

In Vitro Antioxidant and Cytotoxic Activity of *Benincasa hispida* Fruit Extract Against MCF-7 Breast Cancer Cells: Assessment of Apoptosis and Intracellular ROS

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Abstract: *Background:* Plant-derived compounds that combine antioxidant action with specific cytotoxicity toward malignant cells continue to be of interest as breast cancer continues to be the most often diagnosed cancer and a major cause of cancer-related death among women globally. The traditionally used medicinal fruit *Benincasa hispida* (Cucurbitaceae), also referred to as ash gourd or wax gourd, is said to contain flavonoids, phenolics, and triterpenoid cucurbitacin's with antioxidant and anticancer properties. *Objective:* This study assessed the ethanolic *Benincasa hispida* fruit extract's (BHE) *in vitro* antioxidant, cytotoxic, anticancer, and apoptosis-inducing properties. *Methods:* DPPH and nitric oxide radical scavenging assays were used to measure antioxidant capacity; MTT assays were used to measure cytotoxicity in normal Vero cells and MCF-7 breast cancer cells; acridine orange/ethidium bromide (AO/EtBr) dual staining was used to measure apoptosis; and dichlorofluorescein diacetate (DCF-DA) staining was used to measure intracellular reactive oxygen species (ROS). *Results:* DPPH and nitric oxide radicals were scavenged by BHE in a concentration-dependent manner (IC₅₀ 64.3 and 73.16 µg/mL, respectively, compared to 27.74 and 34.18 µg/mL for ascorbic acid). With an IC₅₀ of roughly 568 µg/mL, BHE decreased Vero cell viability, but MCF-7 vitality decreased much more sharply (IC₅₀ = 38 µg/mL), resulting in a selectivity index of roughly 15.0 in Favor of the cancer cell line. Following treatment at the MCF-7 IC₅₀, AO/EtBr staining revealed a roughly 14-fold increase in apoptotic cells along with a noticeable increase in DCF-DA fluorescence, which indicated enhanced intracellular ROS. *Conclusion:* BHE demonstrated moderate free-radical scavenging activity and greater cytotoxicity toward MCF-7 cells than Vero cells, accompanied by apoptosis-like morphological changes and increased intracellular ROS-associated fluorescence and supports additional bioassay-guided fractionation, mechanistic, and *in vivo* assessment of its potential as a breast cancer chemo preventive or adjuvant drug.

Keywords: *Benincasa hispida*, MCF-7, Vero cells, apoptosis, reactive oxygen species

1. Introduction

One of the leading causes of cancer-related mortality in women worldwide, breast cancer is the most common cancer among women[1]. Despite the clinical benefit of surgery, radiotherapy, chemotherapy and hormonal therapy, drug resistance, disease recurrence and high levels of systemic toxicity continue to restrict the clinical usefulness of these treatments and the discovery of safer, more selective and mechanistically informative therapeutic leads from natural sources remains a pursuit. The redox state of cells is in a paradoxical position in this search: cancer cells often operate at a redox level that is higher than what will lead to further increase of ROS that will result in DNA damage, mitochondrial dysfunction and apoptotic cell death. Chronically high levels of ROS and weakened antioxidant defences play a role in the onset and development of malignancy.[2] Pharmacologists are especially interested in compounds that selectively enhance pro-oxidant, apoptosis-inducing effects in transformed cells and have moderate radical-scavenging activity at low concentration. This duality is observed with many extracts of plants as a function of

concentration. *Benincasa hispida* (Thunb.) Cogn. Ash gourd (Cucurbitaceae), also known as ash gourd, wax gourd, winter melon and white pumpkin, is one of the popular vegetables which has been used in traditional medicine systems including Ayurvedic medicine, for various diseases, such as inflammatory, psychological, urinary and haematological disorders, for a long time. Phytochemical analysis of the fruit, seed and peel has demonstrated that it has the following main constituents responsible for its purported antioxidant, anti-inflammatory and cytoprotective properties: flavonoids, phenolic acids, triterpenoids (including the cucurbitacins), glycosides, and sterols. Methanolic seed extract exhibited dose dependent DPPH and hydrogen peroxide scavenging while several *B. hispida* components have been proven to possess antioxidant activity[3]. The aqueous fruit extract scavenged DPPH radicals in a zebrafish neuropharmacology investigation.[4] Both the ethanolic peel extract from the flowers and showed strong inhibitory activity against DPPH, ABTS and lipid peroxidation.[5] *B. hispida* extracts and fractions have been shown to exhibit specific cytotoxic- and apoptosis-inducing activities against breast cancer cell line (MDA-MB-231) associated with cell cycle arrest at S-phase

