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Phytochemical Composition and Pharmacological Activities of *Peperomia pellucida* Methanolic Leaf Extract: Antioxidant, Antidiabetic and Anticancer Potentials

SHYBEY MARIAM ABRAHAM and IWIN C. VARKEY*

Department of Chemistry, CMS College of Science and Commerce, Coimbatore-686001, India

*Corresponding author: E-mail: iwin.c.varkey@gmail.com

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This study explores the phytochemical composition and biological activities of methanolic leaf extracts of *Peperomia pellucida*. Phytochemical screening and GC-MS analysis revealed a wide range of bioactive compounds including alkaloids, flavonoids, tannins, phenols and terpenoids, with the methanolic extract showing the richest profile. Antioxidant activity measured by the DPPH assay demonstrated strong free radical scavenging potential (IC_{50} = 75.4 µg/mL), comparable to ascorbic acid. The extract also exhibited potent antidiabetic effects through inhibition of α-amylase (IC_{50} = 60.8 µg/mL) and α-glucosidase (IC_{50} = 52.8 µg/mL), exceeding the standard drug acarbose. Furthermore, anticancer evaluation on MCF-7 breast cancer cells showed dose- and time-dependent cytotoxicity, inducing apoptotic features such as cell shrinkage, nuclear fragmentation and DNA cleavage, confirmed by AO/EB staining and DNA fragmentation assays. Overall, the methanolic extract of *P. pellucida* demonstrates strong antioxidant, antidiabetic and anticancer potential, supporting its future application in therapeutic development and cancer medicine.

Keywords: *Peperomia pellucida*, Methanolic extract, Antioxidant activity, Antidiabetic activity, Anticancer activity.

INTRODUCTION

Cancer remains one of the most significant global health challenges, marked by uncontrolled cell growth and the ability to spread across different tissues [1]. Its onset and progression are influenced by genetic alterations, environmental exposures and lifestyle-related risk factors such as smoking, diet and infections. Among the diverse cancer types, breast cancer is the most prevalent malignancy in women, accounting for nearly 670,000 deaths annually and affecting approximately 13.1% of women above the age of 40, who are at higher risk [2]. Conventional therapies like chemotherapy, despite being widely used, face limitations such as late-stage diagnosis, tumor resistance and severe side effects, thereby necessitating new, targeted treatment options [3].

Diabetes mellitus is another global health concern, with projections suggesting over 300 million affected individuals by 2025 [4]. Particularly, type 2 diabetes has shown alarming growth, with complications including nephropathy, retinopathy and cardiovascular disease. Among these, impaired wound healing presents life-threatening risks due to chronic ulcers, infections, gangrene and the potential need for limb amputation. Nearly 15% of diabetic patients experience foot ulcers and one in four of these cases progresses to amputation, with post-amputation mortality rates approaching 59% within 5 years [5,6]. The socio-economic and clinical burden highlights the need for innovative and safer therapies [7].

Medicinal plants, long employed in traditional medicine, offer a promising source of bioactive compounds with antioxidant, antibacterial, pro-angiogenic and immunomodulatory properties that support wound healing and broader disease management [6]. *Peperomia pellucida* (family Piperaceae) is a widely used ethnomedicinal plant in Asia, Africa and South America, valued both as a vegetable and as herbal remedy for conditions such as inflammation, diabetes, hypertension and dengue, as well as for promoting hair growth [8-10].

Phytochemical studies reveal that extracts of *P. pellucida* stems and leaves are rich in essential trace elements, including potassium, zinc, iron, manganese and copper, with no evidence of toxic heavy metals [11]. These compounds play key roles in detoxification, protein synthesis and cellular repair. Moreover, the plant exhibits strong antioxidant, antimicrobial, antihypertensive and antidiabetic activity, largely attributed to its ability to inhibit α-glucosidase [12,13]. Bioactive con-

stituents such as dillapiole, phytol and methyl esters have been linked to anticancer and antioxidant effects [14,15], while secondary metabolites including flavonoids, polyphenols, tannins, alkaloids and saponins contribute to wound healing and disease prevention [16].

This study aims to investigates the phytochemical profile of methanolic extracts of *P. pellucida* and evaluates their biological activities *in vitro*. Specifically, the antioxidant and antidiabetic potentials were assessed, along with cytotoxicity and apoptotic effects on MCF-7 breast cancer cells through MTT viability assays, AO/EB dual staining and DNA fragmentation analysis.

EXPERIMENTAL

Sample collection: Fresh leaves of *Peperomia pellucida* were collected in the morning from Aanamooli, Palakkad District, India (latitude 10.784703N; longitude 76.653145E). Healthy, disease-free leaves were selected for the study. The plant was taxonomically identified and authenticated by experts from the Botanical Survey of India, Tamil Nadu, India. A voucher specimen (No. 627) was deposited and preserved for future reference.

Sample preparation: Leaves were thoroughly washed with sterile distilled water to remove dust and surface contaminants. The cleaned leaves were air-dried in the shade at room temperature in a well-ventilated area to prevent degradation of heat- and light-sensitive compounds. The dried plant material was ground into a fine powder using a mechanical grinder. The powdered sample (10 g) underwent sequential Soxhlet extraction with methanol. The extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness at room temperature. Dried extracts were stored in airtight containers at 4 ºC until further use.

Phytochemical screening: Qualitative phytochemical screening of the methanolic extract was carried out using standard procedures described by Oloyede *et al.* [17]. Secondary metabolites tested included carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, steroids, anthraquinones and terpenoids.

Gas chromatography-mass spectrometry (GC-MS) analysis: The phytochemical constituents of methanolic extract of *P. pellucida* were analyzed using GC-MS on an Agilent 7890A gas chromatograph equipped with a 5975 inert mass selective detector and a triple-axis mass spectrometer. Separation was achieved using a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 150 ºC to 300 ºC. One microliter of extract was injected in split mode (10:1 ratio), with helium as the carrier gas at a constant flow rate of 0.8 mL/min. Each run was completed in 26 min. Compounds were identified by matching the obtained mass spectra with those in the NIST library database.

