

Invitro antioxidant and cytotoxic potential of *benincasa hispida*: a narrative review with a comparative perspective on *punica granatum*

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Abstract—*Benincasa hispida* commonly known as ash gourd, wax gourd or winter melon, is a widely cultivated cucurbitaceous fruit with nutritional and traditional medicinal importance. Interest in *B. hispida* has expanded because its fruits, seeds, peel and other parts contain phenolic compounds, flavonoids and other phytochemicals associated with antioxidant and pharmacological effects. The source study reported that fresh *B. hispida* fruit juice had a total phenolic content of 654.6667 µg/ml, total flavonoid content of 27.75 µg/ml and 18.64% DPPH radical-scavenging activity. In the same study, fresh juice produced 100% mortality in the brine shrimp assay after 24 h, indicating cytotoxic potential in this preliminary model. This review reorganizes the available material into a review-article format and critically places these findings in the context of published evidence on *B. hispida*. For comparison, *Punica granatum* L. (pomegranate) is included as an additional antioxidant- and cytotoxicity-associated fruit. Pomegranate is rich in polyphenols including ellagitannins and anthocyanins and has demonstrated antioxidant, antiproliferative and pro-apoptotic effects in preclinical studies. The comparison highlights that *B. hispida* shows promising antioxidant and preliminary cytotoxic activity, whereas pomegranate has a substantially larger body of mechanistic and cancer-cell evidence. However, numerical antioxidant percentages and cytotoxicity values should not be interpreted as directly equivalent unless the plant material, extraction procedure, concentration and assay conditions are standardized.

Index Terms—*Benincasa hispida*; ash gourd; antioxidant activity; DPPH; cytotoxicity; brine shrimp lethality assay; *Punica granatum*; pomegranate; phytochemicals.

I. INTRODUCTION

Cytotoxicity describes the capacity of a substance or biological mediator to damage or kill living cells. In pharmacological and toxicological research, cytotoxicity testing is an important early step because it provides information about how a test material affects cell viability, growth, morphology and proliferation. The response may represent direct cell death or may reflect cytostatic effects in which cellular proliferation is suppressed. Different experimental models are therefore required to understand whether a plant-derived preparation is simply toxic at a particular concentration or possesses selective antiproliferative potential. Cytotoxicity assays are widely used during preliminary screening of natural products because they can identify extracts that merit further investigation in cancer-cell models and mechanistic studies.

Oxidative stress is closely linked to cellular injury and several chronic diseases. It develops when the generation of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defenses. Excessive ROS can damage lipids, proteins and nucleic acids and may contribute to inflammation, metabolic disorders, cardiovascular disease and carcinogenesis. Antioxidants can act by scavenging reactive radicals, chelating pro-oxidant metals, inhibiting oxidative chain reactions or supporting endogenous antioxidant systems. Plant foods are important sources of polyphenols, flavonoids, vitamin C and other compounds that may contribute to antioxidant activity. Consequently, the antioxidant properties of edible fruits have attracted considerable

interest in food science, nutraceutical research and pharmacology.

Benincasa hispida belongs to the family Cucurbitaceae and is commonly called ash gourd, wax gourd, winter melon or white pumpkin. It is an annual climbing plant cultivated extensively in tropical and subtropical regions. The fruit is consumed as food and has also been used in traditional systems of medicine. The source material describes *B. hispida* as having nutritional and medicinal significance and reports traditional uses of the fruit, rind, seeds and root. Published reviews have also described antioxidant, anti-inflammatory, diuretic, antidiabetic, analgesic and other pharmacological activities associated with different parts of the plant.

Among the most relevant biological properties of *B. hispida* is its antioxidant potential. Studies summarized in the source material indicate that different parts of the fruit contain phenolic and flavonoid constituents and can show radical-scavenging or reducing activity. The source experimental study used Folin–Ciocalteu estimation for total phenolic content, an aluminium-chloride colorimetric method for total flavonoids and the DPPH assay for free-radical scavenging. The fresh juice showed a total phenolic content of 654.6667 µg/ml and total flavonoid content of 27.75 µg/ml. Its DPPH antioxidant activity was reported as 18.64%. These findings provide a useful preliminary indication of antioxidant capacity, although assay values are dependent on extraction conditions, reference standards, concentration and experimental design.

The potential cytotoxicity of edible plants is also relevant to natural-product research, particularly because some plant metabolites can interfere with cell proliferation, induce apoptosis or alter signaling pathways. However, preliminary lethality assays should not be equated directly with anticancer efficacy. The source study used the brine shrimp lethality assay and reported 100% mortality of nauplii following exposure to fresh *B. hispida* juice for 24 h. Brine shrimp lethality is useful as an inexpensive preliminary toxicity screen, but it does not identify cancer selectivity or establish a molecular mechanism. Confirmation therefore requires assays using validated cancer cell lines, appropriate normal-cell controls, concentration-response analysis and, ideally, mechanistic endpoints such as apoptosis, cell-cycle distribution and molecular signaling.