Volume 15 Issue 10, October 2026

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and intracellular ROS regulation, separately[6]. The anticancer effects are consistent with those previously reported in these cells for the unique Cucurbitaceae triterpenoid, cucurbitacin B, which induces ROS-dependent DNA damage and autophagy in the same cell line and blocks the proliferation of MCF-7 cells by disrupting microtubules and blocking the G2/M transition[7,8]. Despite this body of evidence, the majority of previous research has focused on either the antioxidant capacity or the anticancer activity of a particular plant part or extract in isolation. Relatively few studies have combined radical-scavenging assays with parallel cytotoxicity screening against a cancer cell line and a normal (non-cancerous) cell line, along with fluorescence-based confirmation of apoptosis and its associated oxidative-stress marker, specifically for an ethanolic *B. hispida* fruit extract. Before such an extract may be considered for bioassay-guided extraction of its active principle or principles or for translational development, it is important to establish this differential (cancer versus normal cell) cytotoxic profile and link it mechanistically to apoptosis and ROS formation. Therefore, the current study was created to assess an ethanolic extract of *Benincasa hispida* fruit is antioxidant (DPPH and nitric oxide scavenging), cytotoxic (Vero cell), anticancer (MCF-7 cell), and apoptosis/ROS-associated (AO/EtBr and DCF-DA staining) activities in an integrated *in vitro* panel.

2. Materials and Methods

Collection and Extraction of Plant Material

Whole fruits of *Benincasa hispida* were collected in January 2026 from local market of Rajapalayam, Tamilnadu and private farm in Muhavur, Tamilnadu and authenticated by Dr. Murugan Assistant Professor, Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi. For washing the fruits, distilled water was used to remove the attached sand and dust and then the fruits were cut in small pieces and dried in a solar dryer (Saravanan Solar Dryer Srivilliputhur). The dried material was further ground to a powder and screened through sieve no. 40. The powder was cold macerated with 1000 mL of ethanol (with continuous stirring) for 72 h at room temperature ($25 \pm 2^\circ\text{C}$) three times and the pooled extract was filtered on muslin cloth and Whatman filter paper. The filtrate was concentrated by vacuum evaporation and the dried *Benincasa hispida* extract (BHE) was kept in a tightly closed container at room temperature until further experiments.[9]

In Vitro Antioxidant Activity

DPPH, or free radical scavenging activity. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique was used to measure BHE is capacity to scavenge free radicals. BHE was added to 1 mL of DPPH solution at concentrations of 20–100 $\mu\text{g/mL}$, A 0.1 mM DPPH solution was prepared in ethanol. After 30 minutes, the absorbance at 517 nm was measured; a lower absorbance indicated more scavenging activity. Ascorbic acid served as the reference standard and was handled in accordance with the samples. A conventional method was used to calculate the IC₅₀ value and percentage inhibition of DPPH radicals.

Activity to scavenge the nitric oxide. Phosphate-buffered saline (PBS pH 7.4) was used to dissolve sodium nitroprusside ($\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$; 10 mM).[10] BHE was

tested at concentrations between 20 and 100 $\mu\text{g/mL}$ by mixing 1 mL of extract with 1 mL of sodium nitroprusside solution, then incubating for 150 minutes at room temperature in the presence of light. Next, 0.5 mL of the reaction mixture was added to 1 mL of the sulfanilamide reagent, and the combination was left to stand for 5 minutes. Next, 1 mL of the naphthyl ethylenediamine dihydrochloride (NEDD) reagent was added, and the mixture was incubated for another 30 minutes at room temperature. At 546 nm, the pink chromophore's absorption was measured. Ascorbic acid served as the standard, and a blank (no extract) was also tested concurrently. [11]

In Vitro Cytotoxicity Studies

culture and cell line. In a humidified CO₂ incubator at 5% CO₂ and 37°C, Vero cells (National Center for Cell Sciences, Pune) were grown in DMEM containing 10% FBS, penicillin (100U/mL), and streptomycin (100 $\mu\text{g/mL}$).

