Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Assessment of the Phytochemical Composition, Antioxidant Activity, and *In-vitro* Cytotoxic Effects of *Begonia malabarica* on Pancreatic Cancer Cell Line

Selvanayahi Sakthivadivel[1], Sabaridasan Arumugam[2], Muralidharan Murugan[1], Soranam Ramaiah[1*]

[1]*Department of Environmental Sciences, Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India.*

[2]*PG and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ulundurpet, Tamil Nadu, India.*

ARTICLE INFO

Article history:

Received: 04 December, 2023

Revised: 04 December, 2023

Accepted: 10 January, 2024

Published: 30 January, 2024

Keywords:

Medicinal plant, Antioxidant activity, Cytotoxicity, PANC-1 cell line.

DOI:

10.25004/IJPSDR.2024.160116

ABSTRACT

The study assesses the biological properties of methanolic extracts derived from the leaves, stems, and roots of *Begonia malabarica*, a native plant species in the mountainous area of southern India. The gas chromatography-mass spectrometry (GC-MS) was used to analyze the phytochemicals in the solvent from *B. malabarica* that had been extracted with methanol. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and as reference solution is ascorbic acid. The cytotoxicity activity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) approach. The GC-MS analysis effectively revealed 35 distinctive phytomolecules. The primary constituents, namely tert-butylbenzene (23%), 2-methylnaphthalene (6.2%), ethyl ester of octadecanoic acid (0.9%), and (Z,Z)-9,12-octadecadienoic acid (0.9%), are noted. The results, the methanolic extracts from *B. malabarica* are antioxidants that have significant levels ($p < 0.05$) of DPPH radical scavenging activities at different doses. The detected radical scavenging activities exhibited a higher proportion in the stem of *B. malabarica*. The IC_{50} values for the methanolic extracts were found to be 0.77, 0.49, and 0.68 mg/mL, respectively. The MTT analysis demonstrated that the methanolic extracts exhibited a considerable increase in cytotoxic activity against the pancreatic cancer (PANC-1) cell line, resulting in a cell viability percentage of 69.63% at a concentration of 31.25 µg/mL. These findings confirm the possible biological effects of *B. malabarica* and its prospective use in different pharmaceutical pursuits in the future.

Introduction

Natural resources and compounds are now recognized as a significant reservoir of potent anticancer drugs.[1] So far, more than 1000 plant species have been identified for their chemopreventive or anticancer characteristics.[2] In animals, the precise regulation of cell cycle regulatory proteins is crucial for maintaining the appropriate size and shape of cells.[3] However, disruptions in these regulatory mechanisms have been observed, causing cells to divide indefinitely and ultimately leading to the development of cancer.[4] According to the World Health Organization (WHO), there will be around 500,000 new cases of pancreatic cancer globally, leading to 470,000 deaths.[5] The global incidence rate for pancreatic cancer is 4.9 per 100,000, with a mortality rate of 4.5 per 100,000. Disturbingly, pancreatic cancer rates have risen significantly in most countries worldwide.[6]

Ethnopharmacology includes the full description of traditional knowledge and its experimental validation against people and domestic animals.[7] It might help with the management, use, and distribution of natural resources for future use.[8] According to studies, more than 80% of the world's population uses complementary and alternative medicines, with natural resources accounting

***Corresponding Author:** Dr. Soranam Ramaiah

Address: Department of Environmental Sciences, Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India.

Email ✉**:** soranamr@gmail.com

Tel.: +91-9486409520

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

for around 60% of clinical pharmaceuticals.[9] Despite breakthroughs in the field of chemotherapy, the use of medicinal plants has recently expanded. One possible reason is that medicinal plants are used to get active pharmaceutical ingredients or as building blocks for the production of chemicopharmaceuticals.[10]

An antioxidant is a molecule that is stable enough to transfer an electron to a free radical and neutralize it, limiting its ability to cause harm.[11] These antioxidants delay or prevent cellular damage primarily by scavenging free radicals.[12] Furthermore, it has been observed that methanol extracts have promising antioxidant activity *in-vitro.*[13] Considering the significant impact of inflammation and oxidative stress on the process of carcinogenesis.[14] Nevertheless, prior research about the cytotoxic effects of this particular plant has not been conducted.[15]

The plant kingdom synthesizes endogenous secondary metabolites that are currently under investigation for their anticancer properties, with the aim of developing novel pharmaceutical medications. The demand for naturally occurring chemicals generated from medicinal plants and their inherent qualities render them attractive as potential therapies for cancer.[16] Plants with medicinal properties pharmacologically active plants generally referred to as herbs; continue to be the primary type of medication in the majority of countries. These plants are considered a significant reservoir of medicinal phytochemicals, specifically phenolic and flavonoids, which have the potential to be used in the creation of groundbreaking cancer therapies.[17] Plant-based antioxidants' pharmacological effectiveness can be judged by testing their cytotoxicity in different cell lines. The need for low cytotoxicity in normal cells is a key factor in choosing these molecules as possible chemotherapeutic agents.[18]

