of cancer cells to apoptosis (18). Our study indicated that administration of Ajwa dates extract can decrease MCL-1 levels, with the combination of this extract and abiraterone acetate showing the lowest average value among groups ($p < 0.001$).

EGFR signalling promotes cancer cell proliferation and survival through the activation of downstream pathways, including PI3K/AKT and MAPK/ERK. Inhibition of this signalling has been shown to decrease cell proliferation, induce apoptosis, and inhibit tumor growth in multiple cancer models (19, 20). This aligns with our findings, which demonstrate that the combination of abiraterone acetate and Ajwa date extract significantly reduces EGFR expression in PC3 cells.

The tumor suppressor p53 plays a central role in orchestrating cellular responses to stress by inducing cell cycle arrest and apoptosis; p53-mediated apoptosis is executed through the transactivation of pro-apoptotic genes, mitochondrial dysfunction, and caspase activation (21). Our findings demonstrate that the abiraterone acetate group as a monotherapy was the most effective in enhancing p53 expression. The findings of our investigation may be elucidated by many factors and possible antagonistic processes. Abiraterone and Ajwa dates are recognized to activate p53 via distinct routes, and their combination is believed to disrupt each other's methods of targeting p53. A study by *Grossebrummel et al.* elucidates the findings of this research, indicating that the presence of active natural compounds may counteract the efficacy of abiraterone in inducing apoptosis via a p53-independent pathway. Consequently, the impact of abiraterone on p53 does not improve when combined with herbal-based crude extracts containing numerous active natural compounds (22).

This work has a notable limitation due to the absence of an in silico analysis and the failure to isolate the active components of Ajwa dates, indicating that these results need further investigation.

The flowcytometry analysis using FACS in this study demonstrated that Ajwa date extract combined with abiraterone acetate and Ajwa dates extract single intervention can induce apoptosis in PC3 cells. These findings align with prior studies by *Mirza et al.* who reported that the *ethyl acetate fraction of Ajwa dates* (EAFAD) induced apoptosis in PC3 cells via mitochondrial membrane depolarization and DNA fragmentation, corroborating our observation of intrinsic apoptotic pathway activation (2). Similarly, *Shahbaz et al.* observed late apoptosis in MDA-MB-231 breast cancer cells at higher concentrations of Ajwa date pulp extract, mirroring the dose-dependent apoptotic response seen in our study (7).

This study has several advantages. First, this study is one of the first in vitro studies to evaluate the effects of Ajwa date extract on prostate cancer in the PC3 cells by analyzing key molecular markers such as MCL-1, EGFR, and p53 with quantitative measurement of apoptosis. The experimental design comparing the single and combined effects of Ajwa date extract with abiraterone acetate allows the identification of potential complementary therapies for prostate cancer. In addition, the use of multivariate analysis methods with multiple linear regression analysis and ridge regression of each treatment group provides an overview of the relative contribution of each molecular parameter to apoptosis induction, thus strengthening the validity of the findings. Thus, this study not only provides new scientific evidence regarding the anticancer potential of Ajwa dates, but also opens up opportunities for the development of safer and more affordable complementary therapies for prostate cancer patients.

This study also possesses many limitations. First, the study design relies on an in vitro model, which cannot adequately recreate the complexity and physiological circumstances of an in vivo model. Secondly, this work has not determined the active biochemical components in Ajwa dates extract, which might elucidate the chemicals involved in the production and manifestation of apoptosis in PC3 cells. Finally, this study has not assessed the potential toxicity effects of Ajwa dates extract on normal cells. Further study should include animal and human models to evaluate the pharmacokinetics, bioavailability, and systemic toxicity, as well as to confirm the anti-cancer efficacy of Ajwa dates demonstrated in this study.

Declarations

Contribution details:

	AA	ASA	HR	IY	LH	SB	AB	AAZ
Concepts	√	√	√	√				
Design	√	√	√	√				
Definition of intellectual content	√							
Literature search	√							
Data acquisition	√	√	√	√				
Data analysis	√							
Statistical analysis	√							√
Manuscript preparation	√							
Manuscript editing	√	√	√	√				
Manuscript review	√	√	√	√	√	√	√	√
Guarantor	√	√	√	√	√	√	√	√

Ethical approval: *Not required.*

Availability of data and material: *Derived data supporting the findings of this study are available from the corresponding author on request.*

Competing interests: *The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.*

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Conference presentation: *This article has not been presented at any conference.*

Declaration of artificial intelligence use: *The use of artificial intelligence is intended for language refinement purposes.*

Conclusions

It can be concluded that Ajwa dates extract can reduce MCL-1 levels as anti-apoptosis, reduce EGFR expression as a proliferation oncogene and increase p53 expression as pro-apoptosis, which contribute to induce apoptosis in PC3 cells. Ajwa dates have the potential as a complementary therapy for prostate cancer, but further research is still needed before clinical testing is carried out.

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