MTT assay. When confluent, cells were exposed to different concentrations of BHE for 24 h and then washed with PBS (pH 7.4) or serum-free DMEM and MTT solution (5 mg/mL) was added at 100 μL and plates were incubated for 4 h. Absorbance at 570 nm was measured after dissolving Formazan crystals in 1 mL DMSO per well.[12] Cell viability was determined by calculating percentage viability: % cell viability = (A₅₇₀ treated/A₅₇₀ control) x 100.

In Vitro Anti-Cancer Activity

culture and cell line. MCF-7 cells were grown in a DMEM medium with 10% FBS, 100U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin, 37°C, 5% CO₂, 95% air, and 100% humidity. The medium was changed twice a week.

Procedure. After 24 h the cells were treated with the various concentrations of BHE (7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 $\mu\text{g/mL}$, prepared in DMSO and diluted in serum-free medium) for 48 h, in triplicate, with medium alone serving as the control[12] MTT (5 mg/mL, 15 μL /well) was added after an additional 4 h, formazan crystals were solubilized in 100 μL DMSO and absorbance read at 570 nm.[13] Percentage cell inhibition was determined as follows: % cell inhibition = $100 - [\text{Abs}(\text{sample})/\text{Abs}(\text{control}) \times 100]$.

Apoptotic Activity

Dual staining with acridine orange and ethidium bromide (AO/EtBr). While EtBr only enters cells with damaged membranes (late apoptosis/necrosis) and produces red-orange fluorescence, AO penetrates all cells and produces green fluorescence in live cells with intact DNA and orange fluorescence in apoptotic cells with fragmented chromatin.[14] MCF-7 cells were seeded onto coverslips in 6-well plates and allowed to attach for 24 h. The cells were then treated with BHE at approximately the MCF-7 IC₅₀ concentration (38 $\mu\text{g/mL}$), fixed in methanol: glacial acetic acid (3:1) for 30 minutes at 37°C, washed with PBS, and stained with AO/EtBr (1:1; 100 μL of 1 mg/mL AO and 100 μL of EtBr in 10 mL PBS). After being cleaned with PBS, stained cells were examined under a fluorescent microscope.[15]

Dichlorofluorescein diacetate staining (DCF-DA). ROS oxidizes DCFH to fluorescent 2',7'-dichlorofluorescein (DCF;

excitation ~488-500 nm, emission ~525 nm), with the quantity of fluorescence directly correlated with the amount of ROS present. DCFH-DA enters cells and is converted by intracellular esterases to non-fluorescent DCFH. Before being examined by fluorescence microscopy, the MCF-7 cells ($\sim 5 \times 10^4$) were grown on coverslips in 6-well plates, cultured for 24 hours with BHE at the estimated concentration for MCF-7 IC₅₀ (approximately 38 $\mu\text{g/mL}$), washed with PBS (pH 7.4), fixed with 4% paraformaldehyde, and incubated for 30 minutes at 37°C in the dark with 10 μM H₂DCF-DA.[13]

3. Results

In Vitro Antioxidant Activity

DPPH radical scavenging. Ascorbic acid scavenged DPPH radicals from $45.2 \pm 1.2\%$ at 20 $\mu\text{g/mL}$ to $88.2 \pm 1.1\%$ at 100 $\mu\text{g/mL}$, while BHE scavenged DPPH radicals from $26.6 \pm 1.5\%$ at 20 $\mu\text{g/mL}$ to $67.2 \pm 1.3\%$ at 100 $\mu\text{g/mL}$. When compared to ascorbic acid (27.74 $\mu\text{g/mL}$), the IC₅₀ of BHE (64.3 $\mu\text{g/mL}$) was greater, indicating reduced but still good scavenging potency. (Table 1, Figure 1A).

