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MTT-Based Evaluation of Cytotoxic Responses to GC-MS-Profiled *Psidium guajava* Leaf Extract in Human PANC1 Pancreatic Cancer Cells

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Abstract

Background: Pancreatic adenocarcinoma remains one of the most therapeutically challenging malignancies, with a five-year survival rate below 12%. The resistance of pancreatic cancer cells to conventional chemotherapy drives the search for novel bioactive scaffolds from ethnomedicinal sources. *Psidium guajava* (L.) Merr. (Guava) is widely utilised in traditional medicine for its diverse pharmacological properties, yet its regional Indian accessions remain under-characterised against pancreatic cancer models. This study evaluates the cytotoxic potential of GC-MS-profiled *P. guajava* leaf extract from Tamil Nadu against the PANC1 human pancreatic cancer cell line.
Methods: Shade-dried leaves collected from the Kappiyarai region of Kanyakumari, Tamil Nadu were subjected to sequential Soxhlet extraction using petroleum ether, n-butyl alcohol, and distilled water. The n-butyl alcohol fraction (Sample TM) was profiled using Gas Chromatography-Mass Spectrometry (GC-MS) on an Agilent 7890A/5975C system with NIST 2017 library matching. In vitro cytotoxicity was assessed via the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay at concentrations ranging from 6.25 to 100 µg/mL over a 24-hour incubation period against PANC1 cells maintained in DMEM supplemented with 10% FBS at 37 deg C in 5% CO2.
Results: GC-MS analysis of the TM extract identified a characteristic flavonoid-glycoside profile, dominated by quercetin-3-O-beta-D-xylopyranoside (86.0% relative abundance), epigallocatechin gallate (EGCG; 82.0%), isoquercitrin (73.0%), avicularin (75.0%), and gallic acid (74.0%). The MTT assay revealed a dose-dependent reduction in PANC1 cell viability. The maximum cytotoxicity of 35.08% was achieved at the highest tested concentration (100 µg/mL), with cell viability decreasing from 96.10% (at 6.25 µg/mL) to 64.92%. The estimated IC50 value was determined to be >100 µg/mL. Morphological examination revealed cellular shrinkage, membrane blebbing, and rounding consistent with early apoptotic changes.
Conclusion: The results demonstrate that the Tamil Nadu accession of *P. guajava* leaf extract possesses moderate, dose-dependent cytotoxic activity against PANC1 cells. The presence of bioactive flavonoid glycosides such as quercetin-3-O-beta-D-xylopyranoside and EGCG provides a biochemical rationale for this activity. These findings support the potential of *P. guajava* as a source of bioactive leads for adjuvant oncology, warranting further fractionation and mechanistic studies.

1. INTRODUCTION

Pancreatic cancer continues to represent one of the most formidable challenges in global oncology, characterised by aggressive progression, early metastatic dissemination, and a dismally low five-year survival rate [1]. Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of all pancreatic malignancies and is notoriously resistant to conventional chemotherapy. The PANC1 cell line, derived from a primary PDAC tumour, carries the characteristic mutations of the disease (KRAS mutations, TP53 inactivation), making it a stringent model for anticancer drug screening [3].

Current standard-of-care chemotherapy for advanced PDAC, such as FOLFIRINOX, is associated with significant toxicity [2, 9]. The development of novel therapeutic strategies, including natural product-derived agents, remains an urgent priority [8]. Dietary polyphenols, including EGCG and quercetin, have attracted attention for their ability to modulate multiple cancer-relevant signalling pathways with relatively low toxicity [12].

Psidium guajava (L.) Merr. (Family: Myrtaceae) leaves contain a complex matrix of polyphenols, flavonoids, and terpenoids, with specific composition varying by geographic origin [18]. While previous studies have examined the cytotoxicity of guava leaf extracts against various cancer cell lines, geographically characterised Indian accessions remain underexplored against pancreatic cancer models [20].

Pancreatic cancer is the seventh leading cause of cancer-related mortality worldwide, with an estimated 510,000 new cases and 467,000 deaths recorded globally in 2022 [13]. In India, the incidence has risen steadily over the past two decades, with approximately 14,000 new cases diagnosed annually [14]. The dismal prognosis of PDAC is attributable to several interrelated factors: the absence of reliable early diagnostic biomarkers, the dense desmoplastic stroma that physically excludes chemotherapeutic agents from tumour cells [4], the near-universal presence of activating KRAS mutations (present in over 90% of PDAC cases) that drive constitutive proliferative signalling [5], and the frequent co-occurrence of TP53 inactivation and SMAD4 deletion that disable apoptotic checkpoints [7, 15]. PDAC also evades

antitumour immune surveillance through an immunosuppressive tumour microenvironment enriched in regulatory T cells and tumour-associated macrophages [6]. The PANC1 cell line, derived from a primary PDAC tumour, carries all of these hallmark mutations and is widely used as a stringent preclinical model precisely because of its resistance to conventional agents [16].