In vitro antioxidant and antidiabetic activity

DPPH radical scavenging assay: The antioxidant potential of the methanolic extract of *P. pellucida* was evaluated using the DPPH radical scavenging method, with slight modifications from Wakhidah *et al.* [18]. A 0.1 mM DPPH solution was prepared in methanol. Different concentrations of plant extracts (25, 50 and 100 μg/mL) were mixed with 2 mL of DPPH solution and incubated in the dark at room temperature for 30 min. The absorbance of the reaction mixtures was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid served as a standard reference antioxidant. The percentage of DPPH radical scavenging activity was calculated using the following equation:

α-Amylase inhibitory assay: The α-amylase inhibitory assay was performed with slight modifications of the method described by Tamil *et al.* [19]. Briefly, 100 μL of plant extract (25, 50 and 100 μg/mL) was mixed with 100 μL of α-amylase enzyme solution and incubated at 37 ºC for 30 min. Subsequently, 1% starch solution was added to each mixture and further incubated for 1 h at 37 ºC. The reaction was terminated by adding 200 μL of dinitrosalicylic acid (DNS) colour reagent, followed by boiling in a water bath for 5 min and cooling to room temperature. The absorbance was measured at 540 nm using a spectrophotometer. Acarbose was used as the standard inhibitor. The percentage inhibition was calculated relative to the untreated control and the IC_{50} values were determined for extract concentrations.

α-Glucosidase inhibitory assay: The α-glucosidase inhibitory activity was assessed following the protocol of Pistia-Brueggeman & Hollingsworth [20] with slight modifications. Plant extract solutions (25, 50 and 100 μg/mL) were prepared and 50 μL of each was added into 96-well microplates along with 10 μL of α-glucosidase enzyme (1 U/mL) and 125 μL of phosphate buffer (pH 6.8). The mixtures were incubated at 37 ºC for 20 min. After incubation, 20 μL of 1 M pNPG (4-nitrophenyl-D-glucopyranoside) was added as the substrate and the mixtures were incubated for 30 min. The reactions were terminated by adding 50 μL of 0.1 N Na_2CO_3. Absorbance of the reaction mixtures was measured at 405 nm using a microplate reader. Acarbose was used as the standard inhibitor. The percentage inhibition of α-glucosidase activity was calculated relative to the control.

$$\text{Inhibition } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100$$

Anticancer activity of methanolic extract of *P. pellucida* against human breast cancer cells

Cell culture: The MCF-7 human breast cancer cell line was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cultures were incubated at 37 ºC in a humidified atmosphere containing 5% CO_2.

MTT assay: The cytotoxic effect of the methanolic extract of *P. pellucida* on MCF-7 cells was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Briefly, cells were seeded into 96-well plates and allowed to form confluent monolayers. After 24 h, the cells were treated with different concentrations of extract (25, 50 and 100 μg/mL). Following incubation, MTT solution was added to each well and incubated for an additional period to allow mitochondrial succinate dehydrogenase enzymes in

viable cells to reduce MTT into insoluble purple formazan crystals. The crystals were solubilized and absorbance was measured at 570 nm using a microplate reader. Each concentration was tested in triplicate. The percentage of cell viability was calculated from the optical density (OD) values using the following formula:

$$\text{Viability (\%)} = \frac{\text{OD value of experimental samples}}{\text{OD value of experimental control}} \times 100$$

Morphological analysis: MCF-7 cells were seeded on sterile glass coverslips at a density of 1×10^5 cells per coverslip and treated with methanolic extracts of *P. pellucida* at concentrations of 20, 50 and 100 μg/mL for 24 h. Following treatment, cells were fixed with ethanol:acetic acid (3:1, v/v). Coverslips were mounted on glass slides and morphological changes were observed using an inverted bright-field light microscope (Nikon, Japan) at 40× magnification. Three independent fields were analyzed per experimental group to assess treatment-induced cellular alterations.

Lactate dehydrogenase (LDH) assay: Cytotoxicity was further evaluated using the LDH leakage assay. MCF-7 cells were exposed to *P. pellucida* extracts (25, 50 and 100 μg/mL) following the same treatment protocol as the MTT assay, except that the conditioned medium was collected for LDH quantification. Briefly, 0.1 mL of conditioned medium was mixed with 1 mL of buffered substrate and incubated at 37 ºC in a water bath. Subsequently, 0.2 mL of NAD^+ solution was added, mixed gently and incubated for 15 min at 37 ºC. DNPH (dinitrophenylhydrazine) reagent was then added, followed by another 15 min incubation. The reaction was terminated by adding 10 mL of 0.4 N NaOH and absorbance was recorded at 440 nm. A standard curve was prepared using sodium pyruvate for calibration. The extent of cell lysis was expressed as a linear function of colour intensity.

AO/EB dual staining for apoptosis: Apoptotic morphological changes were assessed using acridine orange (AO) and ethidium bromide (EtBr) dual staining. MCF-7 cells (1×10^5/well) were treated with *P. pellucida* methanol extract at concentrations of 25, 50 and 100 μg/mL for 24 h. After treatment, the cells were washed twice with PBS (pH 7.2) and incubated with a staining solution consisting of 100 mg/mL AO and EtBr in distilled water (10 μL of dye mix per sample). After 2 min of staining, cells were immediately washed with PBS and observed under a fluorescence microscope. Apoptotic features, including chromatin condensation, nuclear fragmentation and membrane blebbing, were recorded within 20 min of staining. Untreated cells served as negative controls, while 3% H_2O_2-treated cells were used as positive controls.

DNA fragmentation assay: Genomic DNA fragmentation was analyzed to confirm apoptosis induction in MCF-7 cells. Cells (1×10^6/well) were seeded into six-well plates in RPMI-1640 medium containing 10% FBS and antibiotics and allowed to adhere for 24 h. Cells were then treated with *P. pellucida* methanol extract at its IC_{50} concentration. Following incubation, cells were harvested, washed in PBS and lysed in 500 μL lysis buffer for 1 h. DNA was extracted using the phenol:chloroform:isoamyl alcohol method, precipitated with ice-cold isopropanol, air-dried and rehydrated in distilled water. DNA concentration and purity were determined spectrophotometrically. DNA samples were resolved on a 0.8% agarose gel alongside a 100 bp DNA ladder. Banding patterns were visualized using a gel documentation system (Bio-Rad, USA) to detect the characteristic DNA ladder pattern of apoptosis.