Table 1: *In vitro* antioxidant (DPPH) activity of Benincasa hispida extract

S. No	Concentration ($\mu\text{g/mL}$)	Absorbance BH extract	% Inhibition BH Extract	% Inhibition Ascorbic acid
1.	20	0.624 ± 0.018	26.6 ± 1.5	45.2 ± 1.2
2.	40	0.535 ± 0.015	37.1 ± 1.3	57.6 ± 1.1
3.	60	0.448 ± 0.017	47.3 ± 1.4	68.4 ± 1.3
4.	80	0.342 ± 0.014	59.8 ± 1.2	79.1 ± 1.3
5.	100	0.279 ± 0.016	67.2 ± 1.3	88.2 ± 1.1
IC ₅₀	IC ₅₀	IC ₅₀	64.3 $\mu\text{g/mL}$	27.74 $\mu\text{g/mL}$

Nitric oxide scavenging. A similar pattern was observed for nitric oxide scavenging: BHE inhibition rose from $23.3 \pm 1.4\%$ at 20 $\mu\text{g/mL}$ to $63.3 \pm 1.3\%$ at 100 $\mu\text{g/mL}$, versus $41.2 \pm 1.2\%$ to $86.1 \pm 1.1\%$ for ascorbic acid. BHE had an IC₅₀ value of 73.16 $\mu\text{g/mL}$ while that of ascorbic acid was 34.18 $\mu\text{g/mL}$. (Table 2, Figure 1B).

Table 2: *In vitro* antioxidant (nitric oxide) activity of Benincasa hispida extract

S. No	Concentration ($\mu\text{g/mL}$)	% Inhibition BH Extract	% Inhibition Ascorbic acid
1.	20	23.3 ± 1.4	41.2 ± 1.2
2.	40	32.0 ± 1.3	53.6 ± 1.1
3.	60	44.4 ± 1.2	65.8 ± 1.3
4.	80	52.9 ± 1.1	76.4 ± 1.2
5.	100	63.3 ± 1.3	86.1 ± 1.1
IC ₅₀	IC ₅₀	73.16 $\mu\text{g/mL}$	34.18 $\mu\text{g/mL}$

In Vitro Cytotoxicity (Vero Cell Line)

Vero cell viability declined progressively with increasing BHE concentration, from 100% (control) to 91.78% at 7.8 $\mu\text{g/mL}$, 78.18% at 31.2 $\mu\text{g/mL}$, 58.02% at 250 $\mu\text{g/mL}$ and 44.65% at 1000 $\mu\text{g/mL}$. The concentration-dependent decrease indicates relatively lower cytotoxicity toward Vero cells at the lower concentrations tested. (Table 3, Figure 2).

Table 3: Cytotoxic effect of Benincasa hispida extract on the Vero cell line

S. No	Concentration ($\mu\text{g/mL}$)	Absorbance (O.D)	Cell viability (%)
1	1000	0.511	44.65
2	500	0.588	51.20
3	250	0.665	58.02
4	125	0.742	64.78
5	62.5	0.819	71.36
6	31.2	0.896	78.18
7	15.6	0.973	85.94
8	7.8	1.050	91.78
9	Cell control	1.146	100

With cell viability ranging from 91.78% at 7.8 $\mu\text{g/mL}$ to 44.65% at 1000 $\mu\text{g/mL}$, BHE was comparatively less hazardous to Vero cells. In Vero cells, the IC₅₀ for BHE was calculated to be around 568 $\mu\text{g/mL}$. The calculated selectivity index (SI) (Vero IC₅₀/MCF-7 IC₅₀) was approximately 15.0, indicating that MCF-7 cells are significantly more sensitive to BHE than Vero cells.