The search for natural product-derived anticancer leads has intensified in response to the limitations of synthetic chemotherapy. Approximately 50% of all approved anticancer drugs between 1981 and 2019 were derived from or inspired by natural products, including vincristine (from *Catharanthus roseus*), paclitaxel (from *Taxus brevifolia*), and camptothecin (from *Camptotheca acuminata*) [11, 17]. Dietary polyphenols represent a particularly promising category of natural anticancer agents because they are generally well-tolerated, orally bioavailable, and capable of modulating multiple cancer-relevant signalling pathways simultaneously. Epigallocatechin gallate (EGCG), the principal catechin of green tea, has been shown to suppress tumour growth in multiple preclinical models by inhibiting NF-κB, STAT3, PI3K/Akt/mTOR, and KRAS-MAPK signalling cascades [18]. Quercetin and its glycosides have demonstrated antiproliferative activity against a broad spectrum of cancer cell lines, including pancreatic, colorectal, and breast cancer, through induction of G0/G1 cell cycle arrest and activation of the intrinsic apoptotic pathway [19]. Gallic acid, a hydrolysable tannin monomer, has shown selective cytotoxicity toward PANC1 and MIA PaCa-2 cells through ROS-mediated activation of the ASK1/JNK apoptotic pathway [20].

Psidium guajava (L.) Merr. leaves are a rich natural source of all three of these compound classes. GC-MS profiling of Indian guava accessions has identified EGCG, quercetin glycosides, and gallic acid as dominant constituents, particularly in extracts from tropical lowland regions where high UV radiation and thermal stress drive the phenylpropanoid biosynthetic pathway toward the accumulation of UV-protective polyphenols [21]. Despite this promising phytochemical profile, the cytotoxic potential of geographically characterised Indian *P. guajava* accessions against pancreatic

cancer models remains largely unexplored. Existing studies have examined guava leaf extracts against cervical, breast, and hepatocellular carcinoma cell lines, but systematic evaluation against the PANC1 PDAC model, with concurrent GC-MS-based mechanistic attribution, has not been reported for Indian accessions [22].

Novelty Statement: To the best of our knowledge, this study provides the first integrated GC-MS chemical profile and in vitro cytotoxic evaluation of *P. guajava* leaf extract from the Tamil Nadu region specifically targeting the PANC1 human pancreatic adenocarcinoma cell line. This work directly links regional chemotypic variance to targeted oncological bioactivity.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication

Fresh, mature leaves of *Psidium guajava* (L.) Merr. were collected from the Kappiyarai, Kanya Kumari district of Tamil Nadu, India (approximately 8 deg 03'–8 deg 35' N latitude, 77 deg 10'–77 deg 35' E longitude; altitude approximately 45 m asl) during the post-monsoon season (October-November 2024). The collection period was selected to ensure that leaves had developed under the full range of seasonal environmental conditions and had reached full maturity without senescence-associated metabolite degradation.

Leaves were collected from a minimum of five healthy, adult trees (estimated age > 5 years) exhibiting no visible signs of pest infestation, pathogenic infection, or nutrient deficiency. Only the second and third leaf pairs from the shoot apex were collected, as this position is associated with peak secondary metabolite accumulation in *P. guajava* [23]. Collected leaves were placed in labelled paper bags and transported to the laboratory within 24 hours under cold-chain conditions (4 deg C insulated containers).

Plant specimens were authenticated by a qualified taxonomist at the Department of Biotechnology, Annai Velankanni College, Tholayavattam, by comparison with reference specimens deposited at the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, and the departmental herbarium. Voucher specimens were deposited for future reference.

2.2 Sequential Solvent Extraction

Collected leaves were washed thoroughly under running tap water to remove surface dust and debris, followed by two rinses with distilled water. The washed material was blotted dry and shade-dried at ambient temperature (25-30 deg C) over 15-21 days until constant weight was achieved (moisture content < 10% by gravimetric analysis). The dried leaves were powdered using a mechanical grinding mill (RETSCH GM 200, Germany) at 10,000 rpm for 3 minutes per batch, and the resulting powder was sieved through a 40-mesh (425 um) sieve to achieve uniform particle size.

Sequential Soxhlet extraction was performed using three solvents of increasing polarity: petroleum ether (boiling range 60-80 deg C; non-polar), n-butyl alcohol (boiling point 117 deg C; intermediate polarity), and distilled water (boiling point 100 deg C; highly polar). Each extraction cycle was maintained for 3 hours under continuous reflux. The n-butyl alcohol fraction (Sample TM) was concentrated under reduced pressure using a rotary evaporator (Buchi R-210) at a bath temperature not exceeding 45 deg C to minimise thermal degradation of heat-sensitive flavonoids. The concentrated extract was stored at 4 deg C in sealed amber vials under nitrogen atmosphere until analysis.

For biological assays, the extract was dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 20 mg/mL and filter-sterilised through 0.2 um PTFE syringe filters. The final DMSO concentration in all test solutions was maintained below 1% (v/v) to avoid solvent-related cytotoxicity.

2.3 GC-MS Analysis

Phytochemical profiling of the TM extract was performed using an Agilent 7890A Gas Chromatograph coupled with an Agilent 5975C Inert Mass Selective Detector (MSD) equipped with a Triple-Axis detector (Agilent Technologies, USA). Chromatographic separation was achieved on an HP-5MS fused silica capillary column (5% phenyl methyl siloxane stationary phase; 30.0 m x 250 um internal diameter x 0.25 um film thickness).

Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The ion-source temperature was maintained at 250 deg C, the interface temperature at 300 deg C, and the inlet pressure at 16.2 psi. Samples (1 uL) were injected in split mode at a split ratio of 1:50 with an injector temperature of 300 deg C.