Statistical analysis: All *in vitro* experiments were carried out in triplicate and each experiment was repeated independently at least three times. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 17.0. A p-value ≤ 0.01 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical composition of methanolic extract of *P. pellucida*: Phytochemical screening of the methanolic extract of *P. pellucida* leaves revealed a diverse array of bioactive compounds. The extract contained alkaloids, flavonoids, tannins, anthraquinones, glycosides, quinones, saponins, phenols, terpenoids and carbohydrates (Table-1). This comprehensive phytochemical profile highlights the effectiveness of methanol in extracting a wide variety of polar secondary metabolites with potential pharmacological properties.

TABLE-1
PHYTOCHEMICAL RESULTS OF *P. pellucida* LEAVES EXTRACTED WITH METHANOL

Phytochemical	Methanol Extract
Carbohydrates	Present
Tannins	Present
Saponins	Present
Alkaloids	Present
Flavonoids	Present
Glycosides	Present
Quinones	Present
Phenols	Present
Steroids	Absent
Terpenoids	Present
Anthraquinones	Present

Numerous studies highlighted the significant role of phytoconstituents in the diverse biological activities of *P. pellucida*. The plant is recognized as a rich source of natural antioxidants, including polyphenols, tannins, flavonoids, saponins, alkaloids and terpenoids [21]. Fakayode *et al.* [22] identified various bioactive compounds in the methanolic extract such as alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, tannins, xanthoproteins and phlobatannins, corroborating earlier reports on the chemical profile of plant and its extensive traditional medicinal uses, especially for treating dermatitis and related ailments. Additional research confirms the presence of alkaloids, tannins, resins, flavonoids, steroids, phenols and carbohydrates, alongside notable nutritional components like carbohydrates and crude protein [23]. Similarly, these compounds have been reported in the methanolic extracts, whereas ethanolic extracts were found to contain tannins, saponins, flavonoids, reducing sugars and cardiac glycosides [17,24]. Collectively, these findings emphasize the phytochemical richness of *P. pellucida* and underline its considerable therapeutic potential.

GC-MS analysis: The chromatogram (Fig. 1) displayed several significant compounds, with the most abundant appearing as prominent peaks. The identified phytochemicals, along with their retention times (RT), molecular formulas (MF) and molecular weights (MW), are detailed in Table-2. In this study, GC-MS analysis detected a total of 34 compounds, of which 25 phytochemicals were positively identified based on retention indices and relative peak areas. Among these, seven major constituents accounted for the highest relative abundance: chondrillasterol (21.1%), 1-triacontanol (11.5%), β-amyrin (8.9%), β-amyrone (7.05%), 13,27-cycloursan-3-one (6.76%), octacosanol (6.33%) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.81%).

The phytochemical constituents identified in *P. pellucida* exhibit a broad range of significant biological activities, highlighting their versatile applications in therapeutic and agricultural fields. For instance, chondrillasterol demonstrates strong antibacterial activity, positioning it as a promising agent against bacterial infections [25]. Benzamide has been documented for its anti-inflammatory properties, supporting its potential use in managing inflammation-related disorders [26]. In agriculture, 1-triacontanol plays a crucial role in promoting plant growth and accelerating fruit ripening, underscoring its value in enhancing crop production [27]. Furthermore, β-amyrin

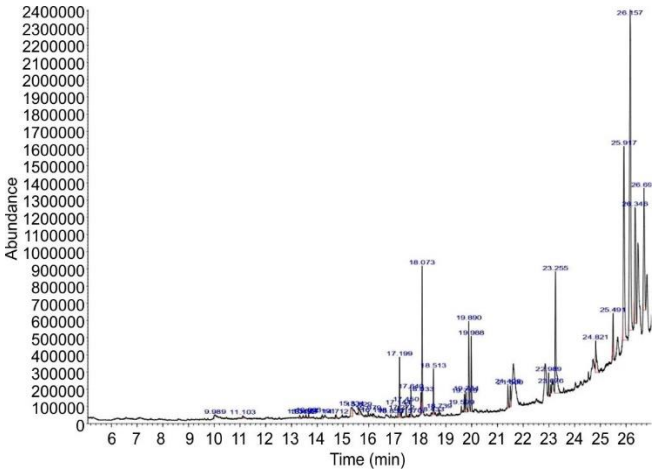

Fig. 1. Gas chromatography-mass spectrometry (GC-MS) chromatogram of methanolic leaf extract of *P. pellucida*

TABLE-2
IDENTIFIED PHYTOCONSTITUENTS OF METHANOLIC LEAF EXTRACT OF *Peperomia pellucida* Based on GC-MS SPECTRUM

