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IN VITRO EVALUATION OF THE ANTIDIABETIC AND ANTICANCER POTENTIAL OF *PANDANUS AMARYLLIFOLIUS* ROXB. LEAF EXTRACTS THROUGH α-AMYLASE INHIBITORY AND MTT CYTOTOXICITY ASSAYS

S. Justin Raj*[1], Mubeena Hamdulla[2]

[1]Assistant Professor and Research Supervisor, Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliyakavilai.

[2]PG Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliyakavilai.

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***Corresponding Author: S. Justin Raj**
Assistant professor and Research Supervisor, Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliyakavilai.
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ABSTRACT

Pandanus amaryllifolius Roxb. is an aromatic medicinal plant rich in bioactive phytochemicals with potential therapeutic applications. The present study evaluated the in vitro antidiabetic and anticancer activities of the methanolic leaf extract of *Pandanus amaryllifolius* using α-amylase inhibition and MTT cytotoxicity assays. Antidiabetic activity was assessed by the DNSA method, while anticancer activity was evaluated against MCF-7 human breast cancer cells at concentrations of 20–100 µg/mL. The methanolic extract exhibited concentration-dependent α-amylase inhibition, with inhibition increasing from 23.95% to 86.00% across the tested concentrations, indicating significant antidiabetic potential. In the MTT assay, the extract produced a gradual decrease in cell viability from 98.19% to 84.23%, demonstrating moderate cytotoxic activity against MCF-7 cells. However, the IC_{50} value could not be determined since cell viability remained above 50% at the highest concentration tested. The observed biological activities are likely associated with the presence of phenolics, flavonoids, and other phytoconstituents. These findings suggest that the methanolic leaf extract of *Pandanus amaryllifolius* possesses promising antidiabetic activity and moderate anticancer potential, warranting further phytochemical characterization and in vivo studies.

KEYWORDS: *Pandanus amaryllifolius*; Antidiabetic activity; α-Amylase inhibition; Anticancer activity; MCF-7 cells; MTT assay; Medicinal plants.

INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and is characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin resistance, or both. According to recent epidemiological reports, the incidence of diabetes continues to increase globally, posing a significant burden on healthcare systems. Long-term complications of diabetes include nephropathy, neuropathy, retinopathy, cardiovascular disorders, and impaired wound healing. Consequently, the search for safer and more effective antidiabetic agents derived from natural resources has become an important area of biomedical research.[1] One of the promising therapeutic strategies for controlling postprandial hyperglycaemia involves inhibition of carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase. These enzymes are responsible for the breakdown of dietary starch into absorbable glucose. Suppression of α-amylase activity delays carbohydrate digestion, thereby reducing glucose absorption and preventing sudden elevation of blood glucose levels after meals. Although synthetic inhibitors such as acarbose are clinically effective, their prolonged use is often associated with gastrointestinal side effects including abdominal discomfort, diarrhoea, and flatulence. Therefore, medicinal plants have gained considerable attention as alternative sources of natural enzyme inhibitors with fewer adverse effects.[2,3]

Medicinal plants synthesize a wide range of secondary metabolites including alkaloids, flavonoids, tannins, phenolic acids, saponins, terpenoids, and glycosides. These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, and hepatoprotective properties. Numerous studies have demonstrated that polyphenolic compounds inhibit carbohydrate-metabolizing enzymes and improve glucose homeostasis through multiple biochemical pathways.[4,5] Cancer is another major global health concern and remains one of the leading causes of mortality despite advances in diagnosis and treatment. Conventional therapeutic approaches such as chemotherapy and radiotherapy frequently produce severe adverse effects and may eventually lead to multidrug resistance. Consequently, there is increasing interest in identifying naturally occurring anticancer compounds from medicinal plants that exhibit selective cytotoxicity toward malignant cells while causing minimal toxicity to normal tissues.[6] The MTT assay is among the most reliable and widely employed in vitro methods for evaluating cytotoxicity and antiproliferative activity of natural products. The assay is based on the reduction of yellow tetrazolium salt into purple formazan crystals by mitochondrial succinate dehydrogenase enzymes in metabolically active cells. Reduction in formazan formation directly reflects decreased cell viability following treatment with cytotoxic agents.[7]