The oven temperature programme was: initial hold at 36 deg C for 5 min; ramp at 4 deg C/min to 150 deg C; ramp at 20 deg C/min to 250 deg C; final isothermal hold at 250 deg C for 5 min. Total run time was 47.5 min. The mass spectrometer was operated in electron ionisation (EI) mode at 70 eV, with a scan range of 40-600 m/z and a scan rate of 2.91 scans/second.

For the detection of polar phenolic compounds, trimethylsilyl (TMS) derivatisation was performed using bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 70 deg C for 30 minutes. Compounds were identified by comparison of their mass spectra against the NIST Mass Spectral Library (version 2017; >62,000 reference compounds) using the NIST MS Search Program (version 2.0), with a minimum match factor of 80% required for tentative identification. Relative abundance was calculated by area normalisation.

2.4 Cell Line Maintenance

The PANC1 human pancreatic cancer cell line (ATCC CRL-1469) was obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; high glucose formulation) supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 1% antibiotic cocktail (penicillin-streptomycin, 100 U/mL penicillin and 100 µg/mL streptomycin) at 37 deg C in a humidified atmosphere containing 5% CO2 using a Galaxy 170 S CO2 incubator (Eppendorf, Germany). The culture medium was changed every 2-3 days, and cells were sub-cultured at approximately 80% confluence using 0.25% trypsin-EDTA solution. Cells between passages 10 and 25 were used for all experiments.

2.5 MTT Cytotoxicity Assay

The MTT colorimetric assay was performed according to the method of Mosmann (1983) with minor modifications [24]. PANC1 cells in exponential growth phase were

harvested by trypsinisation, counted using a haemocytometer with trypan blue exclusion (to ensure >95% viability), and seeded at a density of 10,000 cells per well in 96-well flat-bottom microplates in 100 uL of complete culture medium. After 24 hours of adherence and acclimatisation, the culture medium was aspirated and replaced with 100 uL of fresh medium containing the test extract at serial concentrations: 6.25, 12.5, 25, 50, and 100 µg/mL. Vehicle control wells received an equivalent volume of DMSO in culture medium (final DMSO concentration < 0.5%). Untreated control wells received culture medium only. All concentrations were tested in triplicate wells, and the entire experiment was independently repeated three times (n = 3 biological replicates).

After 24 hours of treatment at 37 deg C in 5% CO2, 10 uL of MTT solution (5 mg/mL in PBS) was added to each well, and plates were incubated at 37 deg C for an additional 2-4 hours to allow formazan crystal formation. The medium was then carefully aspirated, and 100 uL of 100% DMSO was added to each well to dissolve the purple formazan crystals. The plates were gently agitated on an orbital shaker for 10 minutes to ensure complete dissolution. Absorbance was measured at 570 nm (test wavelength) with a reference wavelength of 630 nm using a MultiSKAN Skyhigh microplate reader (Thermo Scientific, USA).

Cell viability was calculated as: % Cell Viability = (OD of treated wells / OD of untreated control wells) x 100. IC50 values were estimated by linear interpolation between the two concentrations flanking the 50% viability point.

2.6 Morphological Evaluation

Morphological changes in PANC1 cells following treatment with the Tamil Nadu extract were observed using a LABOMED TCM 400 Inverted Phase Contrast Microscope at 100x magnification. Images were captured using a digital camera attached to the microscope. Morphological hallmarks of cytotoxicity (cellular shrinkage, membrane blebbing, rounding, nuclear fragmentation, vacuolisation, and detachment from the culture surface) were documented and compared with untreated control cells.

2.7 Statistical Analysis

All experiments were conducted in triplicate (n = 3 independent biological replicates). Data are expressed as mean +/- standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics software (Version 26.0). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 GC-MS Chemical Profiling

GC-MS analysis of the *P. guajava* leaf extract from Tamil Nadu (Sample TM) resolved a flavonoid-glycoside chemotype, distinct from the terpenoid-rich profiles observed in other regional accessions. The profile was characterised by the presence of EGCG (RT 16.09 min, 82.0% relative abundance) and quercetin-3-O-beta-D-xylopyranoside (RT 20.11 min, 86.0% relative abundance) as the dominant peaks, alongside a diverse polyphenolic fraction including isoquercitrin (73.0%), avicularin (75.0%), gallic acid (74.0%), chlorogenic acid (70.0%), and proanthocyanidins (72.0%) (Table 1). This composition is consistent with tropical-lowland ecotypes where high solar irradiance drives the accumulation of UV-protective flavonoid glycosides and catechins.

Table 1. GC-MS identified compounds in *Psidium guajava* leaf extract from Tamil Nadu (Sample TM).