S. No.	RT (min)	Compound	m.f.	Area T (%)	m.w. (g/mol)
1	15.3345	α-Methyl mannofuranoside	$C_7H_{14}O_6$	1.50	194.18
2	15.6294	β-Asarone	$C_{12}H_{16}O_3$	0.65	208.25
3	17.1443	Benzamide, 3,4,5-trimethoxy-	$C_{10}H_{13}NO_4$	0.37	211.21
4	17.1989	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate,	$C_{22}H_{42}O_2$	1.72	338.57
5	17.2717	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.21	268.47
6	17.4502	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	$C_{22}H_{42}O_2$	0.38	338.57
7	17.6395	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	$C_{22}H_{42}O_2$	0.82	338.57
8	18.0328	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	0.60	268.43
9	18.0729	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	4.20	270.44
10	18.5135	1,4-Dibutyl benzene-1,4-dicarboxylate	$C_{16}H_{22}O_4$	1.43	278.34
11	18.7393	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	0.16	298.49
12	19.5987	E-15-Heptadecenal	$C_{17}H_{32}O$	0.23	252.43
13	19.7188	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	0.48	294.47
14	19.7844	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	0.86	292.45
15	19.8900	Phytol	$C_{20}H_{40}O$	2.86	296.53
16	19.9883	Methyl stearate	$C_{19}H_{38}O_2$	2.06	298.51
17	21.4085	*n*-Nonadecanol-1	$C_{19}H_{40}O$	0.75	296.53
18	21.4995	Glycidyl palmitate	$C_{19}H_{36}O_3$	0.65	328.49
19	21.6197	Octacosanol	$C_{28}H_{58}O$	6.33	410.78
20	22.8614	Vitamin E	$C_{29}H_{50}O_2$	2.82	430.72
21	22.9889	3-Octyl-6,10b-dihydro-[1,2,4]thiadiazino[5,4-*a*]isoindol- 4-one 2,2-dioxide	$C_{18}H_{26}N_2O_3S$	0.56	362.47
22	23.0763	4-Methyldocosane	$C_{23}H_{48}$	0.18	324.62
23	23.1273	1*H*-Indene, 1-hexadecyl-2,3-dihydro-	$C_{25}H_{42}$	0.80	346.60
24	23.2547	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	5.81	330.51
25	23.5752	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene- 1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	0.13	495.49
26	24.8205	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	1.28	358.55
27	25.4906	Gomisin L1	$C_{22}H_{26}O_6$	1.63	390.44
28	25.6690	Stigmasterol	$C_{29}H_{48}O$	1.54	412.71
29	25.9166	1-Triacontanol	$C_{30}H_{62}O$	11.5	438.80
30	26.1570	Chondrillasterol	$C_{29}H_{48}O$	21.1	412.71
31	26.3463	β-Amyrone	$C_{30}H_{48}O$	7.05	424.74
32	26.4592	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	6.76	424.74
33	26.6923	β-Amyrin	$C_{30}H_{50}O$	8.90	426.76
34	26.8124	Germanicol	$C_{30}H_{50}O$	3.50	426.76

exhibits antibiofilm and antidiabetic activities, which may aid in controlling persistent infections and metabolic diseases [28].

Several compounds also contribute to eco-friendly pest management as natural alternatives to synthetic pesticides. For example, 13,27-cycloursan-3-one possesses notable antioxidant and antimicrobial properties, providing both protective and therapeutic benefits [29]. Octacosanol is associated with diverse biological effects including anti-fatigue, anti-hypoxia, antioxidant, anti-inflammatory and antitumor activities [30]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester exhibits multifunctional bioactivities combining antioxidant, antimicrobial and anticancer effects, enhancing its pharmacological significance [31]. Moreover, heneicosane is recognized for its antimicrobial capacity, supporting the development of novel natural antimicrobial agents [32]. These results demonstrate the complex and diverse chemical composition of *P. pellucida* methanol extract, underscoring its potential as a rich source of bioactive compounds.

Antioxidant activity

DPPH radical scavenging assay: The methanolic extract of *P. pellucida* demonstrated significant free radical scavenging activity in the DPPH assay, with inhibition percentages of 25.62%, 34.67% and 58.28% at concentrations of 25, 50 and 100 µg/mL, respectively (Fig. 2). The IC_{50} values for ascorbic acid and the methanol extract were 72.1 µg/mL and 75.4 µg/mL, respectively. At the highest concentration tested (100 µg/mL), the extract exhibited its maximum scavenging activity of 58.28%, closely comparable to ascorbic acid's 64.59% inhibition (Table-3). Medicinal plants are renowned for their abundance of natural antioxidants and phenolic compounds, which counteract oxidative stress by neutralizing harmful free radicals [33]. The efficiency of extracting these antioxidant compounds is strongly influenced by the choice of solvent, as evidenced by varying DPPH scavenging activeities reported across solvents [34]. For example, *P. pellucida* extracts prepared using methanol, butanol and ethyl acetate

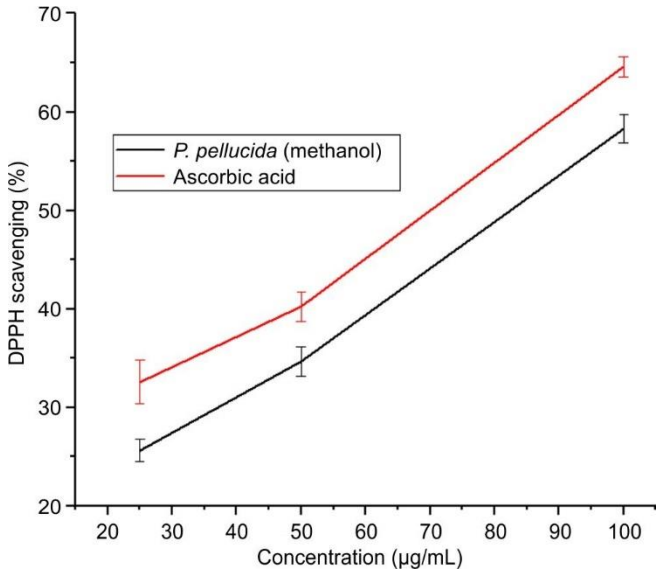

Fig. 2. DPPH radical scavenging activity (%) of *P. pellucida* extracts (methanol) compared to ascorbic acid (control). Values are mean ± SD (n = 3)

TABLE-3
DPPH FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACTS FROM *P. pellucida*

Concentration (µg/mL)	DPPH assay of different solvent extracts	
	Methanol extract	Ascorbic acid
25	25.62 ± 1.1	32.56 ± 2.2
50	34.67 ± 1.5	40.25 ± 1.5
100	58.28 ± 1.4	64.59 ± 1.0
IC_{50} (µg/mL)	75.4	72.1

Values are mean ± SD (n = 3)

exhibited DPPH IC_{50} values of 79.0, 87.3 and 74.0 µg/mL, respectively [35].

