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Enhancing Anticancer Efficacy: The Combined Effects of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) Leaf Extracts with Prayer Water on MCF-7 Breast Cancer Cells Through Phytotherapy and Cultural Practices

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ABSTRACT: This study evaluated the in vitro cytotoxic activity of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) leaf extracts, individually and in combination with prayer-infused water, on MCF-7 breast cancer cells, together with phytochemical screened for alkaloids, flavonoids, saponins, phenols, and tannins, and antioxidant activity was evaluated using DPPH radical-scavenging assay. Cytotoxicity was assessed by MTT assay at 1500 and 2000 µg/mL over 24, 48, and 72 hours of exposure. Prayer-infused water was incorporated at 7% in the reported combination treatment. Qualitative screening detected alkaloids, phenols, and tannins in both extracts, whereas saponins were detected in Siplukan but not in Bidara. Flavonoids were not detected under the qualitative test conditions used. The reported DPPH IC₅₀ values were 12.5 µg/mL for Bidara and 48.5 µg/mL for Ciplukan extracts with 7% prayer-infused water produced a reported viability of 34.6% at 72 hours at the 1500 µg/mL condition. However, the present experimental design does not provide sufficient dose-response information or a formal combination-analysis model to establish pharmacological synergy. The findings are therefore interpreted as preliminary evidence of combined or enhanced activity rather than definitive synergy. Further studies should include expanded dose-response matrices, formal combination analysis, normal-cell controls, chemical characterization, mechanistic assays, and in vivo validation before any therapeutic implications are considered.

1. INTRODUCTION

Breast cancer is a mayor global health challenge, ranking as the most common malignancy among women and accounting for approximately 2.3 million new cases and over 680,000 deaths annually, according to the World Health Organization (WHO) [1,2]. The incidence of breast cancer has been rising, with developing countries bearing a substantial share of this burden due to limited access to early detection and high-cost treatments like chemotherapy, radiotherapy, and surgery [3,4,5]. These traditional treatments, while effective, come with significant drawbacks: side effects, high relapse rates, and limited accessibility in resource-poor settings [6]. These factors underscore an urgent need for innovative, accessible, and culturally sensitive therapeutic options that align with the financial and healthcare infrastructure challenges in such regions.

Natural products, particularly medicinal plants, offer a promising avenue for affordable and effective cancer therapies. Indonesia, a biodiversity hotspot, is home to thousands of medicinal plants used traditionally for their healing properties [7,8]. Among these, Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) have been used for centuries in traditional Indonesian

medicine and are recognized for their potent bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and phenols [9]. Preliminary studies have shown that these compounds possess antioxidative and anti-carcinogenic properties, making them viable candidates for cancer research. For instance, Bidara has demonstrated strong free radical scavenging ability, indicating high antioxidant potential [11,12,13]. This antioxidative capacity is crucial, as oxidative stress is a key driver of cancer progression, promoting cellular damage, inflammation, and tumor growth.

The cultural and symbolic significance of Bidara and Ciplukan in Indonesia further enhances their appeal. Bidara, in particular, holds spiritual value and is often used in traditional healing, especially in Islamic communities. Integrating such culturally relevant practices, including the use of prayer-infused water, into scientific research may offer a dual benefit: it aligns with local traditions, which could improve acceptance and adherence among patients, and it provides psychological comfort, potentially enhancing therapeutic outcomes. Prayer water, while not yet widely studied scientifically, is believed by practitioners to enhance the healing power of treatments, adding a unique dimension to cancer therapy that combines spirituality with medicinal phytotherapy [13,14].