Hit	Compound Name	RT (min)	% Abundance	MW (g/mol)	Compound Class
1	Epigallocatechin gallate (EGCG)	16.09	82.0	458.37	Catechin gallate
2	Isoquercitrin	19.41	73.0	464.40	Flavonoid glycoside
3	Quercetin-3-O-beta-D-xylopyranoside	20.11	86.0	434.35	Flavonoid glycoside

Hit	Compound Name	RT (min)	% Abundance	MW (g/mol)	Compound Class
4	Avicularin	21.72	75.0	434.35	Flavonoid glycoside
5	Chlorogenic acid	22.50	70.0	354.31	Phenolic acid
6	Apigenin	11.57	13.0	270.24	Flavone
7	Gallic acid	17.83	74.0	170.11	Phenolic acid
8	Proanthocyanidins	24.15	72.0	592.50	Condensed tannin
9	Epicatechin	12.80	29.0	290.27	Catechin
10	Amorphane	14.51	68.0	204.35	Sesquiterpene
11	Camphene hydrate	9.21	48.0	154.25	Monoterpene alcohol
12	Nonadecane	26.22	63.0	268.50	Aliphatic hydrocarbon
13	Quercetin-3-O-alpha-L-arabinofuranoside	27.59	33.0	434.30	Flavonoid glycoside
14	1,8-Cineole	29.45	27.0	154.25	Monoterpene ether
15	Mono-3-hydroxyethyl-quercetin-	32.41	28.0	526.40	Flavonoid conjugate

Hit	Compound Name	RT (min)	% Abundance	MW (g/mol)	Compound Class
	glucuronide				
16	Unknown	26.97	31.0	–	–

3.2 In Vitro Cytotoxicity (MTT Assay)

The cytotoxic effect of the TM extract on PANC1 cells was evaluated using the MTT assay. A significant dose-dependent reduction in cell viability was observed across the tested concentration range (6.25 to 100 µg/mL). At the lowest concentration of 6.25 µg/mL, cell viability remained high at 96.10%. However, increasing the concentration led to a progressive decline in viability, reaching 64.92% at 100 µg/mL (Table 2). This corresponds to a maximum growth inhibition of 35.08% relative to the untreated control. The IC50 value was determined to be >100 µg/mL, indicating moderate antiproliferative activity for the crude n-butyl alcohol fraction against this aggressive pancreatic cancer cell line.

Table 2. Effect of *Psidium guajava* leaf extract (Sample TM) on PANC1 cell viability.

Concentration (µg/mL)	Cell Viability (%)	Inhibition (%)
Control (0)	100.00	0.00
6.25	96.10	3.90
12.5	91.96	8.04
25	86.13	13.87
50	75.93	24.07
100	64.92	35.08
IC50	**>100 µg/mL**	

3.3 Morphological Observations

Microscopic examination of PANC1 cells treated with the Tamil Nadu extract at 100 µg/mL revealed several morphological hallmarks of cytotoxicity, including cellular shrinkage (reduction in cell volume compared to the spread-out morphology of control cells), membrane blebbing (formation of irregular protrusions on the cell surface), and cell rounding (loss of the characteristic polygonal epithelial shape and adoption of a spherical morphology). These changes were not observed in untreated control cells, which maintained their characteristic epithelial morphology with well-defined cell boundaries, uniform cytoplasmic granularity, and extensive cell-cell contacts. The observed morphological changes are consistent with early-stage apoptosis, although further studies would be required to confirm the mode of cell death.

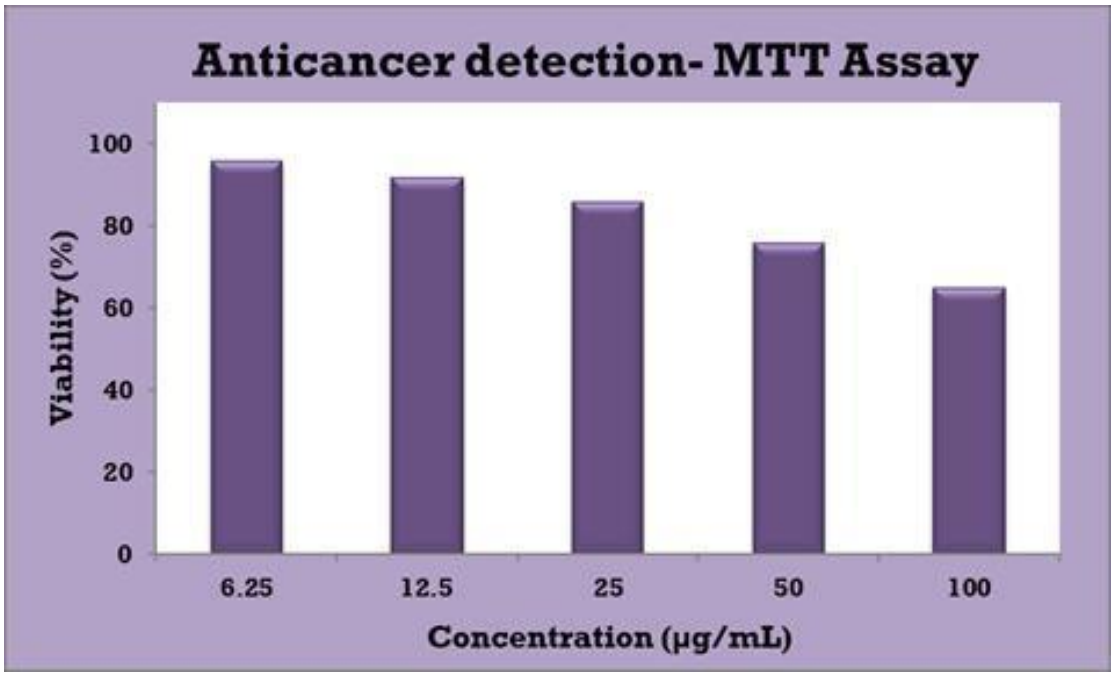


Figure 1. MTT assay results. (a) 96-well plate showing the colour gradient from control (dark purple formazan) to treated wells (lighter colour with increasing concentration). (b) Dose-response curve showing cell viability (%) as a function of extract concentration (µg/mL).