Further confirming its antioxidant potential, Uwaya *et al.* [36] observed activity in both aqueous and methanolic extracts of *P. pellucida* through multiple assays including DPPH, ABTS and hydrogen peroxide scavenging. Similarly, reported high antioxidant activity in methanol extracts of the leaves [37]. Collectively, these studies highlight methanol as a highly effective solvent for isolating antioxidant-rich phytochemicals from *P. pellucida*, reinforcing its promise as a natural antioxidant source.

***In vitro* α-amylase and α-glucosidase inhibitory activity:** The methanolic extract of *P. pellucida* exhibited significant inhibitory effects against α-amylase and α-glucosidase enzymes, with IC_{50} values of 60.8 µg/mL and 52.8 µg/mL, respectively (Table-4). These inhibitory concentrations are comparable to those of the positive control, acarbose, which showed IC_{50} values of 67.4 µg/mL for α-amylase and 68.4 µg/mL for α-glucosidase (Fig. 3). Plants traditionally used for diabetes management frequently contain bioactive compounds that enhance insulin function or inhibit carbohydrate-hydrolyzing enzymes, thereby reducing glucose absorption and exerting hypoglycemic effects. The antidiabetic potential of *P. pellucida* has been documented in earlier studies by Sheikh *et al.* [38] and Kanedi & Mumtazah [39]. Peperochromen-A has been identified as a key bioactive compound underlying this activity [40]. Sultana *et al.* [41] reported antidiabetic properties of methanolic extracts of *P. pellucida*. Moreover, various fractions of *P. pellucida* with the exception of the non-toxic aqueous fraction exhibited stronger α-glucosidase and α-amylase inhibition than the standard drug acarbose [14]. Collectively, these findings underscore the promising potential of *P. pellucida* as a natural source of antidiabetic agents.

***In vitro* anticancer activity:** The cytotoxic effects of the methanolic extracts of *P. pellucida* on MCF-7 breast cancer cells were evaluated at concentrations of 25, 50 and 100 µg/mL using a cytotoxicity assay (Table-5). Untreated control cells (0 µg/mL) exhibited over 99% viability, confirming no inherent cytotoxicity. Treatment with 25 µg/mL of the extract induced moderate cytotoxicity, reducing cell viability to 67.9% (32.3% cytotoxicity). Increasing the concentration to 50 µg/mL significantly enhanced cytotoxicity to 51.6%, corresponding to a viability of 48.6%. At 100 µg/mL, cytotoxicity further increased to 66.9%, with cell viability dropping to 33.3%. The calculated IC_{50} value was 48.5 µg/mL, indicating the concentration required to inhibit 50% of the cells. The

TABLE-4
IC_{50} VALUES FOR α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITIES

Concentration (μg/mL)	α-Amylase inhibitory effect		α-Glocosidase inhibitory effect	
	Methanol extract	Acarbose	Methanol extract	Acarbose
25	27.67 ± 1.8	24.14 ± 1.5	31.17 ± 2.5	28.50 ± 2.1
50	44.15 ± 2.1	38.56 ± 1.7	49.10 ± 1.6	35.61 ± 0.9
100	77.31 ± 1.2	69.50 ± 2.0	80.15 ± 1.1	72.23 ± 1.7
IC_{50} (μg/mL)	60.8	67.4	52.8	68.4

Values are mean ± SD (n = 3).

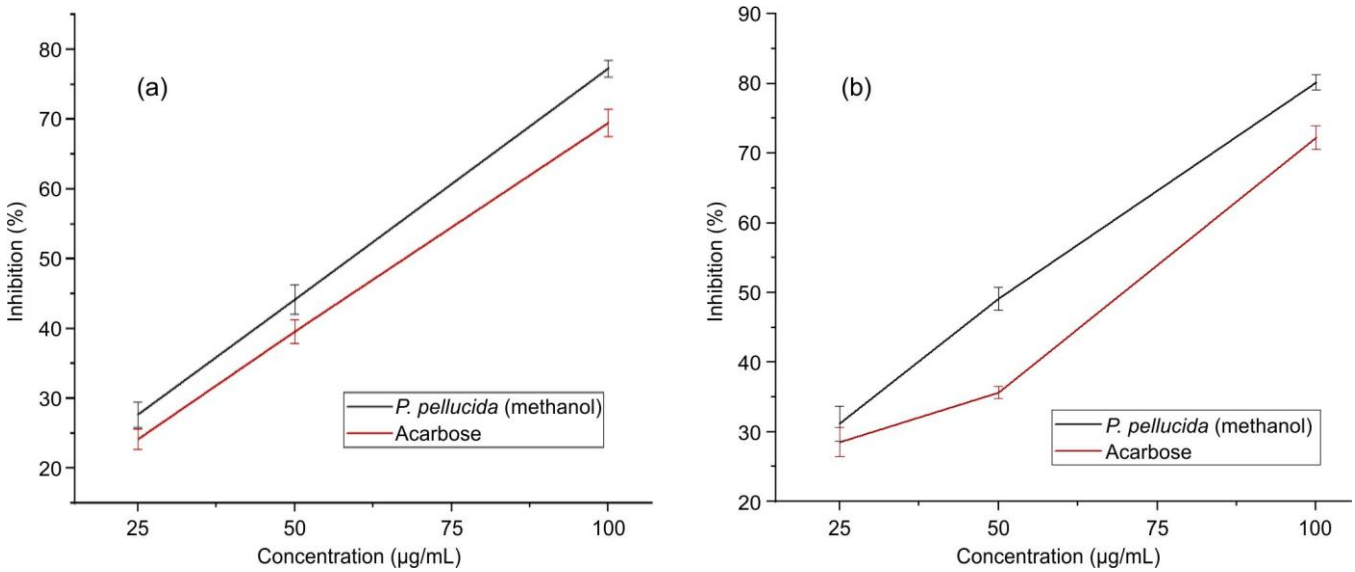

Fig. 3. *In vitro* α-amylase (a) and α-glocosidase (b) inhibitory activities of methanolic extract of *P. pellucida*

TABLE-5
CYTOTOXICITY ASSAY DATA OF *P. pellucida* METHANOLIC EXTRACT ON MCF-7 BREAST CANCER CELLS

Concentration (μg/mL)	Cytotoxicity (%)	Cell viability (%)	IC_{50}
Control	0	> 99	No cytotoxicity
25	32.3 ± 1.25	67.9 ± 0.57	
50	51.6 ± 0.75	48.6 ± 0.75	IC_{50} = 48.5 μg/mL
100	66.9 ± 0.57	33.3 ± 1.25	
10 (Doxorubicin)	68.3 ± 1.25	31.9 ± 0.57	Cytotoxicity

Values are mean ± SD (n = 3).

positive control, doxorubicin (10 μg/mL), demonstrated 68.3% cytotoxicity and 31.9% viability, validating the reliability of assay (Fig. 4).

