RESEARCH ARTICLE

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Evaluating the Efficacy of *Benincasa Hispida* Seed Extract in Inhibiting the Proliferation of a Human Triple Negative Breast Cancer Cell Line

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Abstract

Objective: This study aimed to investigate the possible therapeutic benefits of Benincasa hispida extract as an adjunctive treatment for breast cancer. While previous studies have highlighted the anticancer effects of various components of Benincasa hispida, including its fruit and leaves, there remains a paucity of research concerning the application of Benincasa hispida seeds in the context of cancer treatment, especially breast cancer. **Methods:** The cytotoxic impact of Benincasa hispida on breast cancer cell lines was assessed via in-vitro analysis employing the MTT assay. The apoptosis levels were assessed by applying the Annexin V assay. Furthermore, an analysis of the cell cycle and quantification of intracellular reactive oxygen species (ROS) were conducted. **Results:** The findings of this study demonstrated noteworthy anti-proliferative properties of the Benincasa hispida extract, highlighting its ability to induce apoptotic cell death and to cause cell cycle arrest at the S-phase. Furthermore, the extract exhibited antioxidant characteristics, influencing reactive oxygen species. The aforementioned attributes present significant advantages in the restoration of disrupted physiological mechanisms within breast cancer cells, thereby suggesting potential therapeutic benefits for the treatment of breast cancer. **Conclusion:** In summary, the findings indicate that Benincasa hispida extract presents a noteworthy potential as a supplementary strategy in the treatment of breast cancer. A more thorough examination of the active components and the intricate mechanisms of action is necessary. Such efforts may ultimately promote the advancement of secure and effective therapeutic strategies for the management of breast cancer.

Keywords: Breast cancer-Benincasa hispida- cell cycle- Apoptosis- Reactive Oxygen Species

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Introduction

Cancer, characterized by abnormal cell growth, poses a significant global health challenge with an increasing mortality rate. Breast carcinoma is distinguished as the most common malignancy affecting women globally [1,2], and it holds the position of the second leading cause of cancer-related deaths among females [3]. In India, it represents one-fifth of all female cancers and accounts for seven percent of global breast cancer occurrences. As of 2020, it had overtaken lung cancer to become the most commonly diagnosed cancer, with around 2.3 million new cases documented, accounting for 11.7% of all cancer cases [4]. Forecasts in the field of epidemiology indicate that by the year 2030, the number of global cases of breast carcinoma is projected to surpass two million [5]. The conventional approaches to managing breast carcinoma include radiation, chemotherapy, surgical intervention, and hormone therapy. Nevertheless, the effectiveness of chemotherapy agents is often undermined by obstacles including multi-drug resistance, recurrence of the disease, and significant side effects [3,6-8].

The selection of the most suitable treatment strategy is contingent upon various factors, such as the tumor type, disease stage, and the clinical status of the patient [9]. While chemotherapy continues to play a crucial role in the management of cancer, the considerable side effects and the development of resistance, particularly in hormone therapy, pose significant challenges [9,10]. Consequently, there is a growing fascination with alternative anticancer therapies that utilize naturally occurring substances and extracts characterized by their minimal toxicity [10,11]. Current pharmaceutical research focuses on the development of novel and effective anticancer therapies, particularly those derived from natural sources like plants and certain microbial species. These natural origins

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provide a diverse array of compositions, ecological traits, phytochemical profiles, and ethnopharmacological properties, making them essential in the formulation of anticancer medications.

Belonging to the Cucurbitaceae family, *Benincasa hispida* is known by a multitude of names, including winter melon, ash gourd, winter gourd, white pumpkin, wax gourd, white gourd, animal oil gourd, gourd melon, and Chinese watermelon. This vegetable crop is cultivated extensively. It holds considerable esteem in Asian countries, valued for its applications in both culinary and medicinal contexts.

[12,13]. Historically, the applications of *Benincasa hispida* have encompassed a diverse array of medicinal functions, including its roles as a laxative, diuretic, tonic, aphrodisiac, and cardiotonic agent. They were additionally utilized in the management of urinary stones, hematological conditions, psychological disorders including insanity, epilepsy, and schizophrenia, along with jaundice, dyspepsia, fever, and menstrual irregularities [14,15]. The plant is rich in phytochemicals such as alkaloids, flavonoids, tannins, glycosides, phenolic compounds, amino acids, steroids, triterpenoids, and saponins [16], showcasing a diverse pharmacological profile. The polyphenols present in *Benincasa hispida* exhibit significant antioxidant properties, providing a safeguard against a range of ailments including neurodegenerative disorders, cardiovascular diseases, cancer, liver diseases, and infectious diseases [17]. The tannins found in the plant demonstrate notable inhibitory effects on the activity of pancreatic lipase and the absorption of fats from the intestine [18]. *Benincasa hispida* is widely utilized in culinary applications, serving both as a vegetable and as an ingredient in confectionery production. Since antiquity, the fruit has been esteemed for its therapeutic properties, attributed to its diverse array of chemical constituents. The composition of these fruits is predominantly characterized by a variety of bioactive compounds, including triterpenoids, flavonoids, glycosides, saccharides, carotenes, vitamins, ß-sitosterol, uronic acid, n-triacontanol, tannins, and an array of amino acids [19-21].