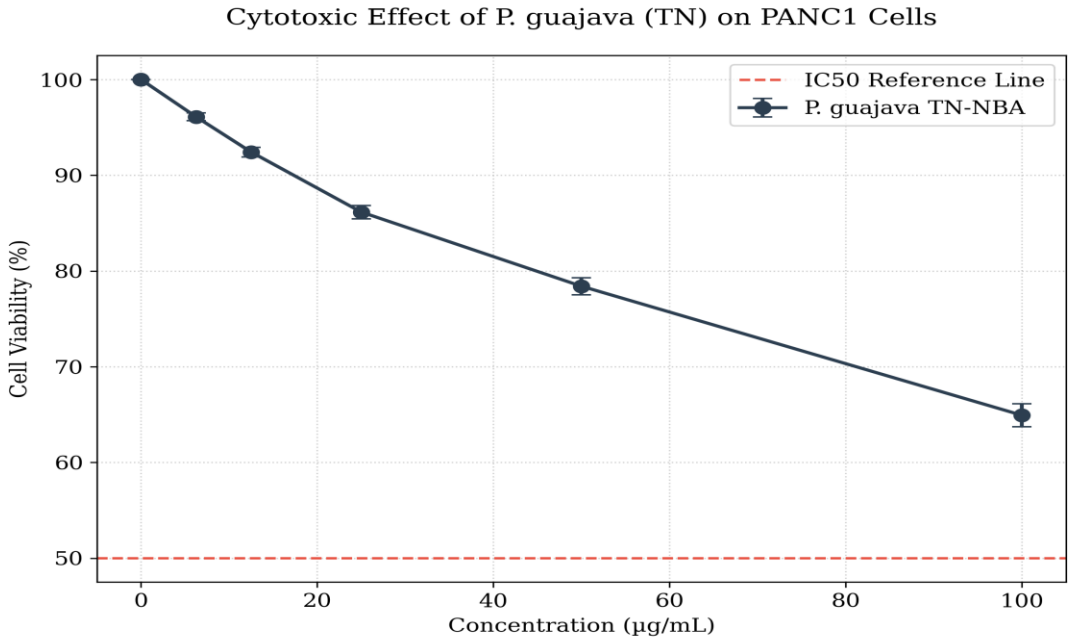
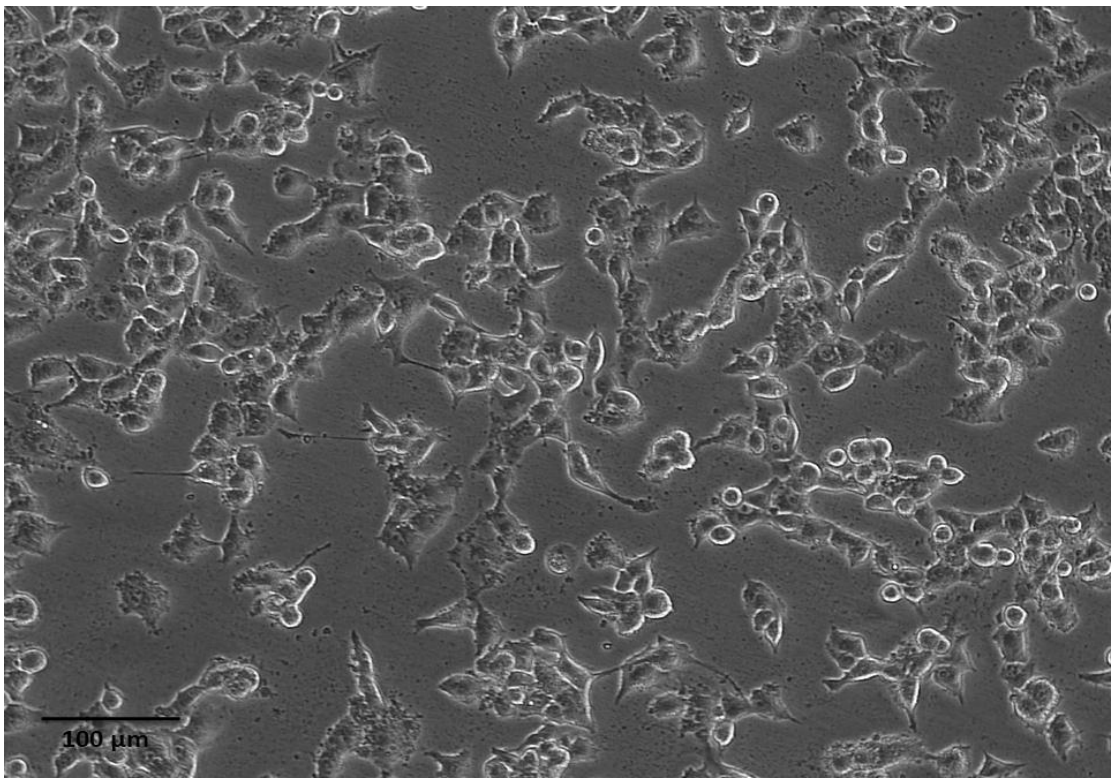
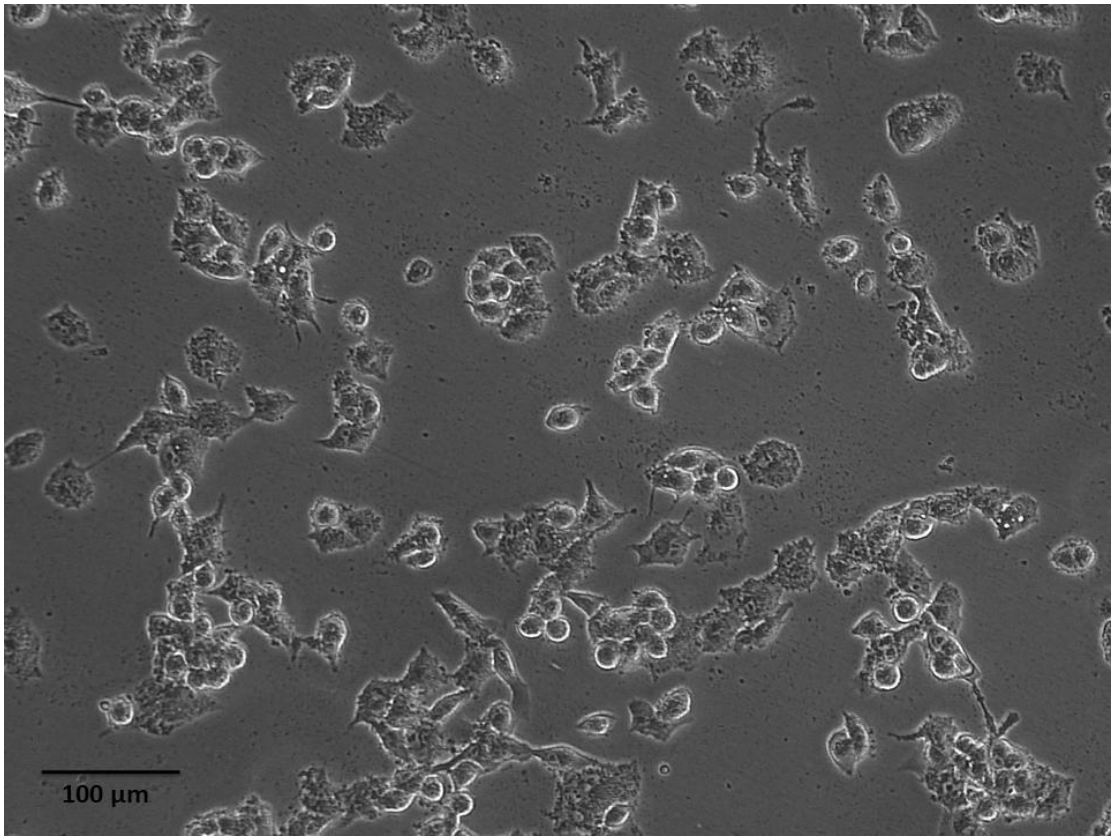