Pandanus amaryllifolius Roxb., belonging to the family Pandanaceae, is an aromatic tropical plant extensively cultivated throughout Southeast Asia. Traditionally, its leaves are used as flavouring agents in food preparation and also employed in folk medicine for treating fever, diabetes, rheumatism, headaches, digestive disorders, and inflammatory conditions. Phytochemical investigations have revealed that the leaves contain abundant phenolic compounds, flavonoids, alkaloids, carotenoids, lignans, volatile oils, and essential minerals that contribute to diverse pharmacological activities.[8] Several investigations have demonstrated that extracts of *Pandanus amaryllifolius* possess significant antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antihyperglycaemic, and anticancer properties. These biological activities are largely attributed to the synergistic action of multiple phytochemicals capable of scavenging reactive oxygen species, modulating inflammatory pathways, and inducing apoptosis in cancer cells.[9]

Methanol is regarded as one of the most efficient solvents for extracting phenolic and flavonoid constituents because of its intermediate polarity. Consequently, methanolic extracts of medicinal plants frequently exhibit greater biological

activity than aqueous or non-polar solvent extracts. Previous phytochemical investigations have shown that methanolic extracts of *Pandanus amaryllifolius* contain comparatively higher concentrations of bioactive phenolics, thereby justifying their use in pharmacological screening.[10]

Therefore, the present study aimed to investigate the in vitro antidiabetic and anticancer activities of methanolic leaf extract of *Pandanus amaryllifolius* by evaluating α-amylase inhibitory activity using the DNSA method and cytotoxic potential against MCF-7 human breast cancer cells using the MTT assay. The findings are expected to contribute valuable scientific evidence supporting the therapeutic potential of this medicinal plant and provide a basis for future isolation and characterization of biologically active compounds.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves of *Pandanus amaryllifolius* Roxb. were collected from local vicinity. The leaves were washed, shade-dried, powdered, and extracted separately with methanol, acetone, chloroform, and distilled water by cold maceration using 20 g of leaf powder in each solvent. The extracts were filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator for organic solvents, while the aqueous extract was air-dried. The dried crude extracts were stored at 4°C until further use. Based on preliminary screening, the methanolic extract exhibited the highest biological activity and was selected for subsequent studies.

In vitro Antidiabetic Activity

The α-amylase inhibitory activity of the methanolic extract was determined by the DNSA method. Briefly, 500 µL of plant extract was mixed with 500 µL of α-amylase solution (0.5 mg/mL) prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 0.006 M NaCl) and incubated at 25°C for 10 min. Subsequently, 500 µL of 1% starch solution was added and incubated for another 10 min. The reaction was terminated with 1 mL DNSA reagent, heated in a boiling water bath for 5 min, cooled, diluted with 10 mL distilled water, and the absorbance was recorded at 540 nm. The percentage inhibition of α-amylase activity was calculated using the standard formula.

The percentage inhibition of α-amylase activity was calculated using the following equation:

$$\text{Percentage Inhibition (\%)} = [(Ac - At) / Ac] \times 100$$

Where:

Ac = Absorbance of the control

At = Absorbance of the treated sample

In vitro Anticancer Activity

The cytotoxic activity of the methanolic extract was evaluated against MCF-7 human breast cancer cells using the MTT assay. Cells (1×10^4 cells/well) were seeded in 96-well plates and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. The cells were treated with extract concentrations of 20, 40, 60, 80, and 100 µg/mL for 24 h. After treatment, 100 µL of MTT solution (0.5 mg/mL) was added and incubated for 2 h. The resulting formazan crystals were dissolved in 100 µL DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to the untreated control. Since cell viability remained above 50% at the highest tested concentration, the IC_{50} value was reported as >100 µg/mL.

The cell viability was expressed using the following formula:

$$\text{Percentage of cell viability} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}} \times 100$$

Statistical Analysis

All experiments were performed in triplicate, Data were analyzed using Microsoft Excel, and concentration-dependent responses were evaluated descriptively.

RESULTS AND DISCUSSION

In vitro Antidiabetic Activity

The α-amylase inhibitory activity of the methanolic leaf extract of *Pandanus amaryllifolius* increased in a concentration-dependent manner, indicating its potential to inhibit carbohydrate digestion. As presented in Table 1 and illustrated in Figure 1, the percentage inhibition increased from 23.95% at 100 µL to 86.00% at 500 µL, while the absorbance values progressively decreased with increasing extract concentration. The highest inhibition (86.00%) observed at 500 µL demonstrates the strong α-amylase inhibitory potential of the methanolic extract.