This study examines the individual and combined effects of Bidara and Ciplukan extracts, along with prayer water, on MCF-7 breast cancer cells. The primary objectives are threefold: (1) to characterize the phytochemical composition of Bidara and Ciplukan leaf extracts, highlighting their bioactive compounds; (2) to quantify the antioxidant potential through DPPH assays, where Bidara's superior antioxidative ability is evidenced by a lower IC₅₀ value, suggestive of a stronger free radical inhibition compared to Ciplukan; and (3) to evaluate cytotoxicity against MCF-7 breast cancer cells, with data revealing that a combination of Bidara, Ciplukan, and prayer water leads to the most pronounced cell viability reduction. In cytotoxicity assays, Bidara alone reduced cell viability by over 50% at concentrations as low as 2000 µg/mL after 72 hours, while Ciplukan showed a similar reduction at the same concentration, with both treatments demonstrating time- and dose-dependent effects. Notably, the combination treatment with prayer water led to a 34.6% viability rate in MCF-7 cells after 72 hours, indicating a synergistic potential that could be harnessed for therapeutic applications.

By bridging phytotherapy with culturally significant practices, this research offers a pathway to holistic cancer treatment that is both scientifically validated and culturally resonant. These findings are not only aligned with the Sustainable Development Goals (SDGs) advocating for health innovation and accessibility but also reflect an integrative approach that supports patient-centered care [15,16]. This study contributes to the emerging field of culturally inclusive cancer therapies, underscoring the importance of accessible, traditional plant-based medicines in modern healthcare settings.

2. MATERIALS AND METHODS

Plant Materials and Authentication

Fresh leaves of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) were obtained from local sources in Indonesia. The plant materials were subjected to botanical identification and authentication at recognized botanical research facility before extraction. The plants parts used were leaves.

Preparation and Extraction of Plants Materials

The fresh leaves were washed with distilled water, air-dried at room temperature for 72 h, and ground into a uniform powder. For each plant, 100 g of powdered material was extracted with 70% ethanol using a Soxhlet extractor at approximately 70 °C for 6 h. After extraction, the solvent was removed under reduced pressure using a rotary evaporator. The resulting crude extracts were stored at -20 °C in amber-colored containers until phytochemical and biological analyses.

Qualitative Phytochemical Screening.

Qualitative phytochemical screening was conducted using standard color- or precipitate-based tests [17,18,19,20] involved the following:

- 1) **Alkaloids:** Detected using Mayer's test, where the formation of a white precipitate indicated the presence of alkaloids.
- 2) **Flavonoids:** Identified by adding a few drops of concentrated hydrochloric acid; a color change to red confirmed their presence.
- 3) **Saponins:** Assessed through the froth test, where the formation of stable foam indicated a positive result.

- 4) **Phenols:** Confirmed through the ferric chloride test, where a dark green or black color indicated the presence of phenols.
- 5) **Tannins:** Identified by the gelatin test, where a dark greenish-black precipitate indicated tannins.

Results were recorded qualitatively as positive (+) or negative (-). Because these assays are qualitative, the symbols do not represent concentrations of individual compound. based on observable reactions, as summarized in *Table 1*.

Antioxidant Activity (DPPH Assay)

DPPH Free Radical Scavenging Assay. The antioxidant activity of the Bidara and Ciplukan extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [21,22,23,24]. Serial dilutions of each extract were prepared in ethanol at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. A solution of 0.1 mM DPPH was prepared in ethanol, and 2 mL of DPPH solution was added to 2 mL of each extract dilution. The mixtures were incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100 \quad (\text{eq 1})$$

The IC₅₀ value, defined as the concentration of extract needed to inhibit 50% of the DPPH radicals, was calculated for each extract using linear regression analysis.

Cell Culture and Cytotoxicity Assay

MCF-7 Cell Line Maintenance. The human breast cancer cell line MCF-7 was obtained from a reputable cell repository. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin [25, 26]. Cell cultures were maintained in a humidified incubator at 37°C with 5% CO₂, and the media were refreshed every 48 hours. Cells were sub-cultured once they reached 80% confluency.

Preparation of Treatment Solutions. The Bidara and Ciplukan extracts were diluted in dimethyl sulfoxide (DMSO) to create stock solutions, which were further diluted in DMEM to achieve final concentrations of 1500 µg/mL and 2000 µg/mL for each treatment. The prayer-infused water was prepared by collecting distilled water that was subjected to specific prayers as per traditional Indonesian practices and added to the treatment combinations at a 7% concentration.

