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Investigating the antioxidant properties and GC-MS profile of Indian native medicinal flower *Pandanus odorifer* and assessing its cytotoxic effects on HT-29 cells

Research Article

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Abstract

This study aimed to investigate the antioxidant potential and bioactive compounds of *Pandanus odorifer* (Forssk.) Kuntze flower. The phytochemical investigation includes both qualitative and quantitative experiments. *In vitro* antioxidant studies were conducted using DPPH, ABTS and hydroxyl methods. The bioactive compounds present in the ethanol extract were identified using GC-MS analysis. The MTT assay was carried out on HT-29 colon cancer cells to investigate the cytotoxic ability of the ethanolic extract. The qualitative phytochemical analysis demonstrated the presence of flavonoids, phenolic compounds, tannins, saponins, steroids, carboxylic acids and cardenolides. The quantitative analysis revealed that the ethanolic extract contains higher level of flavones, flavanones and phenols when compared to the water extract. The total antioxidant capacity revealed 1.6 ± 0.093 mg/g and 1.3± 0.056 mg/g equivalent of ascorbic acid in ethanol and water extracts. The DPPH assay showed IC_{50} values of 35.06 µg/mL in ethanol and 44.82 µg/mL in water extract. Likewise, the ABTS assay revealed an IC_{50} value of 42.20 µg/mL and 47.45 µg/mL in ethanol and water extract. The hydroxyl radical assay showed IC_{50} value of 23.95 µg/mL in ethanol and 36.56 µg/mL in water extract. 22 bioactive compounds were identified using GC-MS analysis based on their similarity index. The ethanolic extract showed greater cytotoxic activity with the IC_{50} value of 28.08 µg/mL on HT-29 colon cancer cells. This study concludes that the *Pandanus odorifer* flower extracts exhibited significant antioxidant and cytotoxic property.

Keywords: *Pandanus odorifer,* phytochemical analysis, antioxidant assays, GC-MS, cytotoxic, HT-29.

Introduction

Colon cancer stands as a significant cause to both illness and fatalities on a global scale. It ranks as the fourth most prevalent form of cancer and third most common cause of cancer-related deaths globally (1). Many researches indicates that being overweight, leading a sedentary lifestyle, smoking cigarettes, consuming alcohol, and unhealthy dietary patterns raise the risk of developing colon cancer (2). The oxidative stress and inflammation are closely connected. It has the potential to induce inflammation, while inflammation can create a harmful cycle (3). Oxidative stress arises when the production and deposition of ROS (reactive oxygen species) in tissues and cells exceeds the capacity of a biological mechanism to eliminate these reactive substances (4). In general, ROS serve as signal transduction molecules, promoting cellular protection and enhancing cellular functions. But if ROS generation increases, they may harm important cellular constituents like lipids, proteins, and nucleic acids which can lead to several disorders (5). Antioxidants are chemicals that neutralize free radicals and ROS in the cell. These antioxidants protect the body from the damaging effects of free radicals that are crucial in the development of many long-term conditions such as aging, cancer, heart disease, inflammation and anemia. Natural antioxidants are mostly derived from plants, and their effectiveness varies with plant species, variety, extraction or processing techniques and growth circumstances. They exist in animal tissues, microbes and in the majority of plants (6).

Medicinal plants are those that contain compounds in one or more of its sections that can be utilized to treat illnesses. They can be applied externally, inhaled, or taken orally. Plant-based medicines are well-known for their affordability, accessibility, and safety (7). Plants are used to

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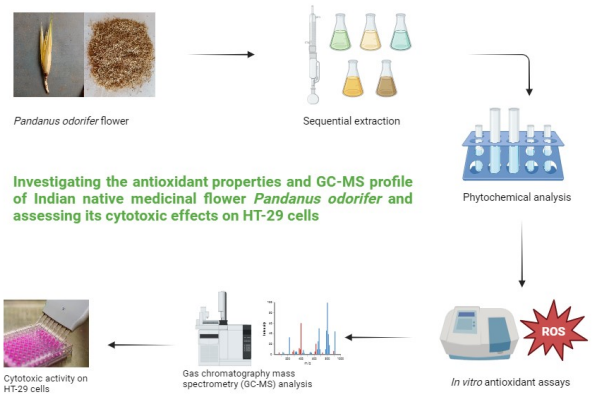
manufacture a large number of medications that are essential in modern medicine. Over the past ten years, the usage of drugs derived from plants has gradually gained support in both developed and developing nations. It is estimated that 80% of people in the world use herbal medicine (8). *Pandanus odorifer* (Forssk.), is also known as *Pandanus odoratissimus,* a small tree in the Pandanaceae family. This plant species is found in South India, Myanmar, Sri Lanka, Cambodia, China, Indonesia and Philippines. Its fruits and leaves are used to flavour the dishes (9). In Taiwan, Sri Lanka and India, traditional medical systems such as Siddha Medicine and Ayurveda employ *Pandanus odorifer* to treat a variety of conditions. The flower of this plant species is used to cure conditions like skin conditions, asthma, urinary tract ailments, and syphilis. Its root is also used to treat thyroid issues, diabetes, fever, constipation, and urinary tract infections. Compared to the root and leaf, the flower has more conventional therapeutic applications. For traditional medical purposes, there are currently very few scientific sources of evidence accessible (10).