The concentration-dependent enzyme inhibition observed in Table 1 and Figure 1 suggests that the extract contains bioactive constituents capable of interacting with the catalytic site of α-amylase, thereby delaying starch hydrolysis and reducing glucose release.[11] Phenolic compounds and flavonoids are well-known natural α-amylase inhibitors and have been reported to contribute significantly to the antihyperglycaemic activity of medicinal plants.[12]

Table 1: Shows the antidiabetic activity of *Pandanus amaryllifolius* in methanol extract by Inhibition assay for α-amylase activity.

	CONTROL	100 µl	200 µl	300 µl	400 µl	500 µl
OD	2.011	1.521	1.277	0.886	0.436	0.272
% of inhibition		23.95%	36.15%	55.70%	78%	86%

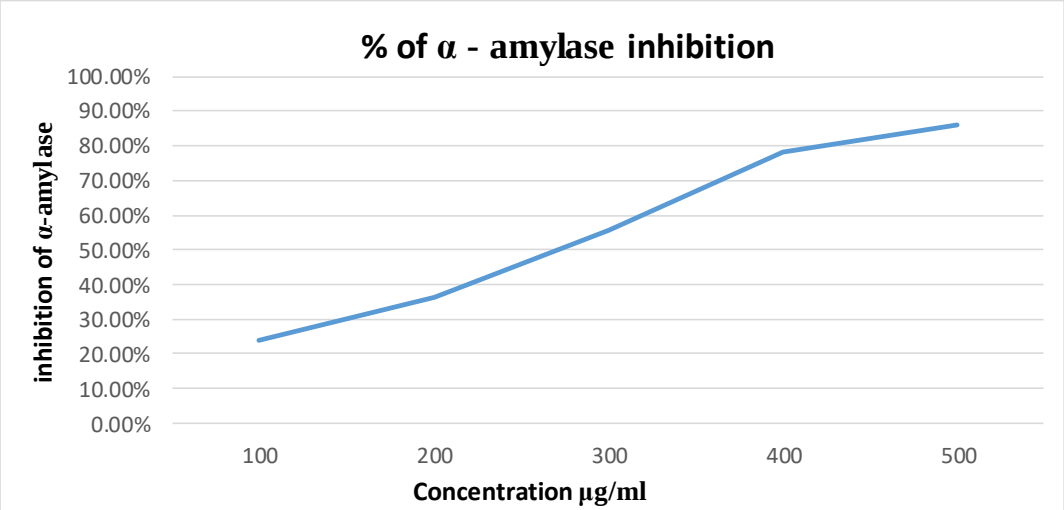

Figure 1: Shows the antidiabetic activity of *Pandanus amaryllifolius*.

Previous investigations have demonstrated similar α-amylase inhibitory activity in phenolic-rich medicinal plants, including *Syzygium cumini*, *Moringa oleifera*, and *Ocimum sanctum*, where increasing extract concentration resulted in enhanced enzyme inhibition.[13,14] Phytochemical studies of *Pandanus amaryllifolius* have also reported the presence of

flavonoids, phenolic acids, tannins, and alkaloids, which are responsible for antioxidant and antidiabetic activities.[15] In addition, the antioxidant properties of these phytochemicals may protect pancreatic β-cells against oxidative stress, thereby contributing to improved glucose homeostasis.[16] Thus, the results presented in Table 1 and Figure 1 support the potential use of *Pandanus amaryllifolius* as a natural antidiabetic agent.

In vitro Anticancer Activity

The cytotoxic effect of the methanolic leaf extract against MCF-7 human breast cancer cells was evaluated using the MTT assay. The raw absorbance values obtained from triplicate experiments are presented in Table 2, while the corresponding percentage cell viability is summarized in Table 3 and graphically represented in Figure 2. A gradual decrease in cell viability was observed with increasing extract concentration, indicating a dose-dependent cytotoxic response. As shown in Table 3 and Figure 2, cell viability decreased from 98.19% at 20 µg/mL to 84.23% at 100 µg/mL. Although the extract exhibited measurable antiproliferative activity, the reduction in cell viability was moderate, and the IC_{50} value could not be determined because cell viability remained above 50% at the highest tested concentration (100 µg/mL). Therefore, the IC_{50} was reported as >100 µg/mL.

