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Phytochemical screening of the fruit extracts of *Manilkara zapota* and *Trichosanthes dioica* Roxb. and the cytotoxicity study on HEC-1 cancer cell line

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Abstract
Cancer continues to be one of the most significant health challenges worldwide, prompting an ongoing search for safer and more effective treatment options. While modern medicine has made remarkable strides in cancer therapy, the adverse effects of synthetic drugs highlight the need for alternative, plant-based treatments. Natural compounds derived from medicinal plants have gained increasing attention for their potential in cancer prevention and therapy. Among these, *Manilkara zapota* (commonly known as Sapodilla) and *Trichosanthes dioica* Roxb. (Pointed Gourd) have long been recognized in traditional medicine for their diverse pharmacological benefits. This study investigates their phytochemical composition, antioxidant potential, and anticancer effects on the HEC-1 endometrial cancer cell line.

Phytochemical screening of the fruit extracts of *M. zapota* and *T. dioica* confirmed the presence of key bioactive compounds such as flavonoids, tannins, saponins, alkaloids, phenolic compounds, and terpenoids. These compounds are known to possess significant medicinal properties, including antioxidant and anticancer activities. To evaluate their free radical scavenging ability, multiple antioxidant assays were conducted, including DPPH free radical scavenging, hydrogen peroxide scavenging, ferric thiocyanate assay, and xanthine oxidase inhibition. The results revealed that both plant extracts exhibited strong antioxidant activity, largely attributed to their rich flavonoid and polyphenol content. These antioxidants play a critical role in neutralizing free radicals, thereby reducing oxidative stress-a key contributor to cancer development.

To assess their anticancer potential, the cytotoxic effects of the fruit extracts were tested against the HEC-1 endometrial cancer cell line using the MTT assay. The findings demonstrated a dose-dependent inhibition of cancer cell growth, suggesting that increasing concentrations of the extracts enhanced cytotoxicity. This indicates that certain bioactive compounds in *M. zapota* and *T. dioica* may effectively suppress cancer cell proliferation. Further, molecular docking studies revealed that phytochemicals such as quercetin, catechin, and zapotin from *M. zapota*, along with carotenoids and flavonoids from *T. dioica*, interact with key cancer-related proteins, potentially disrupting cell survival pathways and promoting apoptosis (programmed cell death).

The significance of these findings extends beyond basic research, offering promising insights into the potential development of natural, plant-based therapeutic agents for cancer treatment. With increasing concerns over the side effects of conventional chemotherapy and radiation therapy, medicinal plants like *M. zapota* and *T. dioica* present a compelling alternative due to their bioavailability, minimal toxicity, and broad pharmacological spectrum. While the study provides strong preliminary evidence, further in-depth research, including *in vivo* studies and clinical trials, is necessary to validate these effects and explore their precise mechanisms of action.

In conclusion, *Manilkara zapota* and *Trichosanthes dioica* emerge as promising sources of natural antioxidants and potential anticancer agents. Their ability to mitigate oxidative stress and inhibit cancer cell growth underscores their medicinal value. As research progresses, these plants could play a crucial role in the development of novel, plant-derived therapies for cancer, offering a more holistic, safer, and potentially effective approach to disease management.

Keywords: *Manilkara zapota*, *Trichosanthes dioica*, antioxidants, phytochemicals, cancer cell inhibition, HEC-1 cell line, flavonoids, natural medicine, plant-based therapy, oxidative stress, molecular docking, mM=Millimole per litre

Introduction

1. Background and Importance of Natural Antioxidants in Medicine

Cancer remains one of the most pressing health challenges worldwide, with millions of new cases diagnosed annually. Despite significant advancements in cancer therapy, including chemotherapy, radiation, and targeted drug therapies, the adverse side effects and high costs of these treatments necessitate the exploration of alternative and complementary strategies [1]. Among the various approaches, the use of natural plant-based compounds has gained immense

attention due to their minimal toxicity, affordability, and potential to target multiple pathways involved in cancer progression [2].