Cytotoxicity Assay (MTT Assay)

Experimental Setup. The cytotoxic effects of the extracts and their combinations on MCF-7 cells were evaluated using the MTT assay [27,28]. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated for 24 hours to allow for attachment. The cells were then treated with each extract at 1500 µg/mL and 2000 µg/mL concentrations, as well as combinations of Bidara, Ciplukan, and prayer-infused water, in triplicate wells for each condition. Untreated cells served as the control group.

MTT Assay Protocol. After 24, 48, and 72 hours of treatment, the medium was replaced with 20 µL of 5 mg/mL MTT solution in phosphate-buffered saline (PBS), and the plates were incubated at 37°C for 4 hours. Following incubation, the MTT solution was removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as follows:

$$\text{Cell Viability (\%)} = \left(\frac{\text{Absorbance of Treated Cells}}{\text{Absorbance of Control Cells}} \right) \times 100 \quad (\text{eq 2})$$

The results were analyzed to determine the cytotoxicity of each extract and combination treatment, with lower absorbance indicating greater cytotoxicity.

Statistical Analysis. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). The statistical significance of differences between groups was evaluated using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons [29]. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Phytochemical Composition of Bidara and Ciplukan Leaves. The extraction and phytochemical analysis of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) leaves revealed the presence of key bioactive compounds, as shown in Table 1. Both extracts contained alkaloids, flavonoids, phenols, and tannins, while saponins were detected only in Ciplukan extract. These compounds are known for their antioxidative and potential anticancer properties, which are crucial in targeting oxidative stress mechanisms in cancer progression

Table 1. Comparison of Phytochemical Constituents in Bidara Leaf Extract and Ciplukan Leaf Extract

Parameter	Bidara Leaf Extract		Ciplukan Leaf Extract	
	Color	Result	Color	Result
Alkaloid	Reddish-brown precipitate formed	+	Reddish-brown precipitate formed	+
Flavonoid	Dark green	-	Dark green	-
Saponin	No foam	-	Foam formed	+
Phenol	Green color present	+	Green color present	+
Tannin	Dark greenish-black	+	Dark greenish-black	+

The morphological characteristics of the leaves, depicted in Figure 1, further underscore their structural integrity and biological readiness for extraction. The presence of these compounds, particularly flavonoids and phenols, aligns with findings on their antioxidative capacities, making them potent candidates for oxidative stress-related therapeutic applications.



Figure 1. Leaf Morphology of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata* L.): (a) Adaxial surface of Bidara leaf, (b) Abaxial surface of Bidara leaf; (c) Whole Ciplukan plant showing leaf, flower, and fruit; (d) Adaxial surface of Ciplukan leaf, (e) Abaxial surface of Ciplukan leaf.

Antioxidant Activity (IC₅₀) Analysis. The antioxidant potential of Bidara and Ciplukan extracts was evaluated using the DPPH assay. As seen in Table 2, the IC₅₀ value for Bidara leaf extract was 12.5 µg/mL, indicating a high capacity to scavenge free radicals [10,30]. In contrast, Ciplukan leaf extract had an IC₅₀ value of 48.5 µg/mL, which, while lower than Bidara, still signifies strong antioxidant properties.

The DPPH assay measures the concentration required to inhibit 50% of free radicals, and a lower IC₅₀ value corresponds to stronger antioxidant activity. As shown in Figure 2, the data indicate that both extracts exhibit concentration-dependent free

radical inhibition. Bidara's superior antioxidative strength could be attributed to its higher flavonoid and tannin concentrations, compounds known for their radical-scavenging properties. The strong antioxidative performance of these extracts suggests potential benefits in mitigating oxidative stress, a key factor in the initiation and progression of cancer.