Begoniaceae is a vast genus of flowering plants, considered one of the largest in the world, with about 1900 species.[19,20] The genus comprises herbaceous plants or climbing vines and is found in tropical and subtropical locations worldwide.[21] There are about 959 species in all, with 19 identified sections in Asia, the majority of which are found in Southeast Asia. *Begonia malabarica* Lam., which has been rendered into Tamil as Narayanachanjeeve, is an herb that is native to the hilly regions of Sri Lanka and southern India.[22] Substituting the leaves for tamarind (*Tamarindus indica* L., Caesalpiniaceae), the Paliyan tribes of the Tirunelveli district in Tamil Nadu ingest the substance after it has been cooked. For the treatment of gastric ulcers, stomachaches, and respiratory issues, they consume boiled leaves.[23] The phytochemical analysis of the leaves has revealed the presence of friedelin, epi-friedelinol, β-sitosterol, luteolin, quercetin, and β-sitosterol-3-β-D-glucopyranoside. Nevertheless, the biological activity of this species has been documented to exhibit antibacterial and antifungal properties in the different extracts derived from *B. malabarica* leaves[23-27] have all documented the antitumor properties of *B. tuberhybrida var. alba, B. plebeja,* and *B. heracleifolia*. Furthermore, the thorough examination could be a powerful reservoir of medication and could function as an efficacious remedy in the future. So, the goal of this study would be to look at the phytochemical composition, antioxidants, and cytotoxicity of the *B. malabarica* methanolic extract in relation to the pancreatic cancer cell line.

Materials and Methods

Plant Material Collection and Identification

B. malabarica's fresh leaves, stem, and roots were procured from the Virudhunagar district in Tamil Nadu, India (11.00' and 12.00'N; 77.28'and 78.50'E). The choice of the plant under investigation was determined by its historical use in traditional medicine. The medicinal plants selected for examination were gathered and preserved in the herbarium of the PG and Research Department of Botany at V.O. Chidambaram College, located in Thoothukudi, Tamil Nadu, India, with the purpose of retaining them for future reference. The verification of the botanical nomenclatures of the specimens was conducted by consulting regional floras (11-13). The leaves that were gathered were subjected to a washing process and afterward dried using filter paper and air.

Preparation of Crude Plant Extracts

The plant samples were brought to the lab, washed well with running tap water, and then rinsed in distilled water to get rid of dirt and debris. The samples were then dried in the shade and ground up. To make the ethanol extracts, 300 g of each of the dry, powdered plant ingredients were soaked in 1 L of ethanol at room temperature for 72 hours. Each of the final extracts went through No. 1 Whatman filter paper. The filtrates were then concentrated on a rotating evaporator at 40°C under a vacuum and kept at 4°C for later use.

The percentage yield of the plant extract was determined using the prescribed formula.

In this context, Cx represents the weight of the plant material after the extraction procedure, while CY represents the weight of the plant material that was first collected for extraction.

GC-MS Analysis

The extracts were dissolved in 1:1 ethyl alcohol. To analyze the crude extract, 2 µL was diluted in HPLC grade methanol and analyzed using JEOL GC mate with a secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC gas chromatography system). The column (HP5) was 50 m×0.25 mm I.D. fused silica. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for the column, 240°C for the injector, helium carrier gas, and 5:4 split ratio. The sample (1 µL)

was evaporated in a split-less injector at 300°C for 22 minutes. These compounds were detected by gas chromatography and mass spectrometry (GC-MS) as described.[28] The NIST database was used to interpret the GC-MS mass spectrum and find out the molecular weight, formula, and structure of the test material compounds.

Determination of DPPH Scavenging Assay

An approach for assessing the DPPH radical scavenging capacity of the extract was described.[29] Mix 0.5 mL of the sample solution dissolved in methanol with 2.5 mL of DPPH-containing 0.5 mM methanolic solution. The solution was subjected to vigorous agitation and thereafter placed in a dark environment at room temperature for 30 minutes. The measurement of absorbance was conducted at a wavelength of 517 nm using a UV spectrophotometer. The positive control used in the experiment was ascorbic acid. The percentage of DPPH free radical scavenging ability was determined through the use of a specific formula.

Where, Ac - absorbance of the control response; As - absorbance of the test sample.