The Sarangadhara Samhita, an esteemed Ayurvedic medical text, discusses the application of *Benincasa hispida* in the treatment of haemorrhage, especially in cases involving lung ulceration and pulmonary complications. Furthermore, the juice derived from the cortical section of the plant has been utilized in the management of diabetes [22]. This investigation explored the anticancer efficacy of an aqueous seed extract derived from *B. hispida*, specifically targeting breast cancer through the utilization of the MDA-MB231 cell line.

Materials and Methods

Authentication and standardisation of B. hispida (Aqueous) seed Extracts

The desiccated seed specimen of *B. hispida* was procured from a certified pharmacy and subsequently validated for its authenticity under the expert supervision of a botanist at the Agharkar Research Institute in Pune, culminating in the issuance of an authentication number [AUTH-23-161]. The aqueous extract was prepared, and the physicochemical analysis was conducted at Sudhatatva Pharmacy, located within Dr. Y Patil Ayurvedic College and Research Centre in Pimpri, Pune-18, in accordance with the guidelines outlined in API Volume 4. The extract underwent a thorough assessment concerning its color, aroma, flavor, and solubility. The analysis included assessments of pH, loss on drying, total ash content, and shelf life.

Extract preparation

In order to generate the aqueous extract through the Soxhlet method (Wation DB 50), 50 grams of *Benincasa hispida* (BH) seed powder were positioned within the thimble of the extractor. Following this, 250 ml of water was introduced into the round-bottom flask (RBF) and subjected to heating for a duration of approximately 6 to 7 hours. The liquid in its condensed form (water) descends from the condenser into the chamber of the Soxhlet extractor, thereby enabling the extraction of soluble constituents from the pulverized seeds. The extraction process ought to persist until the requisite concentration of extract is achieved, which may require multiple cycles. Meticulously detach the thimble that holds the extracted substance from the apparatus. The liquid obtained was subsequently subjected to filtration through filter paper to remove any solid particulates or contaminants. Introduce the filtered extract into a water bath maintained at 80°C to facilitate the acquisition of a dry extract.

Anti-cancer activity of B. hispida
Cell Culture

The human Triple Negative Breast cancer cell line (MDA-MB231) was obtained from the National Centre for Cell Science (NCCS) located in Pune, India. MDA-MB231 cells were cultured as a monolayer in DMEM (GibcoTM), enriched with 10% fetal bovine serum and 1% antibiotic (GibcoTM). The cell lines were cultivated and sustained at a temperature of 37 °C within a humidified environment enriched with 5% CO_2.

Assessment of Cytotoxicity

MDA-MB231 cells, at a density of 1×10^4 cells per well, were introduced into a 96-well plate and subjected to incubation at 37 °C for a duration of 24 hours. Following the incubation period, different concentrations of the aqueous extract of *B. hispida*, specifically 20, 40, 80, 160, 320, and 640 µg/mL, were introduced in triplicate and subjected to incubation at 37 °C for a duration of 24 hours. Subsequently, the assessment of cytotoxicity was conducted utilizing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) from Himedia. The media from the 96-well plates were removed, after which 20 µL of MTT dye (prepared at a concentration of 5 mg/mL in phosphate buffer saline (PBS), (GibcoTM)) was added to each well, followed by incubation at 37 °C for 4 hours. To facilitate the dissolution of the formazan crystals, 150 µL of dimethyl sulfoxide (DMSO) was introduced. A microplate reader (MultiSkan Go by Thermo Scientific)

facilitated the estimation of reduced MTT by quantifying the optical density (OD) at 570 nm [23].

Apoptosis analysis

The FITC Annexin V/Dead Cell Apoptosis Kit (Invitrogen-Molecular Probes®) was employed to perform an apoptosis assay, incorporating the IC_{50} values of BH (142µg/mL) and DOXO (52 µg/mL) [24].

Cell cycle analysis

The MDA-MB231 cells, whether subjected to treatment with BH and DOXO or left untreated, were harvested 24 hours subsequent to being rinsed with 1X PBS (Phosphate Buffered Saline) and subjected to trypsinization. To the cellular samples, 25 µL of RNase A (20 mg/mL from Invitrogen), 2 mM MgCl2 (from Sigma), and 5-10 µL of 100 µg/mL propidium iodide (from Invitrogen) were introduced. The cells were subsequently incubated at ambient temperature for a duration of 10 to 15 minutes prior to analysis utilizing a FACS Caliber (BD Bioscience) [25].

Estimation of generation of intracellular reactive oxygen species

Flow cytometry was employed to examine the generation of reactive oxygen species utilizing Dichloro-dihydro-fluorescein diacetate (DCFH-DA) [26]. In the subsequent experiment, MDA-MB231 cells were cultivated for a duration of 24 hours within 6-well plates. Upon achieving 70-80% confluence, the cells underwent treatment with BH and DOXO at their respective IC_{50} concentrations of BH (142 µg/mL) and DOXO (52 µg/mL), along with 10 µM DCFH-DA. Subsequently, they were incubated in a 5 mM PBS buffer for a duration of 2 hours. Flow cytometry utilizing the Beckman Coulter Cytomics FC 500 (kex = 495 nm and kem = 520 nm) was employed to quantify the fluorescence produced from the hydrolysis of DCFH-DA to DCFH [25].

Statistical analysis

All experiments were performed in triplicate, and the outcomes are presented as mean ± standard deviation. The analysis of the data was conducted utilizing GraphPad Prism 8, employing a "Two-way ANOVA" in conjunction with a suitable post-hoc test, with statistical significance established at $P < 0.05$.