Antioxidants, particularly those derived from plant sources, play a crucial role in maintaining cellular homeostasis by neutralizing harmful free radicals [3]. Free radicals, such as reactive oxygen species (ROS), are unstable molecules that can cause oxidative stress, leading to cellular damage, genetic mutations, and ultimately, the development of chronic diseases, including cancer [4]. Plants naturally produce a variety of phytochemicals that exhibit potent antioxidant properties, making them promising candidates for therapeutic applications.

2. *Manilkara zapota* and *Trichosanthes dioica*: An Overview

Manilkara zapota (Sapodilla)

Manilkara zapota, commonly known as Sapodilla, is a tropical evergreen tree belonging to the Sapotaceae family. It is widely cultivated in tropical and subtropical regions for its sweet, edible fruit. Beyond its culinary value, various parts of *M. zapota* have been traditionally used in folk medicine to treat ailments such as fever, diarrhea, dysentery, pain, hemorrhage, and ulcers [5].

Scientific studies have validated the medicinal properties of *M. zapota*, highlighting its antioxidant, anti-inflammatory, antimicrobial, antihyperglycemic, hepatoprotective, and anticancer activities. Phytochemical analysis has identified several bioactive compounds in *M. zapota*, including flavonoids, tannins, saponins, phenolic acids (such as quercetin, gallic acid, and caffeic acid), triterpenes, and sterols. These compounds contribute to the plant's therapeutic potential by modulating oxidative stress, inflammation, and cellular pathways involved in cancer progression.

Trichosanthes dioica (Pointed Gourd)

Trichosanthes dioica Roxb., commonly known as Pointed Gourd or Parwal, belongs to the Cucurbitaceae family and is cultivated primarily in South and Southeast Asia [10]. It is a rich source of essential nutrients and medicinal compounds, making it a staple in traditional medicine.

The pharmacological properties of *T. dioica* include antihyperglycemic, antihyperlipidemic, anti-inflammatory, antitumor, and cytotoxic effects. The roots and leaves have been particularly noted for their cytotoxic and genotoxic properties, indicating their potential as natural antitumor agents. Phytochemical analysis has identified the presence of alkaloids, flavonoids, carotenoids, tannins, and essential oils, all of which contribute to its therapeutic efficacy.

3. Role of Phytochemicals in Antioxidant and Anticancer Activities

Phytochemicals are bioactive compounds naturally found in plants that have been widely studied for their health benefits. The phytochemical composition of *M. zapota* and *T. dioica* includes a diverse range of antioxidants such as flavonoids, phenolic acids, carotenoids, and tannins [11]. These compounds are known to:

- **Neutralize Free Radicals:** By scavenging ROS and other reactive molecules, phytochemicals prevent oxidative damage to DNA, proteins, and lipids.
- **Modulate Inflammatory Pathways:** Chronic inflammation is a key factor in cancer development. Certain flavonoids and polyphenols in these plants have been shown to inhibit pro-inflammatory mediators.
- **Induce Apoptosis in Cancer Cells:** Many plant-derived compounds trigger programmed cell death (apoptosis) in cancer cells while sparing normal cells.
- **Inhibit Cancer Cell Proliferation:** Some phytochemicals interfere with cancer cell division, preventing tumor growth and metastasis.

4. Antioxidant Assays for Evaluating Plant Extracts

To determine the antioxidant potential of *M. zapota* and *T. dioica*, various *in vitro* assays are commonly used:

DPPH Free Radical Scavenging Assay

This assay measures the ability of plant extracts to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. A decrease in absorbance indicates strong antioxidant activity.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide is a source of ROS that can damage cellular components. This assay evaluates how effectively plant extracts break down hydrogen peroxide to prevent oxidative stress.

Ferric Thiocyanate (FTC) Assay

The FTC assay determines lipid peroxidation inhibition, indicating the ability of antioxidants to prevent fat oxidation and cell membrane damage.

Xanthine Oxidase Inhibition Assay

Xanthine oxidase catalyzes the production of uric acid and superoxide radicals. Inhibiting this enzyme helps in reducing oxidative stress and inflammation.

5. Anticancer Potential of M. zapota and T. dioica on HEC-1 Cell Line

The Importance of HEC-1 Cell Line in Cancer Research

The HEC-1 cell line is derived from endometrial adenocarcinoma, a type of uterine cancer. It is widely used in research to study cancer biology, drug responses, and the potential efficacy of natural compounds in inhibiting cancer cell growth.