Cytotoxicity Assay

PANC-1 cells were used to test the cytotoxicity of methanolic extracts of *B. malabarica*. The cell line was bought from the Pondicherry Centre for Biological Sciences and Educational Trust (Registration Number 2840/B4/2016, Pondicherry). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction test was the initial homogeneous assay designed to assess cell viability in a 96-well format, specifically tailored for high-throughput screening (HTS) purposes.[30] The PANC-1 cell line, which is associated with pancreatic cancer, was cultured individually on 96-well plates. It was plated with 1×10^4 cells per well in EMEM media that had 1X antibiotic antimycotic solution and 10% fetal bovine serum added to it. The culture was maintained in a CO_2 incubator at a temperature of 37°C with a CO_2 concentration of 5%. The cells were rinsed with 200 µL of 1X phosphate-buffered saline (PBS). Subsequently, the cells were exposed to different concentrations of the compound in serum-free media and incubated for 24 hours. The culture medium emerged from the cells upon completion of the treatment duration. A solution of MTT at a concentration of 0.5 mg/mL in 1X PBS was introduced and subjected to incubation at a temperature of 37°C for 4 hours within a CO_2 incubator. Following the incubation period, the media containing MTT was removed from the cells and subsequently washed using 200 µL of PBS. The crystals that had been generated were dissolved by adding 100 µL of dimethyl sulfoxide (DMSO) and ultimately mixed. At 570 nm, the emergence of color intensity was examined. The formazan dye undergoes a color change to a shade of purple-blue. The measurement of absorbance was conducted at a wavelength of 570 nm using a microplate reader.

Statistical Analysis

All the analyses were conducted in triplicate. The mean value and standard deviation (SD) were computed in order to assess the percentage inhibition or percentage antioxidant activity. An analysis of variance (ANOVA) was conducted to compare the data, and the statistical analysis was performed using SPSS version 20. The antioxidant properties of the extracts were assessed using a one-way analysis of variance (ANOVA) and Tukey's test. $p \leq 0.05$ was deemed to be statistically significant.

Results and Discussion

Chemical Composition of Methanolic Extracts by GC-MS Analysis

The chemical composition of *B. malabarica* plant extracts was done to find out which phytomolecules might be responsible for the anticancer effects of plant extracts. In order to assess, compounds in the methanolic extract of *B. malabarica* using GC-MS. Fig. 1 shows the gas chromatograms that were made from the methanolic extract of *B. malabarica*, and Table 1 shows the chemical compounds that have been found. In all, a total of 35 compounds were identified by the utilization of GC chromatogram, retention time, peak area analysis of *B. malabarica* plant extract, and comparison of the mass spectra of each constituent with the entries in mass spectra databases such as NIST and Wiley libraries.

Among the compounds, provisional identifications were made for those that accounted for 98.8% of the total peak area of the methanolic extract of *B. malabarica.* The methanolic extracts of *B. malabarica* were found to contain several prominent phenolic compounds, including benzene, tert-butyl- (23.0%), propane, 1,1,3-triethoxy-(9.1%), ethyl undecyl carbonate (7.6%), 9,10-dimethyltricyclo[4.2.1.1(2,5)] decane-9,10-diol (6.7%), L-alpha-terpineol (6.4%), and naphthalene, 2-methyl (6.2%), as summarized in Table 2. The methanolic extract of *B. malabarica* contained several major components, predominantly esters. These esters included 9,12-octadecadienoic acid, ethyl ester (0.9%), octadecanoic acid, ethyl ester (0.9%), decanoic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-yl ester (0.68%), hexadecanoic acid, ethyl ester (0.65%), and pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.6%). In contrast, the methanolic extract included fatty acid components, including 9,12-octadecadienoic acid (Z,Z)- and octadecanoic acid (0.9%), as indicated in Table 1.

Determination of DPPH Antioxidant Assay

In order to assess the antioxidant capacity of methanolic extracts derived from various parts of *B. malabarica*, we conducted an analysis of the scavenging activity of DPPH radicals. This analysis included methanolic extracts from different parts of *B. malabarica* as well as a standard compound, ascorbic acid. As shown in Table 2, the

Table 1: *B. malabarica* phytochemical compounds are extracted with methanol solvent