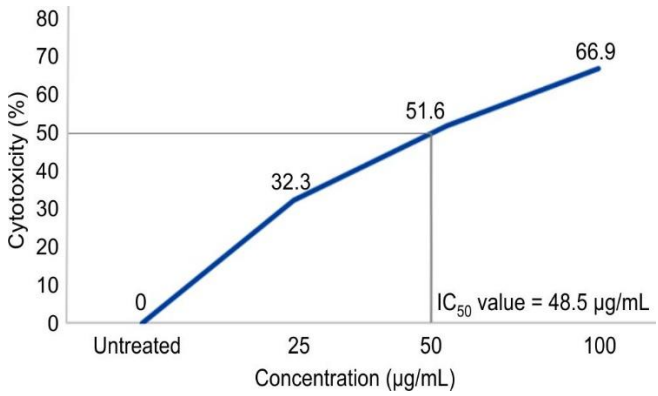

Fig. 4. IC_{50} Determination of *P. pellucida* methanolic extract against MCF-7 breast cancer cell lines

These results align with prior studies demonstrating the anticancer potential of *P. pellucida*. Angelina *et al.* [42] reported IC_{50} values of 96.7 ppm and 2110.8 ppm for *n*-hexane fraction of *P. pellucida* against MCF-7 and Vero cells, respectively. Wei *et al.* [43] observed a reduction of MCF-7 cell viability to 72% at 0.5 μg/mL and further to 54% at 30 μg/mL, with an IC_{50} of 10.4 ± 0.06 μg/mL for the extract. Furthermore, isolated peperomin E from *P. pellucida* leaf extract, which inhibited multiple cancer cell lines, including HL-60, MCF-7 and HeLa, with IC_{50} values ranging from 1.8 to 11.1 μg/mL [44].

Morphological analysis: MCF-7 cancer cells were treated with methanolic extracts of *P. pellucida* at concentrations of 25, 50 and 100 μg/mL for 24 h, inducing significant morphological alterations as shown in Fig. 5. The extent of cellular changes was dose-dependent, with increasing concentrations correlating with enhanced cytotoxicity. Treated cells exhibited hallmark features of apoptosis, including cellular shrinkage,

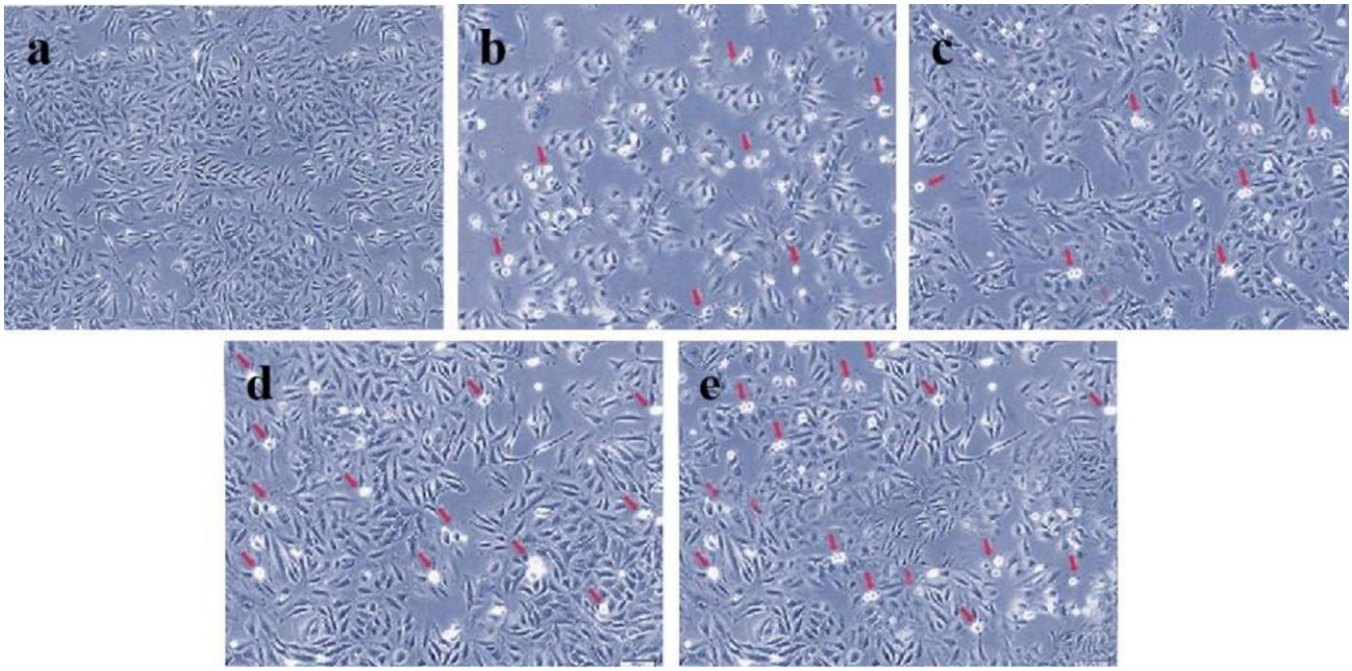

Fig. 5. Morphological analysis of MCF-7 cells treated with methanolic extracts of *P. pellucida* for 24 h: (a) untreated control, (b) 25 µg/mL, (c) 50 µg/mL, (d) 100 µg/mL and (e) positive control (doxorubicin)

membrane blebbing, cytoplasmic condensation and a reduction in the number of viable cells (Figs. 5b-d). In contrast, untreated control cells maintained normal morphology with no signs of cytotoxicity or structural disruption (Fig. 5a). At the highest concentration tested (100 µg/mL), the cytotoxic effect of extract approached that of the positive control, doxorubicin, which induced 66.9 ± 0.57% cytotoxicity (Fig. 5e). These morphological changes are consistent with classical apoptotic processes, beginning with cell rounding and the formation of circular membrane protrusions (blebs), followed by elongation into thin membrane extensions and eventual fragmentation into apoptotic bodies [45,46]. *In vitro*, apoptotic cells that evade phagocytosis later progress to secondary necrosis, displaying features resembling necrotic cells [47].