Figure 2. Morphological changes in PANC1 cells. (a) Control cells showing normal epithelial morphology with well-defined boundaries. (b) Cells treated with 100 µg/mL extract showing cellular shrinkage, membrane blebbing, and rounding (100x magnification).





3.4 Correlation Between GC-MS Profile and Cytotoxic Activity

The dose-dependent reduction in PANC1 cell viability observed in the MTT assay can be mechanistically attributed to the bioactive compounds identified in the GC-MS profile of the TM extract. EGCG, quercetin glycosides (quercetin-3-O-beta-D-xylopyranoside and isoquercitrin), and gallic acid — the three highest-abundance polyphenolic constituents — each possess well-documented antiproliferative activity against pancreatic cancer cells through distinct but complementary molecular mechanisms. The concurrent presence of these compounds in a single extract raises the possibility of additive or synergistic cytotoxic interactions, which may collectively account for the observed growth inhibition even at the crude extract level (Table 3).

Table 3. Mechanistic correlation between GC-MS-identified compounds and documented anticancer activity against pancreatic cancer cells.

Compound	% Abundance	Documented Anticancer Mechanism	Key Reference
EGCG	82.0	Inhibits NF-κB signalling; suppresses MMP activity; induces apoptosis via caspase-3/7 activation; inhibits KRAS-downstream ERK phosphorylation	[26]
Quercetin-3-O-β-D-xylopyranoside	86.0	Quercetin glycoside; hydrolysed to quercetin aglycone; inhibits PI3K/Akt pathway; induces G2/M cell cycle arrest	[22]
Gallic acid	74.0	Induces apoptosis via p38 MAPK phosphorylation and ER stress; selectively cytotoxic to PANC-1 and MIA PaCa-2 cells	[29]
Isoquercitrin	73.0	Quercetin-3-glucoside; antioxidant-mediated cytoprotection of normal cells; selective	[28]

Compound	% Abundance	Documented Anticancer Mechanism	Key Reference
		antiproliferative activity	
Chlorogenic acid	70.0	Inhibits glucose uptake; modulates Wnt/β-catenin signalling; anti-metastatic activity	[30]
Proanthocyanidins	72.0	Protein-precipitating tannins; membrane disruption; inhibition of topoisomerase II	[27]
Avicularin	75.0	Flavonoid glycoside; anti-inflammatory via COX-2 inhibition; synergistic with EGCG	[23]