Results

Benincasa hispida (Aqueous) seed Extracts Preparation

The total amount of 2.7 grams of BH aqueous extract was produced from 50.02 grams of seeds that were in a sticky or sticky-like state. A total of thirteen hours was necessary for the synthesis of the extract (Figure 1).

Physicochemical Examination

The physicochemical analysis revealed that all parameters conformed to the normal range as stipulated by the Ayurvedic Pharmacopeia of India (API) guidelines, demonstrating an absence of adulteration. The extract possesses a white hue, exhibits a distinctive aroma, has a sweet flavor profile, and is soluble in water. The drying loss of 3.48% suggests that the seeds possess an optimal moisture content, facilitating effective extraction and ensuring an extended shelf life. A pH of 6.09 guarantees a state of equilibrium, security, and possible medicinal uses. The total ash content of 1.78% signifies a high level of purity, whereas the acid-insoluble ash at 0.56% corroborates the presence of minimal siliceous impurities. The water-soluble extractive value of 28.13% indicates a substantial presence of bioactive compounds, including phenolics, flavonoids, tannins, and glycosides, thereby endorsing its application in aqueous-based therapies. The extractive value soluble in alcohol, quantified at 23.17%, indicates a notable abundance of bioactive compounds that are soluble in alcohol. The entirety of these findings is catalogued in Table 1.

Antiproliferative activity of Benincasa hispida on MDA-MB231 cell line

The antiproliferative effects of *Benincasa hispida* on the MDA-MB231 cell line are of considerable interest. The aqueous extract of BH was administered at concentrations of 20, 40, 80, 160, 320, and 640 µg/mL, alongside DOXO at concentrations of 5, 10, 20, 40, 50, and 60 µg/mL, to the MDA-MB231 cancer cell line. The cytotoxic impact of BH aqueous extract on the MDA-MB231 cell line exhibited a dose-dependent relationship, yielding an IC_{50} value of 142±7.54 µg/mL, in contrast to DOXO, which presented an IC_{50} value of 52±3.41 µg/mL (Figure 2).

Assessment of the influence of BH on the apoptotic process in MDA-MB231 cells

An analysis of apoptosis was conducted on both control and treated MDA-MB231 cells over a 24-hour period at the IC_{50} concentration of BH, which is 142µg/ml. Upon exposure to the IC_{50} of BH for a duration of 24 hours, 06.51±6.96 % of cells exhibited characteristics of early apoptosis, whereas 60.17±0.04 % of cells demonstrated features indicative of late apoptosis (Figure 3a). DOXO prompted early apoptosis in 21.2 ± 1.61% of cells at the IC_{50} value, while late apoptosis was observed in 37.56 ± 1.37% of cells after a duration of 24 hours (Figure 3b). Upon comparing the effects of BH and DOXO, it was revealed that BH exhibited a more pronounced late

Table 1. Physiochemical Analysis Results of *Benincasa hispida* Seed Aqueous Extract

Sr No	Parameter	Test Observation
1	Description	Colour-white, taste-sweet, Odour- characteristics
2	Solubility	Soluble in water
3	pH	6.09
4	Loss on Drying	3.48%
5	Total ash	1.78%
6	Acid insoluble ash	0.56%
7	Water soluble extractive	28.13%
8	Alcohol soluble extractive	23.17%