LDH assay: Lactate dehydrogenase (LDH) is a cytoplasmic enzyme released into the extracellular environment upon loss of cell membrane integrity, serving as a sensitive indicator of membrane damage [48]. Elevated LDH release is widely recognized as an early marker of necrotic cell death and correlates with increased cellular injury [49]. In this study, treatment of MCF-7 breast cancer cells with methanolic extracts of *P. pellucida* induced a significant, dose-dependent increase in LDH release (Table-6). The IC_{50} value for LDH-mediated cytotoxicity was determined as 48.5 µg/mL, indicating the concentration at which 50% of cells exhibited membrane damage (Fig. 6). These results emphasize the cytotoxic and anticancer potential of the extract, as reflected by elevated LDH levels in the culture medium, signifying membrane rupture and necrotic cell death. Recent reports also suggest that LDH release assays provide a more reliable and precise measure of cytotoxicity compared to other techniques, particularly because cells tend to undergo complete fragmentation during prolonged exposure to cytotoxic agents [50].

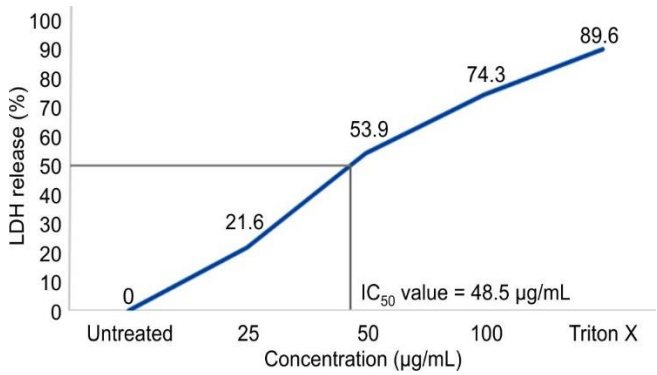

Fig. 6. Lactate dehydrogenase (LDH) release assay of *Peperomia pellucida* methanolic extract in MCF-7 breast cancer cell lines

TABLE-6
LACTATE DEHYDROGENASE (LDH) RELEASE ASSAY DATA OF *P. pellucida* Methanolic EXTRACT ON MCF-7 CELL LINES

Concentration (µg/mL)	LDH cytotoxicity (%)	Cell viability (%)	IC_{50}
Control	0	> 99	No cytotoxicity
25	21.6 ± 0.75	78.6 ± 0.75	
50	53.9 ± 0.57	46.3 ± 1.25	IC_{50} = 48.5 µg/mL
100	74.3 ± 1.25	25.9 ± 0.57	
10 (Triton X)	89.6 ± 0.75	10.6 ± 0.75	Cytotoxicity

Values are mean ± SD (n = 3).

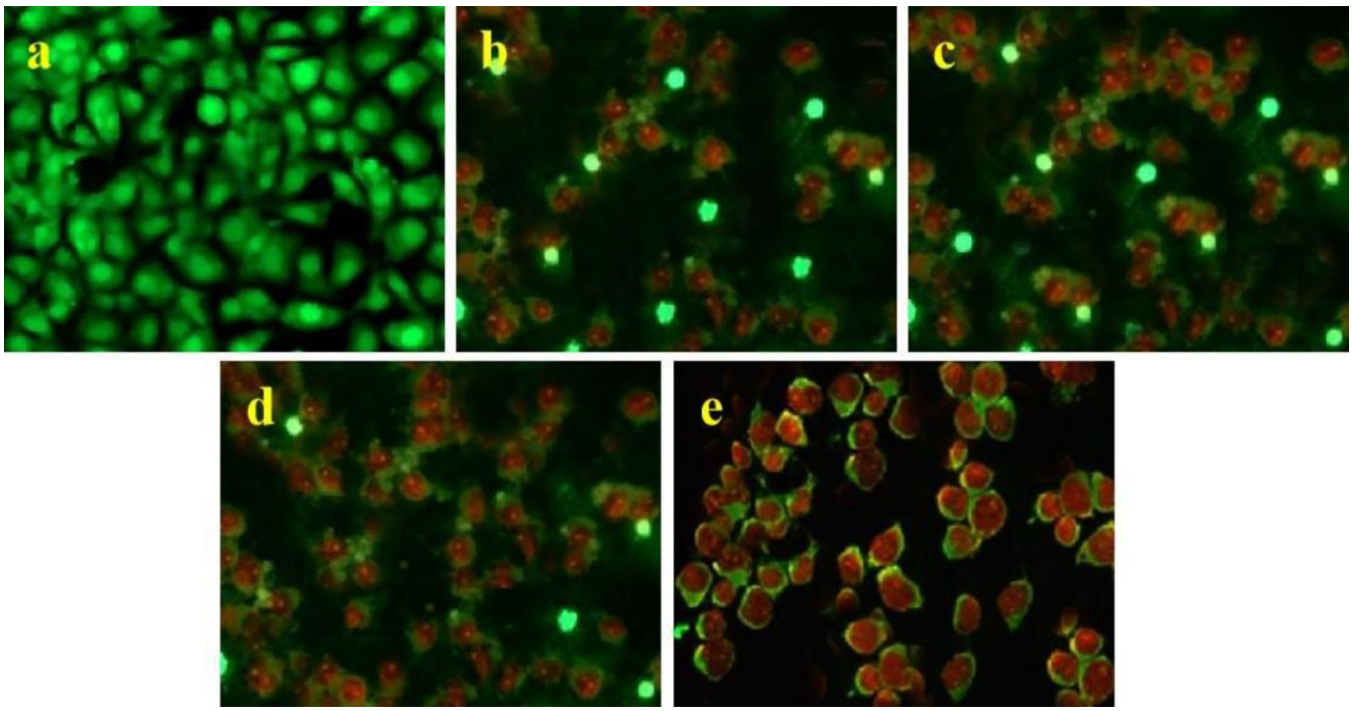

Fig. 7. AO/EtBr staining assay depicting apoptotic changes in MCF-7 cells treated with methanolic extracts of *P. pellucida* for 24 h: (a) untreated control, (b) 25 µg/mL, (c) 50 µg/mL, (d) 100 µg/mL and (e) positive control (3% H_2O_2)