Table 2. Comparison of Inhibitory Activity of Bidara Leaf Extract and Ciplukan Leaf Extract

Sampel	Concentration (ppm)	Average Absorbance	% Inhibition	IC50 (µg/ml)
Bidara Leaf Extract	1000	0,039	95,3	12,5
	500	0,050	94,1	
	250	0,085	89,8	
	125	0,132	84,3	
	62,5	0,185	77,9	
	31,25	0,293	64,9	
Ciplukan Leaf Extract	1000	0,044	94,8	48,5
	500	0,062	92,7	
	250	0,106	87,4	
	125	0,198	76,4	
	62,5	0,291	65,4	
	31,25	0,397	52,7	

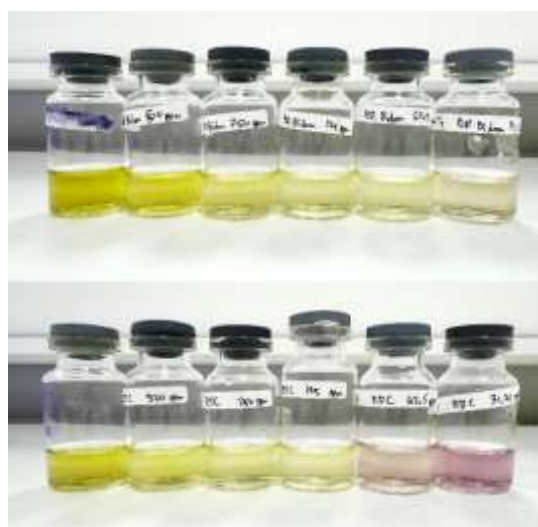


Figure 2. DPPH radical scavenging activity of Bidara and Ciplukan leaf extracts at different concentrations (1000, 500, 250, 125, 62.5, and 31.25 ppm). Bidara leaf extract is shown in the top row, and Ciplukan leaf extract is shown in the bottom row.

Cytotoxicity on Breast Cancer Cells (MCF-7 Line)

Bidara Extract Cytotoxicity. The cytotoxic effects of Bidara extract on MCF-7 breast cancer cells were tested at concentrations of 1500 µg/mL and 2000 µg/mL. Results in Table 3 and Figure 3 demonstrate a time-dependent decrease in cell viability. At 24 hours, the high absorbance (1.79) indicated low cytotoxicity, but this reduced over time, showing significant cell viability inhibition by 48 and 72 hours, with a final reading of 2.43 absorbance at 72 hours.

Table 3. Effect of Bidara and Ciplukan Extracts, and Prayer Water on MCF-7 Cell Viability at Different Concentrations (µg/mL)

No	Sample	Dose	Average			% Viability		
			24h	48h	72h	24h	48h	72h
1	Bidara	1500 µg/mL	1,79	1,90	2,43	137,38	116,64	102,26
		2000 µg/mL	1,88	1,73	1,36	145,66	24,70	48,96
2	Ciplukan	1500 µg/mL	1,90	1,54	1,23	147,32	92,04	42,67
		2000 µg/mL	2,15	1,14	1,54	171,88	61,70	57,87
3	Prayer Water	0,07 (7%)	1,22	1,64	1,87	119,30	150,75	72,76

This time-dependent cytotoxicity implies that Bidara extract may act through cumulative apoptotic mechanisms, gradually inhibiting cell survival pathways in MCF-7 cells. Such delayed action may reflect cellular resistance patterns that become more susceptible to Bidara's active compounds over prolonged exposure [11,31].