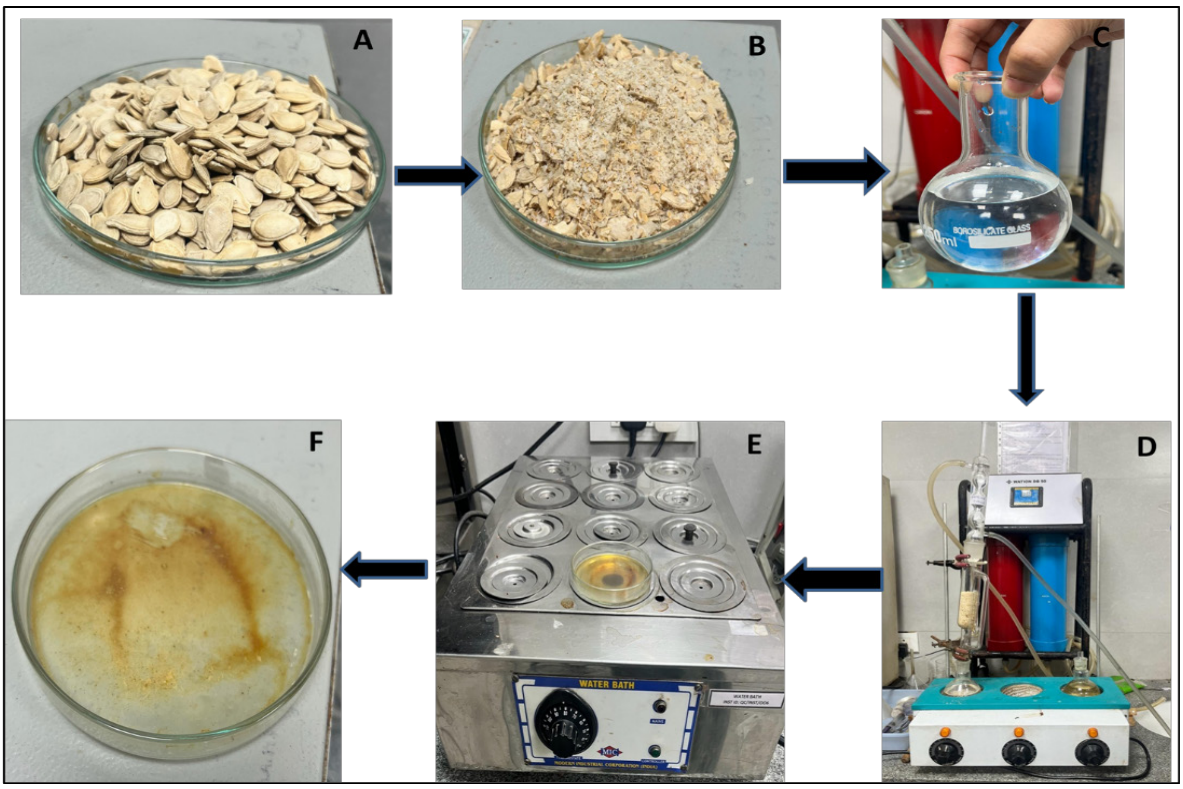

Figure 1. Images (A to F) Shows the Weight of Dry Seeds of BH, its aq. Extraction by Soxhlet Apparatus and Total Extract Obtained.

apoptosis effect than DOXO, while DOXO demonstrated a similar impact in both early and late apoptosis stages (Figure 3c).

Evaluation of the influence of BH on the regulation of the cell cycle in MDA-MB231 cells

The findings indicated that 58.7±6.59% of cells subjected to the BH IC_{50} concentration (142µg/mL) for a duration of 24 hours were halted in the S phase, whereas 9.67±3.74% were allocated to the G1 phase, and 22.0±8.7% were observed in the G2/M phase (Figure 4). DOXO resulted in the accumulation of 23.80±2.81% of cells in the G1 phase, 58.6±1.22% of cells in the S phase, and 17.0±2.35% in the G2/M phase after a 24-hour exposure at the IC_{50} concentration of 52 µg/mL. Ultimately, BH demonstrated cell cycle arrest during the S-phase at the IC_{50} concentration, with DOXO exhibiting analogous results to those of BH. The data presented above illustrates that BH has the capacity to function as an inducer of apoptosis, in addition to serving as a cell growth inhibitor or S-phase blocker in breast cancer.

Measurement of intracellular reactive oxygen species:

Initially, we assessed the distribution of fluorescence intensity both in the presence and absence of BH and DOXO. The administration of BH IC_{50} to MDA-

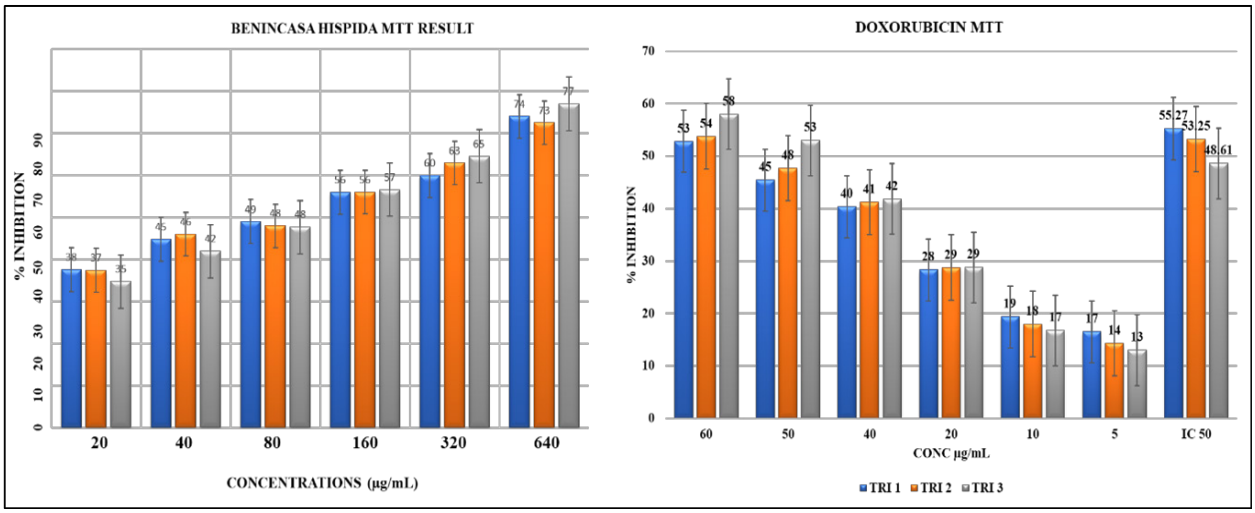

Figure 2. Percent Inhibition of MDA-MB231 by BH Aqueous Extract at Concentrations of 20-640 µg/mL and DOXO at Concentrations of 5 -60 µg/mL