Fluorescence microscopic analysis: The apoptogenic effects of methanolic extracts of *P. pellucida* on MCF-7 cancer cells were evaluated using fluorescence microscopy. Live cells emitted bright green fluorescence due to acridine orange uptake, whereas dead or apoptotic cells showed distinct orange to red fluorescence. Fig. 7a illustrates a predominance of viable cells in the untreated control group. In contrast, MCF-7 cells treated with methanolic extracts exhibited a marked increase in apoptotic cells and apoptotic bodies, evidenced by morphological changes such as nuclear shrinkage, nuclear damage and membrane blebbing, which appeared as orange to red fluorescent bodies (Figs. 7b-d). Cells treated with 3% H_2O_2, serving as a positive control for apoptosis induction, displayed similar morphological features (Fig. 7e). Acridine orange staining revealed bright green fluorescence indicative of early apoptosis characterized by nuclear fragmentation and chromatin condensation. The progression to orange-red fluorescence signified late apoptosis or necrosis, reflecting compromised cell membrane integrity. These observations are consistent with prior studies, which demonstrate a correlation between fluorescence colour changes and distinct stages of cell death [51-53].

DNA fragmentation analysis: DNA fragmentation assay further validated apoptosis induction in MCF-7 cells treated with methanolic extracts of *P. pellucida*. As shown in Fig. 8, DNA from untreated control cells remained intact, indicating no apoptosis. Conversely, treated cells exhibited marked DNA fragmentation, demonstrated by characteristic ladder-like bands alongside continuous smear patterns on the gel. The formation of DNA ladders is a definitive hallmark of apoptosis, reflecting internucleosomal cleavage of genomic DNA, while the smear suggests extensive DNA degradation consistent with systematic cleavage. Together, these patterns strongly support

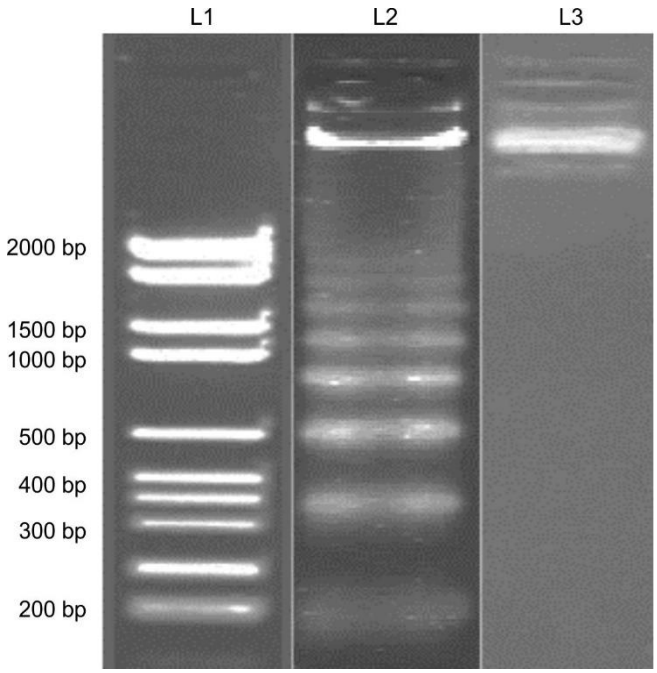

Fig. 8. DNA fragmentation analysis of MCF-7 cells treated with methanolic extracts of *P. pellucida*. L1: 1 kb DNA ladder marker; L2: Fragmented DNA from treated MCF-7 cells; L3: Intact genomic DNA from untreated MCF-7 cells

that *P. pellucida* extracts induce cell death *via* genomic DNA fragmentation in MCF-7 cells.

However, DNA fragmentation alone is not an exclusive marker of apoptosis, as necrotic cell death can also involve internucleosomal DNA cleavage [54,55]. Nonetheless, caspase activation remains a central, conserved hallmark of apoptosis, acting as a pivotal step in orchestrating programmed cell

death [56]. The apoptotic activity here parallels findings by Yang *et al.* [57], who reported dose- and time-dependent apoptosis induction by *Antrodia camphorata* in MCF-7 cells, underscoring the reliability of DNA fragmentation as an apoptosis indicator in cancer cells treated with botanical extracts.

Conclusion

Methanolic extracts of *Peperomia pellucida* are rich in diverse bioactive phytochemicals such as alkaloids, flavonoids, terpenoids and phenolic compounds, which underpin it pharmacological properties. The extract demonstrated significant antioxidant activity, highlighting its ability to scavenge free radicals and donate electrons effectively. Remarkably, it also exhibited strong *in vitro* antidiabetic activity, likely attributable to these bioactive constituents. In addition, the extract exerted a dose-dependent cytotoxic effect on MCF-7 breast cancer cells by inducing apoptosis, as confirmed through AO/EB dual staining and DNA fragmentation assays. This apoptotic induction suggests that *P. pellucida* impairs cancer cell viability by promoting programmed cell death rather than necrosis. These findings collectively position *P. pellucida* methanolic extracts as promising candidates for therapeutic development, particularly in breast cancer treatment. The study lays a foundation for further research into the plant's anticancer potential and supports its role in advancing natural product-based biomedical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

The authors declare that no AI tools were used in the preparation or writing of this research/review article.

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