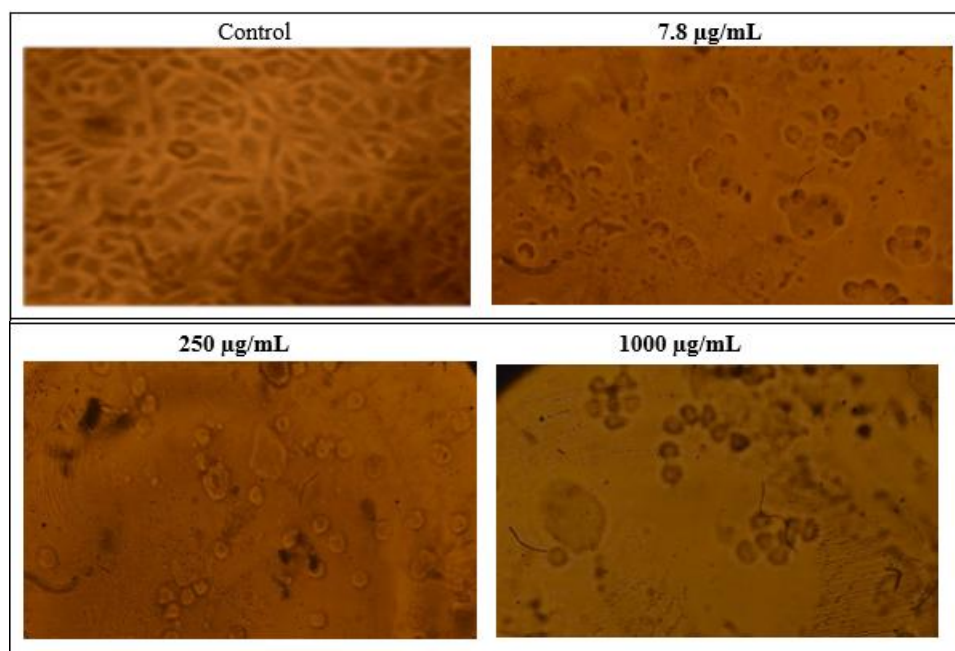


Figure 2: BHE is cytotoxic effects on Vero cells at 7.8 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$, and 1000 $\mu\text{g/mL}$

In Vitro Anticancer Activity (MCF-7 Cell Line)

MCF-7 viability declined more sharply than Vero viability across the same concentration range, from 100% (control) to 63.34% at 7.8 µg/mL, 51.57% at 31.2 µg/mL, 33.66% at 250 µg/mL and 21.89% at 1000 µg/mL. In all matched concentrations, the viability of MCF-7 cells was less than that of Vero cells, suggesting increased cytotoxic sensitivity of the MCF-7 cancer cell model when compared with the Vero cell model. (Table 4, Figure 3).

BHE cytotoxically affected MCF-7 cells in a concentration-dependent manner, reducing cell viability from 63.34% at 7.8 µg/mL to 21.89% at 1000 µg/mL. The IC₅₀ value of BHE against MCF-7 cells was determined using the concentration–response curve to be ~38 µg/mL. The calculated selectivity index (SI) (Vero IC₅₀/MCF-7 IC₅₀) was approximately 15.0, indicating that MCF-7 cells are significantly more sensitive to BHE than Vero cells

Table 4: Anticancer (cytotoxic) effect of Benincasa hispida extract on the MCF-7 cell line

S. No	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1	1000	0.132	21.89
2	500	0.167	27.69
3	250	0.203	33.66
4	125	0.240	39.80
5	62.5	0.276	45.77
6	31.2	0.311	51.57
7	15.6	0.346	57.37
8	7.8	0.382	63.34
9	Cell control	0.603	100