MTT Cytotoxicity Assay

The MTT assay is commonly used to evaluate the cytotoxic effects of plant extracts on cancer cells. It measures cell viability by assessing mitochondrial activity, providing insights into how plant-derived antioxidants affect cancer cell survival.

Molecular Docking Studies

Computational molecular docking studies help predict interactions between phytochemicals and cancer-related proteins. Key bioactive compounds such as quercetin, catechin, and zapotin from *M. zapota*, along with carotenoids from *T. dioica*, have been found to bind effectively to proteins involved in cancer progression, suggesting their potential role in targeted therapy.

Methodology and Qualitative Analysis of *Manilkara zapota* and *Trichosanthes dioica* Roxb. on HEC-1 Cancer Cell Line

1. Collection and Preparation of Plant Materials

Plant Selection and Identification

Fresh fruits of *Manilkara zapota* (Sapodilla) and *Trichosanthes dioica* Roxb. (Pointed Gourd) were collected from authenticated sources.

Cleaning and Drying

The collected fruits were thoroughly washed with distilled water to remove dirt and contaminants. They were then air-dried under shade at room temperature for 10-14 days to preserve their phytochemical integrity.

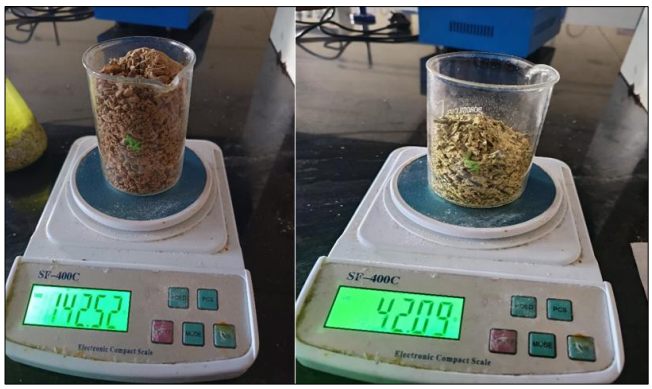
Grinding and Extraction

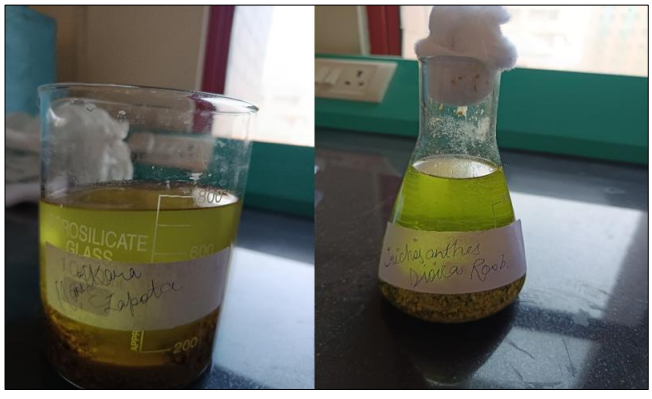
Dried fruit samples were ground into a fine powder using a mechanical grinder. The powder was stored in airtight containers to prevent degradation.

Once dried, the fruit samples were finely ground using a mechanical grinder. The powder was stored in airtight containers to prevent degradation.

Extraction process

- The powdered fruit material was extracted using methanol (99%) via maceration for 48-72 hours with occasional shaking.
- The mixture was then filtered using Whatman No.1 filter paper.
- The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to remove excess methanol.
- The final extract was stored at 4°C for further analysis.





2. Phytochemical Screening

Qualitative phytochemical analysis was performed on the extracts to detect the presence of key bioactive compounds using standard methods.

Test for Alkaloids

- **Mayer's Test:** A few drops of Mayer's reagent (potassium mercuric iodide) were added to the extract. Formation of a creamy white precipitate confirmed the presence of alkaloids.
- **Dragendorff's Test:** A few drops of Dragendorff's reagent (potassium bismuth iodide) were added to the extract. The appearance of an orange or reddish-brown precipitate indicated the presence of alkaloids.