S. No.	*Compounds*	*MF*	*MW*	*RT (min)*	*% Peak area*
1.	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	4.67	0.6
2.	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	$C_{10}H_{16}$	136	6.84	0.61
3.	Ethyl undecyl carbonate	$C_{14}H_{28}O_3$	244	7.40	7.6
4.	Hexane, 2-phenyl-3-propyl-	$C_{15}H_{24}$	204	7.62	1.9
5.	Ethyl 2,2-diethoxypropionate	$C_9H_{18}O_4$	190	7.75	0.57
6.	5-Methyl-2-hexanol, 2-methylpropionate	$C_{11}H_{22}O_2$	186	7.89	6.9
7.	Benzene, 1,2,4-trimethyl-	C_9H_{12}	120	8.13	0.4
8.	2(3H)-Furanone, dihydro-5-methyl-5-phenyl-	$C_{11}H_{12}O_2$	176	8.49	2.9
9.	Hexadecane	$C_{16}H_{34}$	226	8.74	2.62
10.	Benzene, tert-butyl-	$C_{10}H_{14}$	134	9.42	23.0
11.	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	10.92	9.1
12.	3,3-Diethoxy-1-propanol, butyl ether	$C_{11}H_{24}O_3$	204	11.22	5.2
13.	Tetradecane	$C_{14}H_{30}$	198	11.71	2.16
14.	L-alpha-Terpineol	$C_{10}H_{18}O$	154	14.12	6.4
15.	4a,8a-(Methaniminomethano)naphthalene-9,11-dione, 10-phenyl-	$C_{18}H_{13}NO_2$	275	14.24	0.61
16.	Naphthalene, 2-methyl-	$C_{11}H_{10}$	142	17.34	6.2
17.	Pentadecane	$C_{15}H_{32}$	212	17.47	0.6
18.	3-Trifluoromethylbenzhydryl chloride	$C_{14}H_{10}ClF_3$	270	24.62	0.42
19.	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	$C_{12}H_{20}O_2$	196	28.81	6.7
20.	Dibenzo[b,e]7,8-diazabicyclo[2.2.2]octa-2,5-diene	$C_{14}H_{12}N_2$	208	28.96	0.79
21.	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	29.26	1.44
22.	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	$C_{22}H_{42}O_2$	338	30.14	0.68
23.	Carbazole	$C_{12}H_9N$	167	30.23	1.21
24.	Decanoic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-yl ester	$C_{30}H_{58}O_2$	450	31.01	0.68
25.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	32.66	0.9
26.	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282	33.11	0.65
27.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	33.22	0.65
28.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.beta.)-	$C_{10}H_{20}O$	156	35.10	1.75
29.	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	35.39	0.9
30.	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210	35.47	1.16
31.	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	35.75	0.9
32.	n-Propyl 9,12,15-octadecatrienoate	$C_{21}H_{36}O_2$	320	35.83	0.65
33.	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	36.14	0.9
34.	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{18}H_{36}O_4$	316	39.36	0.6
35.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	39.71	0.43

methanolic extracts of *B. malabarica* were able to get rid of DPPH radicals *in-vitro* in a dose-dependent way. The DPPH radical scavenging activities of the standard (L-ascorbic acid) were found to be considerably higher compared to the DPPH radical scavenging activities of all the methanolic plant extracts that were investigated ($p < 0.05$; Table 2). The methanolic extract of *B. malabarica* exhibited substantially higher DPPH radical scavenging capabilities compared to the methanolic extracts of BML, BMS, and BMR at all evaluated doses ($p < 0.05$;). The methanolic extracts of various parts of *B. malabarica* exhibited significantly different percentages of DPPH radical scavenging activities at all concentrations ($p < 0.05$). Notably, the observed percentage of radical scavenging activities was significantly higher in comparison to the stem of *B. malabarica* ($p < 0.05$; Table 2).

In this work, we also established the IC_{50} values, which represent the amounts of plant extracts necessary to scavenge 50% of the DPPH radicals. The IC_{50} values for the BML, BMS, and BMR extracts were determined to be 0.77,

Table 2: Inhibitory effects of different part extracts of *B. malabarica* in DPPH at 517 nm

Concentration in mg/mL	*DPPH Scavenging activity (% inhibition)*			
	Treatments			
	BML	*BMS*	*BMR*	*L-Ascorbic acid*
25	6.77 ± 0.28[a]	25.37 ± 0.62[a]	6.35 ± 0.48[a]	86.36 ± 0.34[a]
50	9.18 ± 0.57[a]	33.10 ± 0.47[a]	14.31 ± 0.45[a]	67.30 ± 0.29[a]
100	12.17 ± 0.52[a]	42.02 ± 0.75[a]	22.35 ± 0.57[a]	49.16 ± 0.40[a]
250	14.47 ± 0.62[a]	52.20 ± 0.57[a]	29.20 ± 0.39[a]	41.07 ± 2.18[a]
500	16.42 ± 0.70[a]	64.91 ± 0.57[a]	34.83 ± 0.63[a]	27.50 ± 1.32[a]
IC_{50}	0.77	0.49	0.68	0.44

The values are expressed as mean±SD, Value with the uppercase superscript letter within the row and column are significantly different ($p < 0.05$)

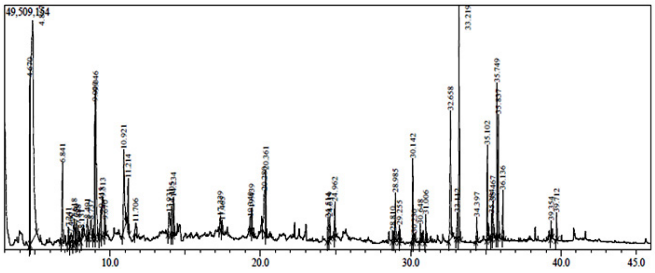

Fig. 1: GC-MS chromatograms of *B. malabarica* samples were made with methanol as the extraction solvent

0.49, and 0.68 mg/mL, respectively (Table 2). In contrast, the IC_{50} value of the reference compound (L-ascorbic acid) was determined to be 0.44 mg/mL.