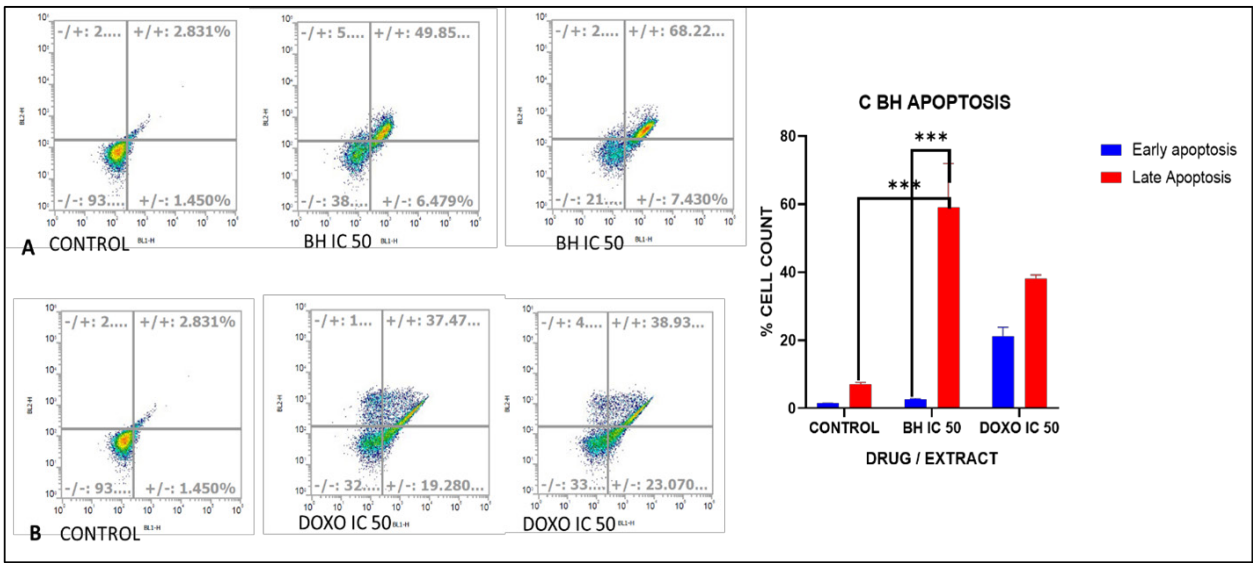

Figure 3. BH-Induced Apoptosis Using Flow Cytometry (A) It depicts cells treated with BH at IC_{50} (IC_{50} (142µg/ml) after 24 hours, and (B) depicts cells treated with DOXO at IC_{50} (52 µg/L) and after 24 hours. (C) Data represented as Mean+SD and, evaluating the apoptotic phases for the respective concentrations of BH and DOXO.

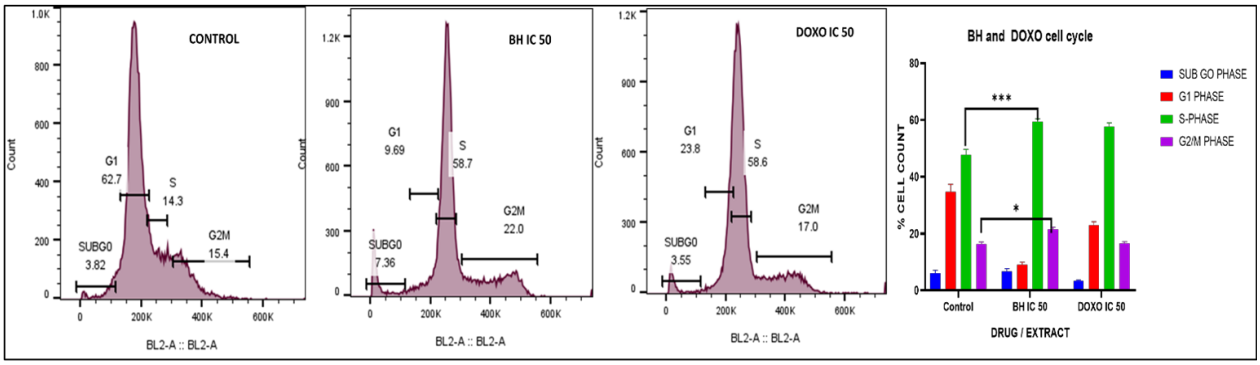

Figure 4. The Regulatory Effect of BH & DOXO on Cell Cycle Distribution in MDA-MB231. The cells were treated with specific concentration of BH & DOXO for 24 h. (A) Cell treated with BH at IC50 (142µg/ml) after 24 h, (B) represents the cells treated with DOXO at IC_{50} (52 µg/L) after 24 h. (C) Data presented as Mean+SD and analyzed, determining the different phases of cell cycle for the respective concentrations of BH and DOXO.

MB231 cells led to a notable reduction in intracellular ROS generation, while the application of DOXO IC_{50} values produced a modest decrease in intracellular ROS generation, suggesting that BH exhibits commendable antioxidant properties (Figure 5).

Discussion

Breast carcinoma is distinguished as the most common cancer affecting women worldwide [1,2], occupying the position of the second leading cause of cancer-related mortality among females. Nonetheless, the effectiveness of chemotherapy agents is often limited by obstacles such as multi-drug resistance, the recurrence of disease, and significant adverse effects [6,7]. As a result, a multitude of scholarly investigations are focusing on both conventional and alternative methodologies. *Benincasa hispida* is widely utilized in culinary applications, serving both as a vegetable and as an ingredient in confectionery manufacturing. The fruit boasts a lengthy history of medicinal use, owing to its diverse array of chemical components.

In their research, Abdullah et al. illustrated that the seed extract of *Benincasa hispida* exhibits inhibitory properties in scavenging activity, reduction of metallic elements, and beta-carotene bleaching assays. Furthermore, it was observed that the seed extract demonstrated the highest total phenolic content when compared to the skin and pulp extracts of *Benincasa hispida* [27]. This indicates that *Benincasa hispida* fruits may represent a valuable source of natural antioxidant compounds, potentially serving as an alternative to synthetic antioxidants [28].