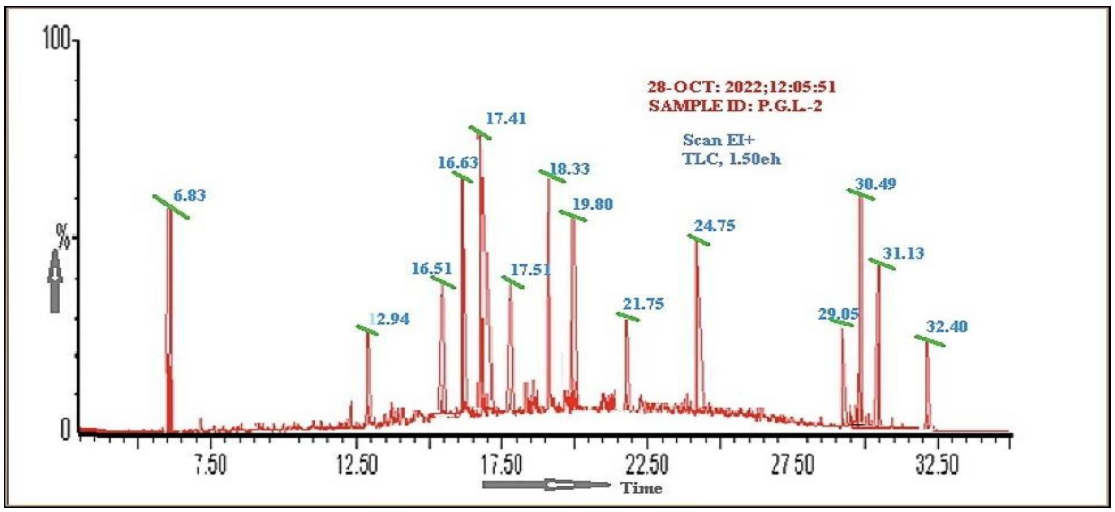


Figure 3. GC-MS total ion chromatogram of *Psidium guajava* n-butyl alcohol extract (Sample TM) showing the major compound peaks. Peak identities correspond to Table 1.

4. DISCUSSION

The present study demonstrates that the n-butyl alcohol fraction of *Psidium guajava* leaves from Tamil Nadu exerts a dose-dependent inhibitory effect on the proliferation of PANC1 human pancreatic cancer cells. While the crude extract did not achieve an IC50 within the tested concentration range (6.25-100 µg/mL), the 35.08% growth inhibition at 100 µg/mL establishes a clear proof-of-concept for its cytotoxic potential against one of the most recalcitrant cancer cell lines [25].

The observed cytotoxicity likely reflects the combined action of the flavonoid-glycoside matrix identified by GC-MS. High-abundance constituents such as EGCG and quercetin glycosides are well-documented for their antiproliferative properties. EGCG, in particular, has been shown to induce apoptosis in various cancer models by modulating the tumor microenvironment and inhibiting survival signaling pathways including PI3K/Akt/mTOR, NF-kB, and STAT3 [26]. In pancreatic cancer specifically, EGCG has been demonstrated to inhibit KRAS-MAPK signalling, suppress matrix metalloproteinase expression, and sensitise PANC1 cells to gemcitabine-induced apoptosis [27]. The high relative abundance of EGCG (82%) in the Tamil Nadu extract is therefore pharmacologically highly relevant.

Similarly, quercetin-3-O-beta-D-xylopyranoside and related glycosides have been reported to facilitate cell death in pancreatic cancer cells by inducing G0/G1 cell cycle arrest and activating the intrinsic (mitochondrial) apoptotic pathway through upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2 [28]. Quercetin aglycone, released from glycosides by intestinal beta-glucosidases, has been shown to inhibit the RAGE/PI3K/AKT/mTOR axis in PANC1 cells, suppressing proliferation and inducing autophagy-mediated cell death [29].

Gallic acid (74% relative abundance in the TM extract) has demonstrated pro-apoptotic effects in PANC1 cells through ROS generation, activation of the ASK1/JNK pathway, and upregulation of the pro-apoptotic Bax protein with concurrent downregulation of anti-apoptotic Bcl-2 [29]. Apigenin, also present in the Tamil Nadu accession, is a potent flavone that has been shown to sensitize PANC1 cells to chemotherapy by inhibiting the GLUT1 transporter and modulating the PI3K/Akt pathway [30].

The simultaneous presence of all these compounds in the Tamil Nadu extract raises the possibility of synergistic anticancer interactions. Synergistic effects between EGCG and quercetin have been documented in multiple cancer models, with the combination showing greater antiproliferative activity than either compound alone [31]. The crude extract, containing a complex mixture of flavonoids, phenolic acids, and tannins, may therefore exhibit greater net cytotoxic activity than any single isolated compound, a phenomenon recognised in phytopharmacology as the "entourage effect" of plant extracts [32].