Test for Flavonoids

- **Shinoda Test:** A few drops of concentrated hydrochloric acid (HCl) and magnesium turnings were added to the extract. The formation of a pink or red color indicated the presence of flavonoids.
- **Alkaline Reagent Test:** Sodium hydroxide (NaOH) solution was added to the extract, leading to the formation of a yellow color, which turned colorless upon the addition of dilute HCl, confirming the presence of flavonoids.

Test for Tannins

Ferric Chloride Test: The addition of ferric chloride ($FeCl_3$) to the extract resulted in a blue-black or greenish-black coloration, indicating the presence of tannins.

Test for Saponins

Foam Test: The extract was vigorously shaken with water, and the formation of a stable froth lasting for more than 10 minutes confirmed the presence of saponins.

Test for Phenolic Compounds

Lead Acetate Test: A few drops of lead acetate solution were added to the extract, resulting in the formation of a white precipitate, which confirmed the presence of phenolic compounds.

Test for Terpenoids

Salkowski Test: The extract was treated with chloroform and concentrated sulfuric acid (H_2SO_4), leading to the formation of a reddish-brown color at the interface, confirming the presence of terpenoids.

Test for Steroids

Liebermann-Burchard Test: The extract was treated with acetic anhydride and concentrated H2SO4, resulting in a greenish-blue coloration, which indicated the presence of steroids.

1)Phytochemical Constituents of *Manilkara zapota* (Sapodilla) -Primary Bioactive Compounds:

- Flavonoids (e.g., quercetin, myricetin) - Antioxidant, anti-inflammatory
- Tannins - Antimicrobial, astringent
- Saponins - Antifungal, immune-boosting
- Terpenoids - Anti-cancer, antimicrobial
- Alkaloids - Neuroprotective, anti-diabetic
- Phenolic Compounds - Strong antioxidants
- Diterpenes - Anti-inflammatory
- Steroids - Hormonal balance, anti-inflammatory
- Glycosides - Cardio-protective properties
- Vitamin C and Carotenoids - Boost immune function

2. Phytochemical Constituents of *Trichosanthes dioica* (Pointed Gourd) -Primary Bioactive Compounds:

- Flavonoids (e.g., kaempferol, quercetin) - Antioxidant, anti-cancer
- Alkaloids - Anti-diabetic, neuroprotective
- Saponins - Antifungal, cholesterol-lowering
- Tannins - Antimicrobial, wound healing
- Steroids - Anti-inflammatory
- Terpenoids - Antiviral, anti-cancer
- Phenolic Compounds - Free radical scavenging properties
- Glycosides - Improve heart health
- Carotenoids - Eye health, anti-aging
- Vitamins (A, C, E) - Essential for immunity

Table 1: Phytochemical screening (qualitative analysis)

Phytochemicals	Name of the test	Methodology	Results
Alkaloids	Mayer's Test	Add 2 ml of Mayer's reagent to 2 ml of extract.	A cream colored precipitate confirms alkaloids.
Flavonoid	Alkaline reagent Test	Add 2 ml of extract, to 2 m of extract.	An intense yellow color disappears with HCL, confirming flavonoid.
Tannins	Ferric chloride test	Add few drops of $Fecl_3$ to solution to 2 ml of extract.	A greenish- black or blue-black color confirms tannins.
Saponins	Froth test	Extract was dil. with distilled water to 20 ml and shaken in a test tube for 15 min.	The formation of foam indicates the presence of saponins.
Terpenoids	Salkowski test	Treat the extract with a few drops of concentrated sulphuric acid or chloroform sol. of sample + conc. h2so4	A reddish-brown ring at the interface confirms Terpenoids.
Glycoside	Borntrager's test	Add 2 ml of dilute h2so4 to 2 ml of extract and heat for 5 minutes. Filter and add 2 ml of ammonia.	A pink or red color confirms glycosides.

Alkaloid	+	++
Flavonoids	+++	++
Tannins	++	++
Saponins	+	+++
Phenolics	+++	++
Terpenoids	+	+
Glycosides	++	++

(+ mild presence, ++ moderate presence, +++ high presence)

Fig 1: Figure for the qualitative test

3. Cytotoxicity and Anticancer Evaluation on HEC-1 Cancer Cell Line (for *Manilkara zapota)*

Cell Culture and Maintenance

HEC-1 cells, derived from human endometrial adenocarcinoma, were obtained from a cell repository and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin-streptomycin). Cells were maintained at 37°C in a humidified atmosphere with 5% CO2.