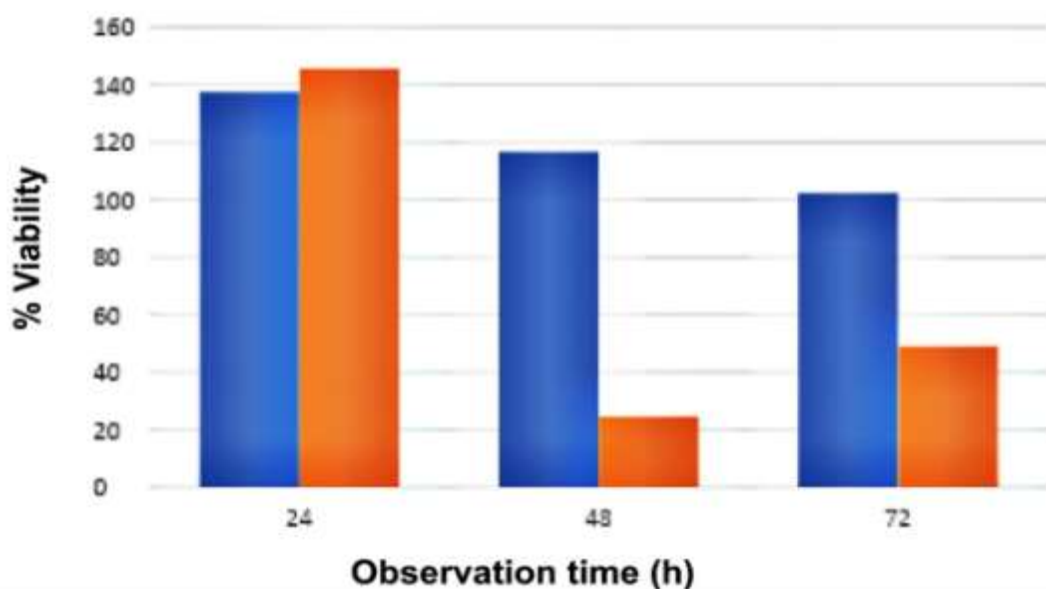


Figure 3. % Viability of MCF-7 Cells with Bidara Leaf Extract

Cytotoxic Activity of Ciplukan Extract. Ciplukan extract showed moderate early cytotoxic effects, with absorbance values indicating a steady reduction in cell viability over time. At 24 hours, cell viability was relatively unaffected (1.90), but by 48 hours, the absorbance dropped to 1.54. This continued reduction, reaching 1.23 by 72 hours, supports the extract's ability to inhibit MCF-7 cells through prolonged exposure, possibly by inducing apoptotic pathways [32,33,34].

Table 4. Effect of Ciplukan Leaf Extract and Prayer Water on MCF-7 Cell Viability at Different Concentrations ($\mu\text{g/mL}$)

Treatment	Dose	Average			% Viability		
		24h	48h	72h	24h	48h	72h
Combination of Ciplukan Extract and Prayer-Infused Water	1500 $\mu\text{g/mL}$	1,53	0,88	1,07	111,79	41,54	34,57
	2000 $\mu\text{g/mL}$	1,92	1,68	1,68	149,86	102,89	64,81

As seen in Figure 4, Ciplukan's efficacy in reducing cell viability aligns with its phytochemical content, notably flavonoids and alkaloids, which are documented for their anti-proliferative effects on cancer cells.