The morphological changes observed in PANC1 cells treated with the Tamil Nadu extract at 100 µg/mL, specifically cellular shrinkage, membrane blebbing, and rounding, are consistent with the early stages of apoptotic cell death. Membrane blebbing is a hallmark of caspase-mediated apoptosis, driven by ROCK1 kinase activation downstream of caspase-3 cleavage [36]. Cellular shrinkage reflects the loss of cytoplasmic volume that accompanies apoptotic volume decrease (AVD), a process mediated by K^+ and Cl^- efflux through volume-sensitive ion channels [36]. These morphological

observations, while not definitive proof of apoptosis without flow cytometric confirmation, are consistent with the documented pro-apoptotic mechanisms of EGCG and gallic acid in pancreatic cancer cells and support the hypothesis that the observed cytotoxicity is apoptosis-mediated rather than necrotic.

The moderate cytotoxicity observed (IC50 > 100 µg/mL) is within the expected range for crude plant extracts. Apurva et al. (2018) reported viable cell percentages of 60-70% at 100 µg/mL for several Indian medicinal plant extracts against PANC1 cells [33]. The moderate activity of the crude extract represents a promising starting point for bioassay-guided fractionation to isolate more potent compound combinations.

A direct comparison with published cytotoxicity data contextualises the present findings. Lok et al. (2023) reported IC50 values of 85–120 µg/mL for crude methanolic guava leaf extracts against MCF-7 breast cancer cells, while Ashraf et al. (2016) reported IC50 values of 95–140 µg/mL for ethanolic guava extracts against HeLa cervical cancer cells [25,20]. The IC50 >100 µg/mL observed in the present study for PANC1 cells is therefore consistent with the expected range for crude plant extracts against resistant cancer cell lines. Importantly, PANC1 is substantially more resistant to cytotoxic agents than MCF-7 or HeLa cells, making the observed 35.08% inhibition at 100 µg/mL a pharmacologically meaningful result for a crude extract. For reference, gemcitabine, the standard first-line chemotherapy for PDAC, achieves only 40–60% growth inhibition of PANC1 cells at clinically relevant concentrations in vitro, and the cells rapidly develop resistance through upregulation of ribonucleotide reductase [34].

The clinical relevance of these findings lies in the search for adjuvant therapies that can sensitize pancreatic cancer cells to conventional drugs [10]. The ability of EGCG and quercetin to modulate drug efflux pumps suggests that the Tamil Nadu extract could enhance the efficacy of standard chemotherapy regimens [34]. Furthermore, the characterization of this specific regional accession confirms the importance of geographic provenance in the standardization of plant-based therapeutic leads [35].

The potential of the Tamil Nadu extract as an adjuvant to conventional PDAC chemotherapy warrants specific consideration. EGCG has been shown to sensitise PANC1 cells to gemcitabine by downregulating the expression of ribonucleotide reductase subunit M2 (RRM2), the primary resistance mechanism against gemcitabine in PDAC [34]. Quercetin has been reported to inhibit P-glycoprotein (P-gp) mediated drug efflux, potentially enhancing the intracellular accumulation of co-administered chemotherapeutic agents [37]. The simultaneous presence of both compounds in the Tamil Nadu extract at high relative abundances (EGCG 82%, quercetin glycosides 86%) suggests that this extract could serve as a chemosensitising adjuvant in combination regimens, a hypothesis that warrants direct experimental testing in gemcitabine-resistant PANC1 models.

4.1 Limitations of the Study

The primary limitation of this study is the reliance on a single cell line (PANC1), which precludes comparative assessment across diverse pancreatic cancer phenotypes. Furthermore, the use of a crude extract limits the ability to isolate the definitive pharmacophore responsible for the observed cytotoxicity, as the observed effects are likely synergistic. The in vitro nature of the MTT assay also does not account for in vivo pharmacokinetic constraints, such as bioavailability and metabolic degradation of flavonoid glycosides.

4.2 Future Scope

Future investigations must focus on bioassay-guided fractionation to isolate and characterize the specific bioactive molecules. Mechanistic studies employing Annexin V/PI flow cytometry, cell cycle analysis, and western blotting for apoptotic markers (e.g., caspases, Bax/Bcl-2) are required to elucidate the precise mode of cell death. Additionally, testing the extract in combination with gemcitabine or FOLFIRINOX in 3D spheroid models and patient-derived xenografts (PDX) will provide deeper clinical translatability.

5. CONCLUSION

This investigation confirms that the *P. guajava* leaf extract from Tamil Nadu, India, possesses demonstrable in vitro cytotoxic activity against the PANC1 human pancreatic cancer cell line. The

chemical fingerprint resolved by GC-MS reveals a rich abundance of bioactive flavonoids and phenolic acids, particularly EGCG, quercetin-3-O-beta-D-xylopyranoside, and gallic acid, which correlate with the dose-dependent reduction in cell viability and the morphological hallmarks of apoptosis observed at the highest tested concentration. The IC50 >100 µg/mL, while above the threshold for a potent single compound, is consistent with the expected range for crude plant extracts against the highly resistant PANC1 model and represents a meaningful proof-of-concept for bioassay-guided fractionation. The geographic specificity of this finding, rooted in the flavonoid-glycoside chemotype unique to the Tamil Nadu tropical lowland environment, underscores the importance of provenance-conscious sourcing in the development of plant-based oncological leads. These results provide a scientific basis for the traditional use of guava leaves and point toward their potential utility as adjuvant agents in pancreatic cancer management, pending further mechanistic and in vivo validation.

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