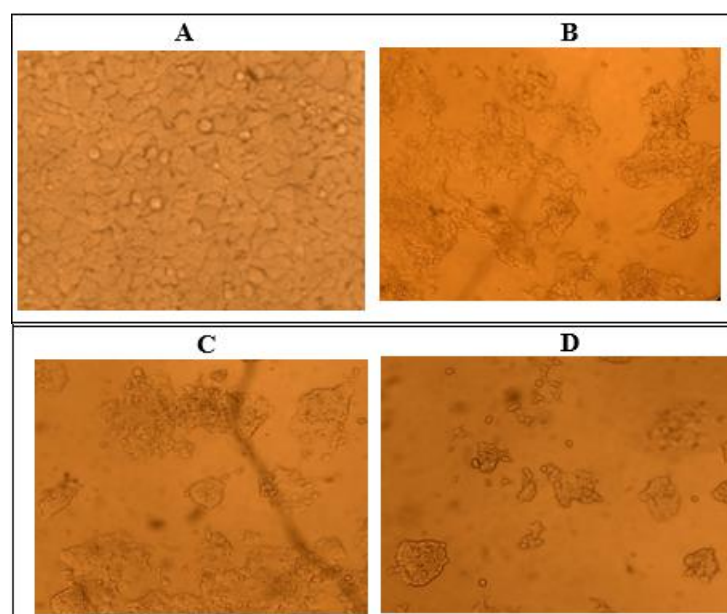


Figure 3: Anticancer effect of BHE on MCF-7 cells (A. control, B.7.8, C.31.2 and D.1000 µg/mL)

In Vitro Apoptotic Activity

AO/EtBr dual labelling was used to measure the number of cells undergoing apoptosis, and the results showed a considerable increase from 5 ± 2 apoptotic cells per 100 cells (control) to 73 ± 3 apoptotic cells per 100 cells (treatment) (almost a 14-fold increase) (Table 5, Figure 4). Following this, the treated cells' DCF-DA fluorescence intensity increased in comparison to the control. The concurrent increase in DCF-DA fluorescence and AO/EtBr-positive cells

suggests an association between increased intracellular ROS and apoptosis-like cell death. (Fig. 5).

Table 5: Apoptotic cell counts by AO/EtBr dual-staining technique

S. No	Technique	Control	Treated with BHE
1	Ao/EtBr Staining	5±2	73±3

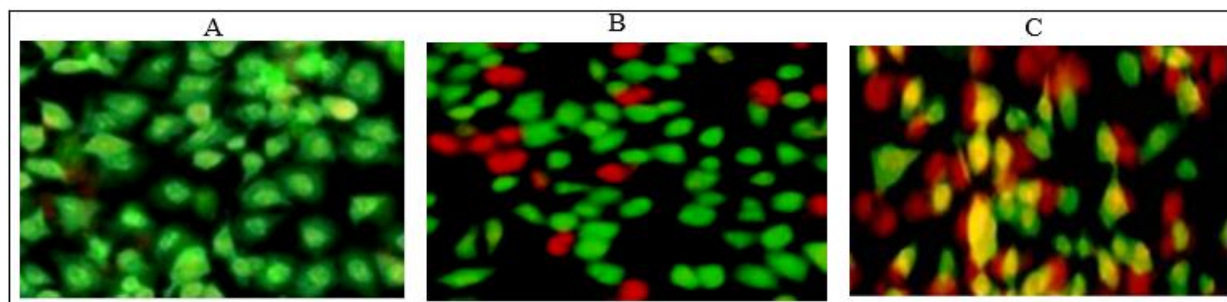


Figure 4: AO/EtBr dual staining of MCF-7 cells. (A) Control cells showing predominantly green fluorescence; (B) BHE-treated cells showing increased orange/red fluorescence and apoptotic morphological changes; (C) representative higher-magnification field, if applicable

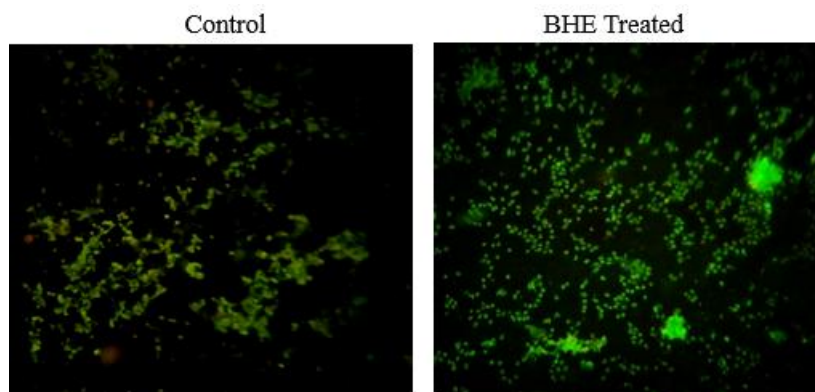


Figure 5: DCF-DA staining of MCF-7 cells — control versus BHE-treated, showing increased ROS-associated fluorescence