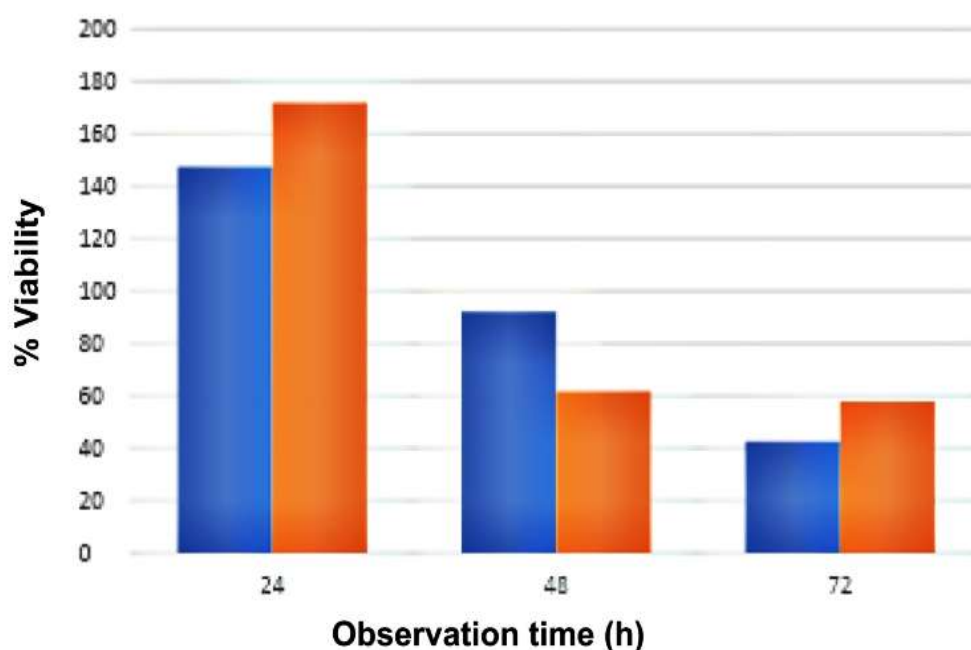


Figure 4. Effect of Ciplukan Leaf Extract on the Viability of MCF-7 Cancer Cell

Combined Treatment of Bidara, Ciplukan, and Prayer Water. The combined use of Bidara and Ciplukan extracts (1:1 ratio, 1500 $\mu\text{g/mL}$) with 7% prayer water showed promising results, as illustrated in Table 5 and Figure 5. At 24 hours, the initial absorbance was 1.53, which significantly reduced to 0.88 at 48 hours, and further to 1.07 by 72 hours. This consistent decrease indicates enhanced cytotoxic effects due to potential synergistic interactions between Bidara and Ciplukan compounds when used together with prayer water [35,36].

Table 5. Effect of Combined Bidara, Ciplukan, and Prayer Water Extracts on MCF-7 Cell Viability at Different Concentrations ($\mu\text{g/mL}$)

Treatment	Dose	Average			% Viability		
		24h	48h	72h	24h	48h	72h
Combination of Bidara and Ciplukan Leaf Extracts and Water Treated with Prayer	1500 $\mu\text{g/mL}$ + 7%	1,81	1,15	1,07	138,6	62,2	34,6
	2000 $\mu\text{g/mL}$ + 7%	2,30	1,72	1,49	186,1	105,9	55,3

The mechanism behind this synergy may involve the complementary actions of bioactive components from both plants on different cellular targets within the MCF-7 cells. Moreover, the inclusion of prayer water, traditionally believed to possess healing properties, may enhance perceived efficacy in patients by incorporating psychological and cultural dimensions into the treatment. Scientific investigations could further explore this combination's molecular impact on cell signaling pathways to uncover novel anticancer applications.

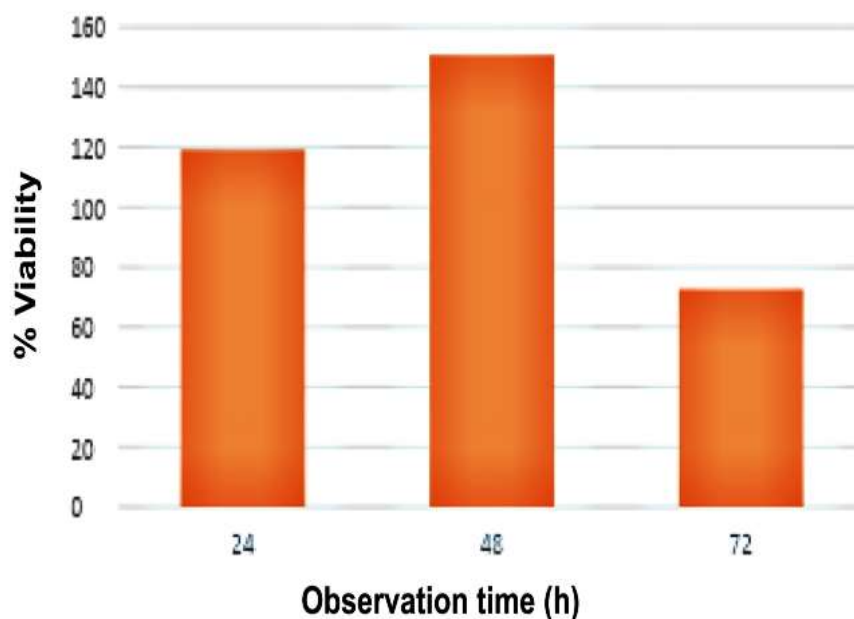


Figure 5. Effect of Prayer Water on the Viability of MCF-7 Cell

Prayer Water Alone. The effect of prayer water alone, as seen in Table 3 and Figure 6, revealed minimal cytotoxicity on MCF-7 cells at 7% concentration. Absorbance readings at 24 hours were 1.22, slightly increasing by 48 hours to 1.64. At 72 hours, variability was evident, with values between 1.1823 and 2.1878. This variability suggests that, while prayer water alone may offer minimal direct cytotoxic effects, its role may lie more in enhancing the efficacy of other treatments.