Cytotoxicity Effects of *B. malabarica* Extracts

The cytotoxicity of the methanolic extract of *B. malabarica* against the PANC-1 pancreatic cancer cell line was assessed using the MTT assay. Fig. 2 illustrates the cytotoxicity impact of the methanolic extract on the PANC-1 cell line, which is associated with pancreatic cancer. The methanolic extract exhibited a substantial impact on the viability of PANC-1 cells across all tested doses. In Fig. 3, it can be observed that the cell viability was significantly high at a concentration of 31.25 µg/mL, with a corresponding cell viability percentage of 69.63%. As illustrated in Fig. 3, the extract demonstrated substantial suppression of the proliferation and viability of pancreatic cancer cells in a dose-dependent manner. The selectivity of the cytotoxic activity exhibited by the extract of *B. malabarica* was assessed by conducting a comparative analysis of its cytotoxic activity (IC_{50}) against pancreatic cancer cells and normal cell lines.

Medicinal plants have been shown to play a significant role in contemporary health care by recent advances in alternative medicine and ethnopharmacological studies. Preserving medicinal plants and ensuring a steady supply of them is an important aspect of the future of medicine and public health around the world.[31] The tropical plant *B. malabarica* has been used to treat a variety of medical conditions, including stomach ulcers, stomach pain, and

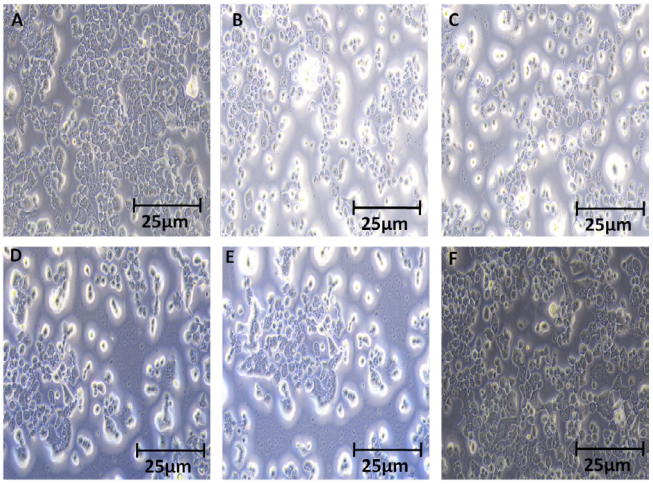

Fig. 2: Cell viability as a function of methanolic extract cytotoxicity against PANC-1 cells (A- Normal cell culture without ME, B-F- Culture treated with ME at different concentrations of 500, 250, 125, 62.5, & 31.25 µg/mL)

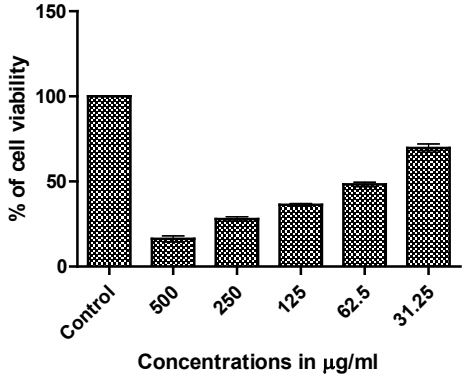

Fig. 3: Cytotoxicity efficacy of a methanolic extract of *B. malabarica* against a pancreatic cancer cell line (PANC-1) was dose-dependent

respiratory issues.[23] Additionally, the Palliyan tribe people in the Tirunelveli District of Tamil Nadu consume the leaves of this plant to treat venereal infections and provide a cooling effect to the body.[32] The Kani Tribe, residing in the Kanyakumari district in Tamil Nadu, India, utilizes leaves as a source of wild edible.[33] These

leaves are known to possess a significant concentration of phenolic and flavonoid components, which contribute to their ability to scavenge free radicals and exhibit antioxidant activity.[34] Hence, the current investigation is centered on the assessment of the phytochemical content, antioxidant capacity, and *in-vitro* cytotoxicity effects of *B. malabarica* methanolic extracts on the pancreatic cancer cell line.