4. Discussion

The current results provide evidence that the ethanolic fruit extract of *Benincasa hispida* (BHE) possesses concentration-dependent free-radical scavenging activity, highly cytotoxic effect on MCF-7 breast cancer cells compared to normal Vero cells and the ability to induce apoptosis and ROS production, which all indicate a selective anticancer action via a redox linked mechanism. Despite the difficulties in comparing the results of different investigations due to variations in plant component, solvent and antioxidant reference standard used, the DPPH and nitric oxide scavenging values (IC_{50} 64.3 and 73.16 $\mu\text{g/mL}$ respectively) falls within the range of antioxidant activity previously reported for this species. An aqueous fruit extract has been found to inhibit DPPH at 300 $\mu\text{g/mL}$ by 79.8% [3]. DPPH scavenging in the same low-to-mid micromolar range was achieved by methanolic seed extract [11]. The ethanolic peel extract containing high flavonoids and phenolics was strongly inhibitory with both DPPH and ABTS assays [9]. The scavenging activity of the current ethanolic fruit extract was expected as for any crude extract and was less potent than ascorbic acid at the same concentration, but the moderate but repeatable scavenging activity of the current extract was consistent with literature and most likely attributed to its flavonoid and phenolic content. The most significant observation of this study is the difference in cytotoxicity found between the Vero cells (IC_{50} = 568 $\mu\text{g/mL}$) and the MCF-7 cells (IC_{50} = approximately 38 $\mu\text{g/mL}$) which gave a selectivity index of approximately 15.0. Selective activity is generally considered to be at least two (IC_{50} of normal cells/ IC_{50} of cancer cells). These selectivity indices are similar to those used elsewhere as criteria for a selectively cytotoxic plant extract [16]. At the same time, a methanolic *Elaeis guineensis* extract tested in the same

combination of MCF-7 and Vero cells showed a similar preferential cytotoxicity against the cancer cell line, despite having a lower absolute MCF-7 IC_{50} value (15 $\mu\text{g/mL}$) than the Vero IC_{50} value (22 $\mu\text{g/mL}$) [16]. The MCF-7 IC_{50} was also lower than the IC_{50} recently reported for an aqueous B (142 $\mu\text{g/mL}$), and is therefore more potent. The seed extract of *Hispida* has been shown to have anti-tumor properties in breast cancer cells that are triple-negative [13]. That the anti-cancer activity of *B. hispida* preparations is significantly affected by the solvent used and the part of the plant used, ethanolic fruit extract used in this study was found to have favourable results in this regard. These data suggest a plausible molecular basis for this selective cytotoxicity, namely, ROS and apoptosis. The combined effect of both DCF-DA fluorescence and the AO/EtBr positive apoptotic cells show that DCF-DA was induced in the death of MCF-7 cells, which does not appear to be a direct necrosis pathway. This pattern is similar to mechanistic studies of a pure form of cucurbitacin B, a unique triterpenoid found in Cucurbitaceae, which affects the polymerization of microtubules, the induction of G2/M arrest and apoptosis in MCF-7 cells, as well as the induction of autonomous autophagy and ROS-dependent DNA damage in MCF-7 cells [8]. The current study did not find or measure the particular compounds responsible, but it is believed that the cucurbitacin type triterpenoids reported in *B. hispida* could play part in the cytotoxic and ROS-related activity. The direct contribution of their activities, however, could not be confirmed in the present study. This is because cucurbitacin's are known phytoconstituents of *Benincasa hispida*. Together, the findings demonstrate the context-dependent and concentration-dependent dual properties of the redox active phytochemicals, BHE being an antioxidant agent at low concentration in the DPPH and nitric oxide assays, and a pro-