Natural fruits with both antioxidant and cytotoxic or antiproliferative properties provide an important area for pharmacological investigation. *Punica granatum* L. (pomegranate) is included in this review as a comparative fruit because it is particularly well documented for both activities. Pomegranate contains anthocyanins, ellagitannins and other polyphenols and has demonstrated antioxidant effects in chemical, cellular and clinical research. Preclinical literature also reports antiproliferative, anti-invasive, anti-metastatic and apoptosis-associated effects in several cancer models. This makes pomegranate a useful comparator for interpreting the current evidence for *B. hispida*, while recognizing that the two fruits have been investigated using different preparations and assay systems.

The purpose of this review is therefore to consolidate the relevant information on the phytochemical basis, antioxidant activity and preliminary cytotoxic potential of *B. hispida*; summarize the experimental findings reported in the supplied research paper; and compare these properties with those of *P. granatum*. The review also identifies methodological limitations and future research needs, particularly the requirement for standardized extracts, validated cytotoxicity models and mechanistic anticancer studies.

Benincasa hispida: botanical and pharmacological overview

Botanical profile

Benincasa hispida is an annual climbing plant of the Cucurbitaceae family. The supplied paper lists the taxonomy as Kingdom Plantae, Phylum Tracheophyta, Class Magnoliopsida, Order Cucurbitales, Family Cucurbitaceae, Genus *Benincasa* and Species *Benincasa hispida*. The fruit is known by several common names including ash gourd, wax gourd and winter melon. The source material reports that the plant grows under warm conditions, prefers well-drained soil and can be cultivated in tropical and subtropical environments.

Traditional and reported medicinal uses

The supplied paper describes traditional uses involving the fruit, rind, seeds and root. The rind is described as diuretic, while the seeds are reported to possess anthelmintic, anti-inflammatory, demulcent, diuretic and expectorant properties. The fruit is traditionally associated with antiperiodic, aphrodisiac,

diuretic, laxative and tonic effects. Modern reviews have additionally summarized antioxidant, anti-inflammatory, analgesic, antidiabetic, nephroprotective, hypolipidemic and antimicrobial activities. These activities should be considered as reported pharmacological evidence rather than proof of clinical efficacy.

Phytochemical basis of antioxidant activity

Phenolic compounds and flavonoids are important contributors to the antioxidant behavior of plant-derived foods. In *B. hispida*, published investigations have evaluated antioxidant capacity in the fruit, skin and seeds using radical-scavenging and reducing assays. The supplied study quantified total phenolics using gallic acid as the reference standard and total flavonoids using quercetin as the reference standard. The reported values for fresh fruit juice were 654.6667 µg/ml total phenolics and 27.75 µg/ml total flavonoids. The source paper also describes free-radical scavenging and potential effects on lipid peroxidation and antioxidant enzymes as relevant biological features of *B. hispida*.

The DPPH assay is a chemical free-radical scavenging method in which reduction of the DPPH radical is reflected by a decrease in absorbance. In the supplied experiment, the control absorbance was 1.362 and the sample absorbance was 1.108 at 517 nm. Using the equation $[(AC-AS)/AC] \times 100$, the reported antioxidant activity was 18.64%. This value is best interpreted as activity under the exact conditions used in that experiment and should not be used as a universal potency value for *B. hispida*.

Cytotoxic potential of benincasa hispida

Plant-derived cytotoxicity is of interest because phytochemicals may affect cell survival, proliferation, oxidative balance and apoptosis. The supplied study evaluated fresh *B. hispida* juice using the brine shrimp lethality assay. Thirty nauplii were distributed into three tubes, with ten nauplii per tube, and exposed to a preparation containing fresh juice and saline. After 24 h, the study reported 100% mortality. The finding

supports the presence of preliminary cytotoxic or general toxic activity in the tested preparation.

Importantly, brine shrimp lethality is a screening assay rather than a cancer-specific test. A high mortality rate does not demonstrate selective killing of malignant cells, nor does it establish a therapeutic window. Future work should therefore investigate *B. hispida* extracts using standardized concentrations and human cancer cell lines, alongside non-malignant cells. Appropriate endpoints could include MTT/SRB or equivalent viability assays, apoptosis markers, caspase activation, mitochondrial membrane potential, ROS generation and cell-cycle analysis.

Punica granatum as an additional antioxidant and cytotoxic fruit

Punica granatum L, commonly known as pomegranate, is included as the additional fruit requested for this review. It is a polyphenol-rich fruit containing anthocyanins, ellagitannins and hydrolysable tannins. Reviews and experimental studies have consistently described antioxidant activity associated with pomegranate juice, peel and other fruit fractions. Pomegranate preparations have been evaluated by DPPH and other radical-scavenging methods, and clinical trial meta-analysis has reported improvements in selected oxidative-stress biomarkers following pomegranate consumption.