Some studies indicate that the liquid and methanolic extracts of *Benincasa hispida* exhibit significant activity in a dose-dependent manner when compared to ascorbic acid. The pronounced eliminative efficacy of *B. hispida* liquid extract was observed at 87% concentration (100 µg/mL), while the alcoholic extract demonstrated a slightly higher efficacy at 88% concentration (100 µg/mL) [29,30]. The current investigation demonstrated anti-proliferative activity at a concentration of 142µg/mL. BH demonstrated anti-proliferative activity primarily through mechanisms

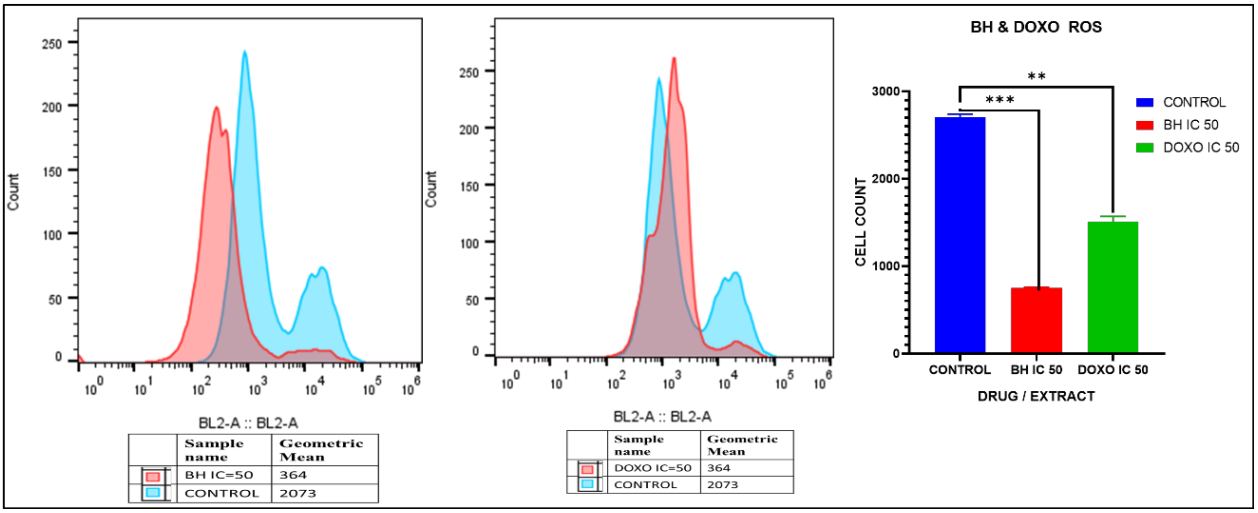

Figure 5. Effect of BH on Intracellular ROS Production, Determining Production of Intracellular ROS for the Respective Concentrations of BH and DOXO. (A) Cell treated with BH at IC_{50} (142µg/ml) after 24 h, (B) represents the cells treated with DOXO at IC_{50} (52 µg/L) after 24 h. (C) Data presented as Mean+SD and analyzed, determining the Intracellular ROS production for the respective concentrations of BH and DOXO.

associated with late apoptosis. BH predominantly exhibited cell cycle arrest at the S-phase.

Gill et al. investigated the capabilities of the methanolic extract of *Benincasa hispida* seeds (MEBH) in neutralizing free radicals, as well as its anti-inflammatory and analgesic effects. The evaluation of the free radical scavenging capacity of MEBH was conducted through the DPPH (1,1-diphenyl-2-picryl-hydrazyl) and hydrogen peroxide (H2O2) methodologies, demonstrating notable activity in a dose-dependent fashion when juxtaposed with ascorbic acid. MEBH demonstrated its peak radical scavenging activity of 79.8% at a concentration of 300 µg/mL, alongside an H2O2 scavenging effect of 63.7% at a concentration of 200 µg/mL. The evidence indicates that MEBH may function as a natural antioxidant in the management of inflammation and pain [31]. The findings presented here substantiate previous research, illustrating the antioxidant properties of BH through its capacity to diminish Reactive Oxygen Species (ROS).

Nadira Binte Samad and colleagues conducted an investigation into the effects of water extracts obtained from the dried seeds of *Benincasa hispida* on antioxidant activity, examining its relationship with the total phenolic and flavonoid contents in vitro. The researchers determined that the dried seeds of *Benincasa hispida* may serve as promising natural antioxidants within the food industry [32]. The results of these findings are consistent with those observed in our own study.

Hamouda et al. undertook a study aimed at evaluating the toxicity of biosynthesized AgNPs on the human cervical neoplastic cell line (HeLa) as well as on traditional human primary osteoblasts cell line. The characterization of the AgNPs was conducted through the application of UV–Vis spectrometry, dynamic light scattering (DLS), FTIR, and various microscopy techniques. Their findings underscored the therapeutic potential of AgNPs synthesized from *B. hispida*, attributed to their minimized side effects, indicating a range of possible therapeutic applications [33].

At concentrations between 10 and 1000µg/mL, proteins derived from the fruit, seed, and root demonstrated a cytotoxic effect on Artemia salina that was contingent upon the concentration levels. The median lethal concentration (LC50) values for the extracts of fruit, seed, and root were determined to be 44, 41, and 50 µg/mL, respectively [34]. Furthermore, this study demonstrated that the root proteins inhibited the proliferation of HeLa and K-562 cells by 28.50% and 36.60%, respectively. A further investigation revealed that the methanolic extract derived from the entire plant (5-50µg/mL) displayed cytotoxic effects on A. salina, yielding an LC50 value of 45.187µg/mL [35]. Furthermore, the aqueous seed extract (20-800µg/mL) exhibited no cytotoxic effects on HUVECs and normal fibroblast (NIH/3T3) cells. In a distinct study, the aqueous extract (1–20µg/mL) demonstrated a reduction in the activation of cell adhesion molecules by impeding monocyte adhesion, lowering Reactive Oxygen Species (ROS) levels, and inhibiting the nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) in high glucose (25mM) induced HUVECs cells [36].