Table 2: Shows the MTT assay results for varying concentration of *Pandanus amaryllifolius.*

Volume (µL)	Triplicate values			Average OD
	OD1	OD2	OD3	
Control	0.833	0.838	0.821	0.831
20	0.813	0.815	0.819	0.816
40	0.800	0.804	0.806	0.803
60	0.784	0.788	0.792	0.788
80	0.746	0.748	0.735	0.743
100	0.702	0.706	0.691	0.700

The MTT assay is widely employed for assessing mitochondrial metabolic activity and cell viability following treatment with bioactive compounds.[17] The gradual decline in absorbance values observed in Table 2, together with the reduction in percentage viability shown in Table 3 and Figure 2, indicates that the methanolic extract exerts moderate cytotoxic effects on MCF-7 cells. The anticancer activity may be attributed to phenolics, flavonoids, alkaloids, and terpenoids present in *Pandanus amaryllifolius*. These phytochemicals have been reported to induce apoptosis, arrest the cell cycle, inhibit oxidative stress, and modulate signalling pathways involved in tumour progression.[18,19] Flavonoids, in particular, are known to activate caspase-dependent apoptosis and suppress proliferative pathways such as PI3K/Akt and MAPK, thereby reducing cancer cell survival.[20]

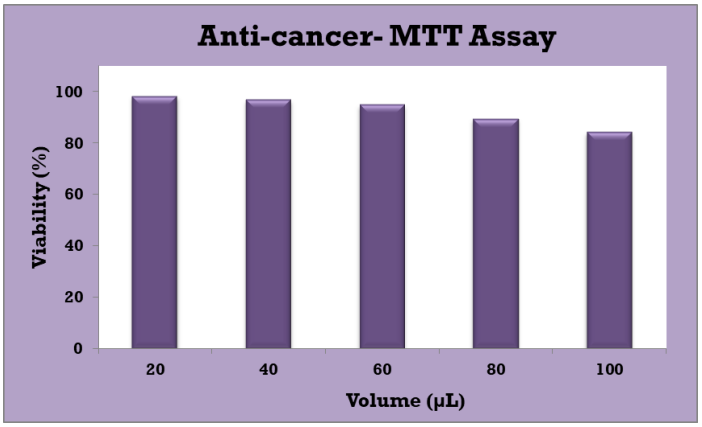

Figure 2: Shows the MTT assay of *Pandanus amaryllifolius*.

Table 3: Percentage of viability for varying concentration of *Pandanus amaryllifolius*.

Volume (µL)	Percentage of viability
20	98.19
40	96.71
60	94.86
80	89.45
100	84.23
IC 50	>100 µL

Previous studies have demonstrated antioxidant and cytotoxic activities of *Pandanus* species against various cancer cell lines, supporting the moderate antiproliferative activity observed in the present study.[21] However, the relatively high cell viability shown in Table 3 suggests that the crude methanolic extract exhibits only moderate cytotoxicity at the tested concentrations. Fractionation and purification of the extract may concentrate the active constituents and enhance its anticancer efficacy.[22] The inability to determine an IC_{50} value is expected during preliminary screening when the maximum tested concentration does not reduce cell viability below 50%. According to accepted screening criteria, crude plant extracts should be evaluated at higher concentrations or subjected to bioassay-guided fractionation before definitive conclusions regarding cytotoxic potency are drawn.[23] Therefore, future investigations should include higher extract concentrations together with apoptosis assays, cell-cycle analysis, reactive oxygen species estimation, and molecular studies to elucidate the mechanism of action.[24] Overall, the findings presented in Tables 1–3 and Figures 1–2 demonstrate that the methanolic leaf extract of *Pandanus amaryllifolius* possesses strong α-amylase inhibitory activity and moderate cytotoxic activity against MCF-7 breast cancer cells. These pharmacological effects are likely due to the synergistic action of multiple phytochemicals present in the extract [25], highlighting its potential as a promising source of natural antidiabetic and anticancer compounds.

CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Pandanus amaryllifolius* possesses promising in vitro antidiabetic and moderate anticancer activities. The extract exhibited a concentration-dependent inhibition of α-amylase, achieving a maximum inhibition of 86%, indicating its potential to regulate postprandial hyperglycaemia through inhibition of carbohydrate digestion. The MTT assay further revealed a dose-dependent reduction in the viability of MCF-7 human breast cancer cells, although the cytotoxic effect was moderate, with an IC_{50} value greater than 100 µg/mL. The observed biological activities are likely associated with the presence of phenolics, flavonoids, tannins, alkaloids, and other bioactive phytochemicals in the methanolic extract. Overall, the findings scientifically support the traditional medicinal use of *Pandanus amaryllifolius* and suggest that it represents a valuable natural source of compounds with antidiabetic and anticancer potential. Further studies involving phytochemical isolation, mechanistic investigations, toxicity evaluation, and in vivo experiments are recommended to validate its therapeutic efficacy and facilitate the development of novel plant-based pharmaceuticals.

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