Evidence for cytotoxic or antiproliferative activity is also extensive, although results depend strongly on the plant part, extraction solvent, concentration and cancer model. Pomegranate extracts and constituents such as punicalagin and ellagic acid have been associated with inhibition of cancer-cell proliferation, induction of apoptosis, cell-cycle effects and modulation of signaling pathways including NF-κB and PI3K/AKT/mTOR. Primary studies have also reported antioxidant and antitumor activity of pomegranate-derived fractions. These findings provide a stronger mechanistic evidence base than the preliminary brine-shrimp result available for *B. hispida* in the supplied study.

Comparative analysis of benincasa hispida and punica granatum

Table 1. Qualitative comparison of antioxidant and cytotoxic evidence for *B. hispida* and *P. granatum*.

Parameter	Benincasa hispida	Punica granatum	Interpretation
Plant part / preparation	Fresh fruit juice in the supplied study; other fruit/seed/peel studies also reported	Juice, arils, peel and isolated fractions have been studied	Different preparations limit direct numerical comparison.
Phenolic compounds	High total phenolic content reported: 654.6667 µg/ml in the supplied study	Rich in ellagitannins, anthocyanins and other polyphenols	Both fruits have polyphenol-associated antioxidant potential.
Flavonoids	27.75 µg/ml reported in the supplied study	Flavonoids and anthocyanins contribute to antioxidant activity	Assay standards and units differ across studies.
Antioxidant assay evidence	DPPH activity reported as 18.64%	DPPH and multiple antioxidant assays have been reported; clinical studies also assessed oxidative-stress biomarkers	Pomegranate has broader evidence; percentages are not directly comparable without matched protocols.
Cytotoxicity model	Brine shrimp lethality; 100% mortality after 24 h in the supplied study	Cancer-cell studies report antiproliferative/cytotoxic effects in several models; some studies also use Artemia	Pomegranate has more cancer-specific mechanistic evidence.
Mechanistic evidence	Mechanism not established by the supplied brine-shrimp assay	Apoptosis, cell-cycle and signaling-pathway modulation reported in preclinical studies	<i>B. hispida</i> requires mechanistic validation.
Overall evidence	Promising preliminary antioxidant and cytotoxic activity	Broader preclinical evidence for antioxidant and anticancer potential	Neither should be considered an established anticancer treatment based on these data alone.

II. DISCUSSION

The supplied research paper provides preliminary evidence that *B. hispida* fresh fruit juice contains measurable phenolic and flavonoid constituents and can scavenge DPPH radicals. The reported 18.64% DPPH activity, together with the phenolic and flavonoid content, supports the presence of antioxidant-associated constituents. The reported 100% brine shrimp mortality indicates biological activity in a simple invertebrate toxicity model. Nevertheless, the current evidence is insufficient to conclude that *B. hispida* is an anticancer agent. A

review article should distinguish screening evidence from therapeutic evidence, particularly when cytotoxicity is measured in a non-cancer model.

In comparison, pomegranate has been investigated across a broader range of experimental systems. Published reviews describe antioxidant, anti-inflammatory, antiproliferative, anti-invasive and anti-metastatic properties, while individual studies have examined cancer-cell viability, apoptosis and molecular signaling. The greater depth of evidence for pomegranate does not mean that it is clinically established as a cancer treatment; rather, it indicates a more developed preclinical research base. The

comparison therefore suggests that *B. hispida* is a worthwhile candidate for further natural-product research, especially because it is an edible fruit with a history of traditional use.

Another important consideration is standardization. Total phenolic content, total flavonoid content and DPPH activity are affected by cultivar, maturity, storage, extraction solvent, temperature, sample concentration and assay protocol. Similarly, cytotoxicity varies according to cell type, exposure time, extract composition and concentration. Future comparative work should therefore use matched extraction procedures and standardized concentrations for both fruits. Such studies could determine whether *B. hispida* has selective cytotoxicity and whether its antioxidant-associated constituents contribute to the observed biological effects.

III. CONCLUSION

Benincasa hispida is a nutritionally important fruit with reported antioxidant and pharmacological properties. The supplied research study reported 654.6667 µg/ml total phenolic content, 27.75 µg/ml total flavonoid content, 18.64% DPPH radical-scavenging activity and 100% mortality in a brine shrimp cytotoxicity assay. These findings support further investigation but should be regarded as preliminary. *Punica granatum* provides a useful comparative example because its antioxidant and antiproliferative activities have been investigated more extensively, including mechanistic studies involving polyphenols, apoptosis and cancer-related signaling. Overall, *B. hispida* has promising research potential, but standardized and mechanistically informative in vitro, in vivo and eventually clinical studies are needed before conclusions about anticancer efficacy can be made.

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