The present study evaluated the antioxidant activity of methanolic extracts derived from various components of *B. malabarica* through the use of the DPPH assay. The findings of the study indicated that the plant extracts demonstrated a dose-dependent ability to scavenge DPPH radicals, although their effectiveness was comparatively lower than that of the conventional antioxidant, ascorbic acid. Numerous studies conducted in the realm of natural antioxidants have consistently demonstrated that plant extracts, particularly those abundant in phenolic compounds, possess notable antioxidant characteristics.[35,36] These molecules possess the ability to counteract detrimental free radicals within the human body, which have been linked to a range of ailments such as cancer and the aging process.[37]

The DPPH assay is a frequently employed technique for assessing the antioxidant capacity, and the outcomes of our investigation are consistent with previous studies conducted on antioxidants produced from plants.[29,38] Chemical composition analysis was done on the methanolic extract of *B. malabarica*. This showed that it contained a number of different substances, including phenolic compounds, esters, and fatty acids. Numerous prior investigations have examined the phytochemical makeup of various plant extracts, leading to the identification of a diverse array of bioactive chemicals.[35] These substances frequently play a part in the biological activity that the extracts exhibit.[39]

The presence of 5-methyl-2-hexanol and 2-methylpropionate, which are volatile chemicals, has been discovered in a variety of plant species.[40] These molecules are particularly associated with the aromatic and flavor characteristics exhibited by specific fruits and flowers.[41] The aforementioned chemicals are constituents of the wider domain of plant secondary metabolites, which fulfill diverse ecological and biological roles.[42] L-alpha-terpineol is frequently encountered in essential oils derived from many aromatic plant species.[43] According to Mohamed and Alotaibi,[33] it has a significant role in enhancing the fragrance and taste of various botanical specimens, often accounting for their distinctive aromas. However, certain terpenes, such as terpineol, exhibit antibacterial capabilities that contribute to the defense mechanism of plants against viruses and illnesses.[45]

Phenolic chemicals, including flavonoids and polyphenols, have gained recognition for their antioxidative characteristics and possible implications for human health.[46,47] The study presented findings that indicate the methanolic extract of *B. malabarica* showed cytotoxic properties when tested against the PANC-1 pancreatic cancer cell line. The investigation of the cytotoxic properties of plant extracts against cancer cells is an increasingly prominent subject within the realms of natural products and cancer research.[48,49] Several studies have been conducted to examine the capacity of plant-derived chemicals to inhibit the proliferation of cancer cells and trigger programmed cell death, also known as apoptosis.[15,50] The observed selectivity of cytotoxic action in our investigation, wherein the extract exhibited more toxicity towards cancer cells in comparison to normal cells, is a favorable attribute for prospective cancer therapies.

The quantification of IC_{50} values in our investigation offers a numerical assessment of the concentration of the extract necessary to impede 50% of DPPH radicals or the proliferation of cancer cells. In the fields of pharmacology and toxicology, IC_{50} values are frequently employed as a means of evaluating the efficacy of various drugs.[51] Lower IC_{50} values indicate more potency or efficacy.[29] The evaluation of the relative efficiency of natural products often involves comparing the IC_{50} values of plant extracts to those of conventional chemicals, such as ascorbic acid, as demonstrated in this work. The presence of several secondary metabolites resulted in dose-dependent cytotoxic effects on specific cancer cell lines.[52,53] There are a lot of bioactive compounds in *B. malabarica*, and it has great antioxidant properties and helps pancreatic cancer cell lines survive and migrate. This means that the substances that can be extracted are very valuable and have a lot of potential for use in pharmaceutical products.

Conclusion

The findings of this study are consistent with a larger body of research that suggests plant extracts, particularly those containing high levels of phenolic chemicals, exhibit antioxidant properties and demonstrate cytotoxic effects on pancreatic cancer cell lines. Nevertheless, although these findings show promise, they only serve as a first step in comprehending the possible therapeutic uses of *B. malabarica* extracts. Additional investigation, encompassing mechanistic inquiries and clinical trials, would be imperative to thoroughly examine their efficacy in the realm of medicine and healthcare.

Acknowledgment

The authors are grateful thanks to the authorities of Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University at Alwarkurichi, Tirunelvlei, Tamil Nadu for providing facilities.