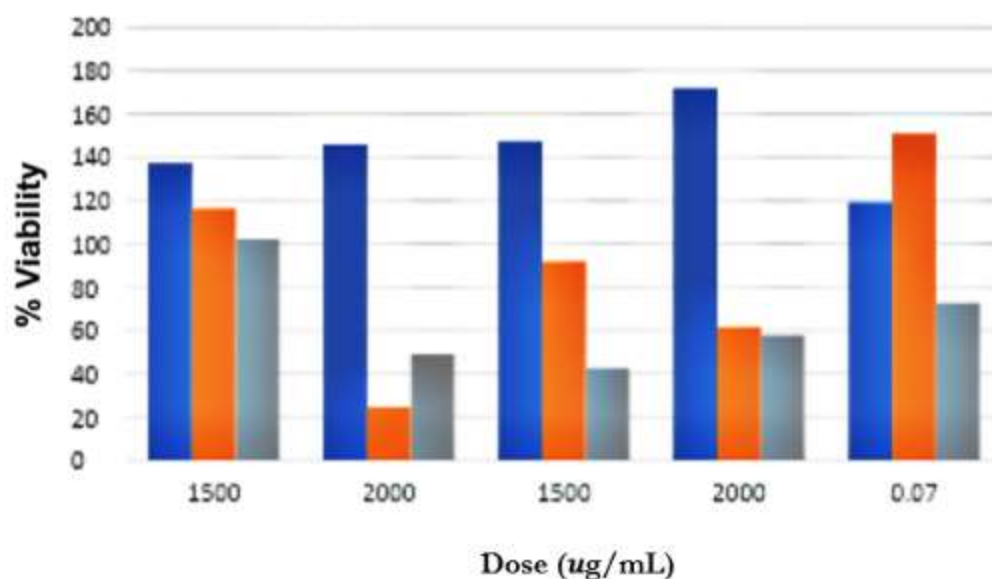


Figure 6. Effect of Bidara and Ciplukan Leaf Extracts, and Prayer Water on the Viability of MCF-7 Cell

Discussion and Implications. The comprehensive analysis of data presented in Tables 3–5 and Figures 3–6 reveals compelling evidence for the significant cytotoxic effects of Bidara (*Ziziphus mauritiana*) and Ciplukan (*Physalis angulata*) extracts against MCF-7 breast cancer cells, both as independent agents and in synergistic combinations. The observed time-dependent reduction in absorbance values strongly suggests these extracts' capacity to modulate cellular viability through potential apoptotic pathways or anti-proliferative mechanisms. Most notably, the combined treatment protocol, particularly when augmented with prayer water, demonstrated superior cytotoxicity compared to individual treatments, indicating a potentially powerful synergistic interaction.

The cytotoxic effects observed in our study align with previous research on *Ziziphus* species [37,38], it is reported that significant antiproliferative activity against various cancer cell lines. Similarly, our findings regarding *Physalis angulata* complement the work of Pillai et al. (2024) [39], who identified specific bioactive compounds responsible for its anticancer properties. The enhanced efficacy of the combined treatment suggests a novel therapeutic approach that merits further investigation within the broader context of natural product-based cancer therapeutics.

The molecular basis for the observed synergistic effects might be attributed to the diverse phytochemical profiles of both plants targeting multiple cancer-related pathways simultaneously. Bidara contains various bioactive compounds, including triterpenes and flavonoids, known for their anticancer properties [40,41]. Similarly, Ciplukan's physalins and withanolides have demonstrated significant antineoplastic activities in previous studies [42,43]. The combination of these distinct phytochemical profiles creates a multi-targeted approach to cancer cell elimination, potentially explaining the enhanced efficacy observed in our combination treatments.