In summary, this research indicates that *Benincasa hispida* seeds could represent a valuable adjunctive therapeutic avenue for breast cancer treatment. Additional investigation is necessary to explore the underlying mechanisms of action, possible adverse effects, and ideal dosage protocols of *Benincasa hispida* seed extract in relation to breast cancer therapy. This investigation may ultimately enhance the array of alternatives accessible to healthcare professionals and individuals facing this illness.

Author Contribution Statement

Monali Kanase: Methodology, Data curation, Software, Formal analysis, Vaibhav Ladke: Conceptualization, Formal analysis, Investigation, Validation, Writing

original draft. Nilima Dharkar: Methodology, Data curation, Formal analysis, Writing - review & editing. All authors approved the submitted manuscript.

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Conflict of interest

All authors declare that they have no known competing financial interests.

Abbreviations

ANOVA: Analysis of Variance
BC: Breast Carcinoma
BH: *Benincasa hispida*
DCFH: Dichlorodihydrofluorescein
DMSO: Dimethyl Sulfoxide
KEGG: Kyoto Encyclopaedia of Genes and Genomes
MTT: 3-(4,5-dimethythiazol-2-yl)-2,5- diphenyl tetrazolium bromide
ROS: Reactive Oxygen Species
TLC: Thin-layer chromatography

References

1. Li Y, Li S, Meng X, Gan RY, Zhang JJ, Li H Bin. Dietary Natural Products for Prevention and Treatment of Breast Cancer. Nutrients. 2017;9(7):728. https://doi.org/10.3390/NU9070728.
2. Iqbal A, Sharif M. BTS-ST: Swin transformer network for segmentation and classification of multimodality breast cancer images. Knowledge-Based Syst. 2023;267:110393. https://doi.org/10.1016/J.KNOSYS.2023.110393.
3. Kamaruzman NI, Tiash S, Ashaie M, Chowdhury EH. siRNAs Targeting Growth Factor Receptor and Anti-Apoptotic Genes Synergistically Kill Breast Cancer Cells through Inhibition of MAPK and PI-3 Kinase Pathways. Biomedicines. 2018;6(3):73. https://doi.org/10.3390/BIOMEDICINES6030073.
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. https://doi.org/10.3322/CAAC.21660.
5. sofi S, Mehraj U, Jan N, Almilaibary A, Ahmad I, Ahmad F, et al. Clinicopathological Significance and Expression Pattern of Bcl2 in Breast Cancer: A Comprehensive in silico and in vitro Study. Saudi J Biol Sci. 2024;31(2):103916. https://doi.org/10.1016/J.SJBS.2023.103916.
6. Chen L, Li CI. Racial disparities in breast cancer diagnosis and treatment by hormone receptor and HER2 status. Cancer Epidemiol Biomarkers Prev. 2015;24(11):1666–72. https://doi.org/10.1158/1055-9965.EPI-15-0293.
7. Ko EY, Moon A. Natural Products for Chemoprevention of Breast Cancer. J Cancer Prev. 2015;20(4):223–31. https://doi.org/10.15430/JCP.2015.20.4.223.
8. Singh SK, Singh S, Wlillard J, Singh R. Drug delivery approaches for breast cancer. Int J Nanomedicine. 2017;12:6205–18. https://doi.org/10.2147/IJN.S140325.
9. Li N, Wang Z, Zhang Y, Zhang K, Xie J, Liu Y, et al. Curcumin-loaded redox-responsive mesoporous silica nanoparticles for targeted breast cancer therapy. Artif Cells, Nanomedicine, Biotechnol. 2018;46(sup2):921–35. https://doi.org/10.1080/21691401.2018.1473412.
10. Sen GS, Mohanty S, Hossain DMS, Bhattacharyya S, Banerjee S, Chakraborty J, et al. Curcumin enhances the efficacy of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer. J Biol Chem. 2011;286(49):42232–47. https://doi.org/10.1074/JBC.M111.262295.
11. Bimonte S, Barbieri A, Palma G, Rea D, Luciano A, D'Aiuto M, et al. Dissecting the role of curcumin in tumour growth and angiogenesis in mouse model of human breast cancer. Biomed Res Int. 2015;2015:878134. https://doi.org/10.1155/2015/878134.
12. Nimbal S.K, Venkatrao N LS and PB. Anxiolytic evaluation of *Benincasa hispida* (Thumb) Cogn. fruit extracts. Int J Pharm Pharm Sci Res. 2011;1:93–7.
13. Zaini NAM, Anwar F, Hamid AA, Saari N. Kundur [*Benincasa hispida* (Thunb.) Cogn.]: A potential source for valuable nutrients and functional foods. Food Res Int. 2011;44(7):2368–76. https://doi.org/10.1016/J.FOODRES.2010.10.024.
14. Bishen Singh Mahendra Palsingh. Indian Medicinal Plants. 2 vol. 1975.
15. Jayasree T, Kishore KK, Vinay M, Vasavi P, Chandrasekhar N, Manohar VS, et al. Evaluation of the diuretic effect of the chloroform extract of the benincasa hispida rind (Pericarp) extract in guinea-pigs. J Clin Diagnostic Res. 2011;5(3):578–82.[16. Hussain I, Ullah R, Ullah R, Khurram M, Ullah N, Baseer A, et al. Phytochemical analysis of selected medicinal plants. African J Biotechnol. 2011;10(38):7487–92.
17. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A. 1993;90(17):7915. https://doi.org/10.1073/PNAS.90.17.7915.
18. Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H, et al. Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. Int J Obes (Lond). 2007;31(6):1023–9. https://doi.org/10.1038/SJ.IJO.0803502.
19. Nadkarni K. M . Indian Materia Medica. Vol 1. Bombay: Popular Prakashan; 1976.
20. Wollen weber E FR and GEM. A rare triterpene as major constitutes of the "wax" on fruits of *Benincasa hispida*. Indian Drugs. 1991;28:458–60.
21. Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J, Yoshikawa M. [Medicinal foodstuffs. XI. Histamine release inhibitors from wax gourd, the fruits of *Benincasa hispida* Cogn]. Yakugaku Zasshi. 1998;118(5):188–92. https://doi.org/10.1248/YAKUSHI1947.118.5_188.
22. Lee KH, Choi HR, Kim CH. Anti-angiogenic effect of the seed extract of *Benincasa hispida* Cogniaux. J Ethnopharmacol. 2005;97(3):509–13. https://doi.org/10.1016/J.JEP.2004.12.008.
23. Deng X, Yin F, Lu X, Cai B, Yin W. The apoptotic effect of brucine from the seed of Strychnos nux-vomica on human hepatoma cells is mediated via Bcl-2 and Ca2+ involved mitochondrial pathway. Toxicol Sci. 2006;91(1):59–69. https://doi.org/10.1093/TOXSCI/KFJ114.
24. Wyreogonekbska A, Gach K, Lewandowska U, Szewczyk K, Hrabec E, Modranka J, et al. Anticancer Activity of New Synthetic α-Methylene-δ-Lactones on Two Breast Cancer Cell Lines. Basic Clin Pharmacol Toxicol. 2013;113(6):391–400. https://doi.org/10.1111/BCPT.12120.