For *in vitro* studies, the inhibition effect of proliferation on HEC-1B cell by triptolide was determined by MTT assay;

- Endometrial Cancer (Stephen Charnock-Jones, 1993) Four species of VEGF were expressed by the endometrial carcinoma cell lines Ishikawa, HEC 1-A, and HEC 1-B. Estradiol increased steady-state levels of mRNA encoding VEGF in a dose- and time-dependent manner in HEC 1-A cells. Conditioned medium from these cells possessed angiogenic activity that was depleted by passage through a heparin affinity column. None of the cell lines demonstrated mRNA for acidic or basic fibroblast growth factor (FGF), despite previous reports of the identification of immune reactive basic FGF in HEC 1-A and HEC -B cells. These findings show that VEGFs, not FGFs, are the principal angiogenic growth factors secreted by these cells and that human endometrium expresses a secreted angiogenic growth factor whose site of expression changes during the menstrual cycle.
- MTT assay for cell proliferation HEC-1B cells were diluted to 5x104 /ml and 100ul of the solution was seeded in wells of a 96-well plate. The cells were cultured at 37°in 5% CO2 for 24 hours, then the medium was replaced with that containing different concentrations of triptolide for another 24, 48, or 72 hours. 5 duplicates were set for each experiment, and equal volume of MEM medium containing 0.01% (V/V) DMSO was used as negative control and medium without drug or cell as blank control. At the end of culture, 20uL of MTT

solution (5g/L) was added to each well, incubated for another 4 hours, then the medium was aspirated and replaced with 150ul of DMSO, followed by low-speed oscillation utill all purple crystals were completely dissolved. Absorbance of the solution at 490nm was read with a microplate reader, and growth inhibition rate was calculated as: Inhibition rate (%) = [1- (Atest - Ablank)/ (A negtive - Ablank)] x 100%, and IC50 value was calculated using the modified Kou formula.

Result & Discussion

The growth inhibition effect of triptolide on HEC-1B cells The growth inhibition effect of triptolide on HEC1B cells was time and dose-dependent. The Inhibition Effect of Triptolide on Human Endometrial Carcinoma Cell Line HEC-1B: A *in vitro* and *in vivo* Studies 5-10ng/ml triptolide for 24h would exerted significant inhibitory effect, which became more potent as treatment time extended. When administered for 72 hours, the inhibition effect of triptolide on HEC-1B cells reaches 73.3-79.3%, but higher dose or longer treatment time than that produced no significantly better efficacy.

Table 2: Cell Cycle Change Resulted from Different Concentrations of Triptolide (%, x ±s, n=3) for Chikkoo

S. No.	Group G0/G1	S	G2/M
control	69.12±4.73	21.15±4.77	7.73±1.74
5ng	58.48±4.06**	33.52±3.30*	6.99±3.54
10ng/ml	59.25±2.21**	28.76±2.09	11.99±0.60
20ng/ml	55.51±4.94**	29.89±2.75	13.60±3.51
40ng/ml	56.12±4.77**	28.19±4.18	17.49±1.56**
80ng/ml	54.56±1.92**	28.92±3.03	16.52±1.13** *

Compare to control group: *p<0.01

Conclusion

This study highlights the promising antioxidant and anticancer potential of *Manilkara zapota* and *Trichosanthes dioica* fruit extracts against HEC-1 endometrial cancer cells. Phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, phenolics, tannins, and terpenoids, which are known for their therapeutic efficacy. Antioxidant assays demonstrated strong free radical scavenging activity, while cytotoxicity tests revealed dose-dependent inhibition of cancer cell growth. Molecular docking further supported the interaction of key phytochemicals with cancer-related proteins, indicating their role in promoting apoptosis and suppressing tumor progression. These findings suggest that *M. zapota* and *T. dioica* could serve as natural, plant-based alternatives in cancer therapy, offering effective bioactivity with minimal toxicity. Further *in vivo* and clinical research is needed to validate their efficacy and mechanisms of action, but this study lays foundational support for their development as potential anticancer agents.

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