The integration of prayer water into the treatment protocol represents a novel approach to bridging traditional healing practices with modern scientific methodology. This integration aligns with WHO's 'Traditional Medicine Strategy 2014-2023, which emphasizes the importance of incorporating traditional healing practices into modern healthcare systems [44,45]. Our findings demonstrate that such cultural integration not only respects traditional knowledge systems but may also enhance treatment outcomes. This approach could particularly benefit communities where traditional medicine plays a significant role in healthcare delivery, potentially improving treatment adherence and outcomes through culturally resonant therapeutic options.

The research contributes significantly to sustainable healthcare development by exploring accessible cancer treatment options using locally available plants. This aligns with global initiatives for developing cost-effective and culturally appropriate healthcare solutions, particularly relevant for regions where access to conventional cancer treatments may be limited. The successful demonstration of synergistic effects between these natural compounds suggests possibilities for reduced dosage requirements, potentially minimizing side effects while maintaining therapeutic efficacy.

However, several important considerations must be addressed before these findings can be translated into clinical applications. Detailed molecular studies are needed to elucidate the exact pathways involved in the observed cytotoxicity and to understand the specific cellular targets and signaling cascades underlying the synergistic effects. Furthermore, *in vivo* studies will be essential to validate these findings in animal models, followed by comprehensive pharmacokinetic and pharmacodynamic studies to determine optimal dosing strategies. The development of standardized extraction methods and quality control measures for ensuring consistent bioactive compound content represents another crucial step toward clinical application.

Our findings also highlight the potential for developing more comprehensive cancer treatment approaches that integrate natural products with existing therapeutic strategies. The demonstrated synergistic effects suggest possibilities for combination therapies that could enhance treatment outcomes while potentially reducing side effects commonly associated with conventional cancer treatments. This approach could be particularly valuable in developing personalized treatment strategies that consider both biological efficacy and cultural preferences.

Future research directions should focus on conducting comprehensive apoptotic assays and cell cycle analyses to better understand the mechanisms of action. Investigation of specific molecular targets and signaling pathways will be crucial for optimizing these treatments and predicting potential interactions with other therapeutic agents. Clinical trials will ultimately be necessary to evaluate therapeutic efficacy in human subjects and establish appropriate dosing protocols that maintain the observed synergistic benefits while ensuring patient safety.

This study provides substantial evidence for the anticancer potential of Bidara and Ciplukan extracts, particularly when used in combination and integrated with cultural practices such as prayer water. These findings not only contribute to the growing body of evidence supporting natural product-based cancer treatments but also demonstrate the value of integrating traditional medical knowledge with modern scientific approaches. This research provides a foundation for developing accessible, culturally appropriate, and potentially more effective cancer treatment strategies that could significantly impact global healthcare practices, particularly in regions where traditional medicine plays a crucial role in healthcare delivery.

4. CONCLUSION

Bidara and Ciplukan leaf extracts demonstrated qualitative phytochemical constituents and DPPH radical-scavenging activity under the experimental conditions examined. The MTT experiments showed changes in MCF-7 cell viability following exposure to the individual extracts, Ciplukan combined with prayer-infused water, and the combined Bidara–Ciplukan treatment containing 7% prayer-infused water. The combined Bidara–Ciplukan treatment with 7% prayer-infused water produced a reported viability of 34.6% at 72 h at the 1,500 µg/mL condition. These findings provide preliminary *in vitro* evidence of differential or enhanced observed activity, but they do not establish pharmacological synergy or clinical anticancer efficacy. Further research using expanded dose-response designs, formal combination analysis, normal-cell controls, chemical characterization, mechanistic assays, and *in vivo* models is required before therapeutic implications can be considered.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

No human participants or animals were involved in this study. The study used an established MCF-7 human breast cancer cell line for *in vitro* experiments.

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