25. Changade J, Thanvi H, Raut C, Chavan M, Prasad P, Ladke V. In vitro Evaluation of Anti-Cancer Potential of Different Solvent Extracts Derived from Clerodendrum Infortunatum Linn against Cervical Cancer. Asian Pac J Cancer Prev. 2024;25(3):1065–75. https://doi.org/10.31557/APJCP.2024.25.3.1065.
26. Brandt R, Keston AS. Synthesis Of Diacetyldichlorofluorescin: A Stable Reagent For Fluorometric Analysis. Anal Biochem. 1965;11:6–9. https://doi.org/10.1016/0003-2697(65)90035-7.
27. Abdullah N, Wan Saidatul SWK, Samicho Z, Zulkifli KS, Aziman N. Study on antioxidant capacity and phenolic content of various parts of wax gourd (*Benincasa hispida*). World Appl Sci J. 2012;19:1051–6. https://doi.org/10.5829/idosi.wasj.2012.19.07.2900.
28. Kabra A, Parashar B, Badhani S, Kainth A. Evaluation of Antioxidant Activity of *Benincasa hispida* Fruit Extracts. Am J PharmTech Res. 2013;3.
29. Rana S, Suttee A. Phytochemical investigation and evaluation of free radical scavenging potential of *Benincasa hispida* peel extracts. Int J Curr Pharm Rev Res. 2012;3(3):43–6.
30. Alsaadi SARA, Abass KS. *Benincasa hispida* is an antioxidant of possible physiological importance: A comparative review. Plant Arch. 2020;20(2):2833–8.
31. Gill NS, Dhiman K, Bajwa J, Sharma P, Sood S. Evaluation of free radical scavenging, anti-inflammatory and analgesic potential of *Benincasa hispida* seed extract. Int J Pharmacol. 2010;6:652–7. https://doi.org/10.3923/IJP.2010.652.657.
32. Samad NB, Debnath T, Jin HL, Lee BR, Park PJ, Lee SY, et al. Antioxidant activity of *Benincasa hispida* seeds. J Food Biochem. 2013;37:388–95. https://doi.org/10.1111/j.1745-4514.2011.00643.x.
33. Hamouda RA, Hussein MH, Abo-elmagd RA, Bawazir SS. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica. Sci Rep. 2019;9(1):13071. https://doi.org/10.1038/S41598-019-49444-Y.
34. Darusman LK. Bioactive Proteins from *Benincasa hispida* (Thunb.) Cogn. Hayati Journal of Biosciences. 2009;16(4):161-4. https://doi.org/10.4308/HJB.16.4.161.
35. Ullah MO, Haque M, Urmi KF, Abu AH, Anita ES, Begum M, et al. Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. Asian Pac J Trop Biomed. 2013;3(1):1–7. https://doi.org/10.1016/S2221-1691(13)60015-5.
36. Moon MK, Kang DG, Lee YJ, Kim JS, Lee HS. Effect of *Benincasa hispida* Cogniaux on high glucose-induced vascular inflammation of human umbilical vein endothelial cells. Vascul Pharmacol. 2009;50(3-4):116–22. https://doi.org/10.1016/J.VPH.2008.11.007.