References

1. Momtazi AA, Askari-Khorasgani O, Abdollahi E, Sadeghi-Aliabadi H, Mortazaeinezhad F, Sahebkar A. Phytochemical Analysis and Cytotoxicity Evaluation of Kelussia odoratissima Mozaff. *JAMS J Acupunct Meridian Stud* 2017;10(3):180–186.
2. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. *Curr Med Chem* 2001;8(12):1467–1486.
3. Rowbotham SP, Kim CF. Don't Stop Re-healin'! Cancer as an Ongoing Stem Cell Affair. *Cell* 2017;169(4):563–565.
4. Verma AK, Singh S. Phytochemical analysis and *in-vitro* cytostatic potential of ethnopharmacological important medicinal plants. *Toxicol Reports* 2020;7(February):443–452.
5. International Agency for Research on Cancer. *Cancer incidence in five continents, vol. VI*. 2017.
6. Ilic I, Ilic M. International patterns in incidence and mortality trends of pancreatic cancer in the last three decades: A joinpoint regression analysis. *World J Gastroenterol* 2022;28(32):4698–4715.
7. Kroes BH. The legal framework governing the quality of (traditional) herbal medicinal products in the European Union. *J Ethnopharmacol* 2014;158:449–453.
8. Verma AK, Prasad SB. Antitumor effect of blister beetles: An ethno-medicinal practice in Karbi community and its experimental evaluation against a murine malignant tumor model. *J Ethnopharmacol* 2013;148(3):869–879.
9. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod* 2016;79(3):629–661.
10. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. *Phytomedicine Plus* 2021;1(2):100029.
11. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 2010;4(8):118–126.
12. Sharifi-Rad M, Anil Kumar N V., Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol* 2020;11(July):1–21.
13. Kalpanadevi V, Mohan VR. *In-vitro* antioxidant studies of *Begonia malabarica* Lam. and Begonia floccifera Bedd. *Asian Pac J Trop Biomed* 2012;2(3, Supplement):S1572–S1577.
14. Li G, Ding K, Qiao Y, Zhang L, Zheng L, Pan T, et al. Flavonoids regulate inflammation and oxidative stress in cancer. *Molecules* 2020;25(23). doi:10.3390/molecules25235628.
15. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S, et al. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules* 2021;26(4):1–29.
16. Greenwell M, Rahman PKSM. Medicinal Plants: Their Use in Anticancer Treatment. *Int J Pharm Sci Res* 2015;6(10):4103–4112.
17. Vakele Y, Odun-Ayo F, Reddy L. *In-vitro* antioxidant and cytotoxicity activities of selected indigenous South African medicinal plants. *Afr Health Sci* 2022;22(1):395–403.
18. Susmi TF, Rahman Khan MM, Rahman A, Mahmud S, Saleh MA, Reza MA, et al. *In-vitro* antioxidant and cytotoxicity activities and in silico anticancer property of methanolic leaf extract of Leucas indica. *Informatics Med Unlocked* 2022;31:100963.
19. Maw MB, Ding H-B, Yang B, Win PP, Tan Y-H. Taxonomic studies on Begonia (Begoniaceae) in Myanmar I: three new species and supplementary description of Begonia rheophytica from Northern Myanmar. *PhytoKeys* 2020;138:203–217.
20. Hughes M, Aung MM, Armstrong K. AN UPDATED CHECKLIST and A NEW SPECIES of BEGONIA (B. RHEOPHYTICA) from MYANMAR. *Edinburgh J Bot* 2019;76(2):285–295.
21. Moonlight PW, Ardi WH, Padilla LA, Chung KF, Fuller D, Girmansyah D, et al. Dividing and conquering the fastest-growing genus: Towards a natural sectional classification of the mega-diverse genus begonia (Begoniaceae). *Taxon* 2018;67(2):267–323.
22. Tebbitt MC. Taxonomy of Begonia longifolia Blume (Begoniaceae) and related species. *Brittonia* 2003;55(1):19–29.
23. Ramesh N, Viswanathan MB, Saraswathy A, Balakrishna K, Brindha P, Lakshmanaperumalsamy P. Phytochemical and antimicrobial studies of *Begonia malabarica*. *J Ethnopharmacol* 2002;79(1):129–132.
24. Doskotch RW, Hufford CD. Hexanor–cucurbitacin D, a degraded cucurbitacin from Begoniatuberhybrida var. alba. *Can J Chem* 1970;48(11):1787–1788.
25. Doskotch RW, Malik MY, Beal JL. Cucurbitacin B, the cytotoxic principle of Begonia tuberhybrida var. alba. *Lloydia* 1969;32(2):115–122.
26. Fuller RW, Cardellina JH 2nd, Cragg GM, Boyd MR. Cucurbitacins: differential cytotoxicity, dereplication and first isolation from Gonystylus keithii. *J Nat Prod* 1994;57(10):1442–1445.
27. Frei B, Heinrich M, Herrmann D, Orjala JE, Schmitt J, Sticher O. Phytochemical and biological investigation of Begonia heracleifolia. Planta medica. 1998;64(4):385–386.
28. Baeshen NA, Almulaiky YQ, Afifi M, Al-Farga A, Ali HA, Baeshen NN, et al. GC-MS Analysis of Bioactive Compounds Extracted from Plant Rhazya stricta Using Various Solvents. *Plants (Basel, Switzerland)* 2023;12(4). doi:10.3390/plants12040960.
29. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules* 2022;27(4). doi:10.3390/molecules27041326.
30. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65(1–2):55–63.
31. Alnuqaydan AM, Rah B. Comparative assessment of biological activities of different parts of halophytic plant Tamarix articulata (T. articulata) growing in Saudi Arabia. *Saudi J Biol Sci* 2020;27(10):2586–2592.
32. Mini Shobi T, Gowdu Viswanathan MB. Antibacterial activity of di-butyl phthalate isolated from *Begonia malabarica*. *J Appl Biotechnol Bioeng* 2018;5(2):97–100.
33. Ayyanar M, Ignacimuthu S. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. *J Ethnopharmacol* 2011;134(3):851–864.
34. Thenmozhi K, Anusuya N, Ajmal Ali M, Jamuna S, Karthika K, Venkatachalapathi A, et al. Pharmacological credence of the folklore use of Bauhinia malabarica in the management of jaundice. *Saudi J Biol Sci* 2018;25(1):22–26.
35. Yu M, Gouvinhas I, Rocha J, Barros AIRNA. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Sci Rep* 2021;11(1):1–14.
36. Hoang HT, Moon J-Y, Lee Y-C. Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives. *Cosmetics* 2021;8(4). doi:10.3390/cosmetics8040106.
37. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen* 2022;2(2):48–78.
38. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int J Mol Sci* 2021;22(7). doi:10.3390/ijms22073380.
39. Stéphane FFY, Jules BKJ, Batiha GE-S, Ali I, Bruno LN. Extraction of Bioactive Compounds from Medicinal Plants and Herbs. In: El-Shemy HA, editor. *Natural Medicinal Plants*. IntechOpen: Rijeka, 2021 doi:10.5772/intechopen.98602.
40. El Hadi MAM, Zhang FJ, Wu FF, Zhou CH, Tao J. Advances in fruit aroma volatile research. *Molecules* 2013;18(7):8200–8229.
41. Mostafa S, Wang Y, Zeng W, Jin B. Floral Scents and Fruit Aromas: Functions, Compositions, Biosynthesis, and Regulation. *Front Plant Sci* 2022;13(March):1–23.
42. Gu I, Howard L, Lee SO. Volatiles in Berries: Biosynthesis, Composition, Bioavailability, and Health Benefits. *Appl Sci* 2022;12(20). doi:10.3390/app122010238.
43. Thamkaew G, Sjöholm I, Galindo FG. A review of drying methods for improving the quality of dried herbs. *Crit Rev Food Sci Nutr* 2021;61(11):1763–1786.
44. Mohamed AA, Alotaibi BM. Essential oils of some medicinal plants and their biological activities: a mini review. *J Umm Al-Qura Univ Appl Sci* 2023;9(1):40–49.

45. Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran T Bin, et al. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chem X* 2022;13(January):100217.
46. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Reports* 2019;24:e00370.
47. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines* 2018;5(3):93.
48. Khan T, Ali M, Khan A, Nisar P, Jan SA, Afridi S, et al. Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules* 2020;10(1). doi:10.3390/biom10010047.
49. Campoccia D, Ravaioli S, Santi S, Mariani V, Santarcangelo C, De Filippis A, et al. Exploring the anticancer effects of standardized extracts of poplar-type propolis: *In-vitro* cytotoxicity toward cancer and normal cell lines. *Biomed Pharmacother* 2021;141:111895.
50. Esmeeta A, Adhikary S, Dharshnaa V, Swarnamughi P, Ummul Maqsummiya Z, Banerjee A, et al. Plant-derived bioactive compounds in colon cancer treatment: An updated review. *Biomed Pharmacother* 2022;153:113384.
51. Muthoni Guchu B, Machocho AKO, Mwihia SK, Ngugi MP. *In-vitro* Antioxidant Activities of Methanolic Extracts of Caesalpinia volkensii Harms., Vernonia lasiopus O. Hoffm., and Acacia hockii de Wild. *Evidence-based Complement Altern Med* 2020;2020. doi:10.1155/2020/3586268.
52. Lay MM, Karsani SA, Banisalam B, Mohajer S, Abd Malek SN. Antioxidants, Phytochemicals, and Cytotoxicity Studies on *Phaleria macrocarpa* (Scheff.) Boerl Seeds. *Biomed Res Int* 2014;2014:410184.
53. Gudasi S, Gharge S, Koli R, Patil K. Antioxidant properties and cytotoxic effects of Oxalis corniculata on human Hepatocarcinoma (Hep-G2) cell line: an *in-vitro* and in silico evaluation. *Futur J Pharm Sci* 2023;9(1):25.

HOW TO CITE THIS ARTICLE: Sakthivadivel S, Arumugam S, Murugan M, Ramaiah S. Assessment of the Phytochemical Composition, Antioxidant Activity, and *In-vitro* Cytotoxic Effects of *Begonia malabarica* on Pancreatic Cancer Cell Line. Int. J. Pharm. Sci. Drug Res. 2024;16(1):119-126. **DOI:** 10.25004/IJPSDR.2024.160116