oxidant agent at high concentration in the MCF-7 cell apoptosis assay, where it was found to increase ROS levels sufficiently to trigger apoptosis. The antioxidant/pro-oxidant effects have been demonstrated for a number of plant polyphenols and triterpenoids, where the antioxidant activity enables the normal cell metabolism and the pro-oxidant action is able to induce apoptosis in more vulnerable cancer cells. This behaviour is similar to that of ROS homeostasis to regulate cell fate, as described above[2]. This study has limitations which might impact the interpretation of the results. Because BHE is a crude extract with ethanol instead of pure, isolated molecule, the current data cannot be used to determine the relative contributions of specific ingredients (e.g. flavonoids versus cucurbitacin's) to the observed cytotoxic and antioxidant effects. Cytotoxicity and apoptosis were assessed in one breast cancer cell line (MCF-7) and one cell line (Vero) as a reference. mechanistic conclusions are based on morphological (AO/EtBr) and fluorometric (DCF-DA) endpoints and not molecular confirmation such as caspase activity, Bax/Bcl-2 expression and flow cytometry quantification of apoptosis and cell-cycle distribution. Since there is no *in vivo* evidence, it is uncertain whether the selective action observed in cell culture is reflected in a favourable therapeutic window in the whole organism. Future studies should include bioassay-guided fractionation and identification of the components responsible for the activity by chromatography/spectroscopy, particularly quantifying cucurbitacin-type triterpenoids; evaluation of the apoptotic mechanism by flow cytometry, caspase activity assays, and expression analysis of pro- and anti-apoptotic proteins; cytotoxicity testing in various breast cancer subtypes and other non-cancerous lines to validate the selectivity index established herein; and, finally, *in vivo* safety and efficacy. Based on these confirmatory studies, it can be concluded that the fruit extract of *Benincasa hispida* is a promising plant origin Chemo preventive or as an adjunct anticancer agent, considering the constant antioxidant activity, the favourable selectivity index and the potential apoptotic and pro-oxidant effects of the extracts on MCF-7 cells.

5. Conclusion

In the present study, a higher concentration-dependent free-radical scavenging activity was observed for BHE compared to normal vero cells, while a significant selective cytotoxicity towards MCF-7 breast cancer cells was observed, with a selectivity index of approximately 15.0. BHE selectively induced apoptosis in MCF-7 cells without causing any non-specific toxic effects, as the increase in apoptotic cells (as determined by AO/EtBr dual labelling) was approximately 14-fold and there was a corresponding increase in the DCF-DA fluorescence. The strength and direction of these effects is supported by the published phytochemical profile of *B. hispida*, and by the known ROS-dependent apoptosis-inducing activity of the unique cucurbitacin-type triterpenoids of this species. All of these findings suggest that BHE is a low concentration (LC) plant extract that has a protective antioxidant effect and, at higher concentrations (HC), it has a selective pro-apoptotic and pro-oxidant effect on malignant cells. This association is increasingly recognized as being pharmacologically desirable in future anticancer phytotherapies. The observed selectivity index in the present study surpassed the preliminary selective values

for cytotoxicity, further testing on other cancer and non-cancerous cell lines is warranted, and visible morphological and fluorometric indicators of apoptosis indicate that the fruit is a potential source for breast cancer chemo preventive and/or therapeutic leads. These results, however, should be viewed with caution as hypothesis generating as they have been obtained with a simple extract, in one cancer cell line, using endpoint measurements of morphology and fluorometry. Further logical steps required before the therapeutic potential of *Benincasa hispida* revealed by this work can be translated further include confirmation of the specific active constituent, molecular validation of the apoptotic pathway (caspase activation, Bax/Bcl-2 expression and flow cytometry), extension to other breast cancer cell lines and *in vivo* efficacy and safety studies. Given the above findings, the natural source of *Benincasa hispida* is worth further investigation in the context of its selectively cytotoxic anticancer activity, as an *in vitro* proof of this.

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