Phytochemicals are simply plant chemicals. They are non-nutritive chemical substances of plants that provide numerous health advantages and disease prevention characteristics. More than a thousand phytochemicals have been identified and classed as primary and secondary metabolites depending on their function in plant metabolism (11). The most common secondary metabolites are alkaloids, flavonoids, phenols and so on. Alkaloids are primarily produced from amino acids resulting in a wide range of chemical structures with the majority being extracted from plants. They are crucial in both human treatment and an organism's natural defence (12). Flavonoids are a broad family of polyphenols classified according to their basic structure. They have a variety of biological processes, such as antioxidant, antiviral, disinfectant, cardioprotective, anticancer, anti-inflammatory and neuroprotective actions. Recent flavonoid study and research developments focus on recognition, extraction, separation, physicochemical characterisation, and health-related applications (13). Phenolics are the most prominent secondary metabolites present in plants, and their existence is evident during the metabolic process. These phenolic molecules, or polyphenols, include numerous types of substances (14). Plant bioactive substances are important for human health because of their numerous biological consequences (15). The study was aimed to carry out the phytochemical screening of *Pandanus odorifer* extracts, which includes both qualitative analysis and quantitative estimation using various extracts. In addition, this research intended to evaluate the antioxidant and cytotoxic capacity of these extracts and identify the bioactive compounds through Gas Chromatography-Mass Spectrometry (GC-MS) analysis as shown in (Fig. 1).

Figure 1: Graphical representation of the overall study



Materials and Methods

Materials

All the chemicals were bought from Sigma Aldrich and Hi Media was employed in this study.

Collection of flower sample

Pandanus odorifer flower was collected from Pollachi, Coimbatore and was authenticated by Dr. M. U. Sharief, (BSI/SRC/5/23/2023/Tech-800), Botanical Survey of India, TNAU campus, Coimbatore.

Preparation of sample extract

Pandanus odorifer flowers were washed thoroughly and shade-dried. Subsequently, the dried sample was ground into powder and kept in an airtight container for future use. The sample extract was prepared using soxhlet method. The powdered sample was sealed in the soxhlet and extracted using different solvents (Petroleum ether, Toluene, Chloroform, Ethanol, and Water) in the ratio of 1:5. The extra solvents were allowed to evaporate and the dried extracts were stored.

Preliminary phytochemical analysis

The preliminary phytochemical study was conducted on all the extracts using the method followed by (16) with some alterations.

Test for resin

To 1mL of each selective extract, acetic anhydride and 1mL of conc. H_2SO_4 were added. The change in colour from orange to yellow indicates the presence of resins.

Test for alkaloids

To the few mL of each selective extract, 1-2 drops of Dragendorff's reagent was added. The formation of reddish brown precipitate indicates the presence of alkaloids.

Test for carbohydrates

To 2mL of each selective extract, 2 drops of an alcoholic α –naphthol and 1mL of conc. H_2SO_4 were added. The formation of violet ring shows the presence of carbohydrates.

Test for cardenolides

To 0.2mL of each selective extract, a few drops of Kedde reagent was added. The development of a blue or violet colour that faded out in1 to 2 hrs confirms the presence of cardenolides.

Test for amino acids and proteins

To 2mL of each selective extract, Millon's reagent (few drops) was added. The formation of white precipitate shows the presence of amino acids and proteins.

Test for flavonoids

To 1mL of each selective extract magnesium turnings and few drops of HCl were added. The formation of pink, orange, or red to purple confirms the presence of flavonoids.

Test for phenols

To 1mL of each selective extract, few drops of few drops of 1% ferric chloride ($FeCl_3$) was added. The formation of red, blue, violet, purple and green colour specifies the presence of phenols.

Test for tannins

To 1mL of each selective extract, 5mL of distilled water and 1% gelatin were added. Further, 10% NaCl was added. The formation of white precipitate shows the presence of tannins.

Test for carboxylic acids

To 1mL of each selective extract, 1mL of Na_2CO_3 was added. The formation of effervescence shows the presence carboxylic acids.

Test for steroids

To each selective extract, a few drops of acetic anhydride and concentrated sulphuric acid were added. The formation of green colour indicates the presence of steroids.

Test for saponins

To 2mL of each selective extract, 2mL of Benedict's reagent was added. A blue-black precipitate confirms the presence of saponins.

Determination of Flavone and Flavonol content

The quantification of total flavone and flavonol was conducted using the aluminium chloride method (17) with certain modifications. Distinct volumes of quercetin (0.2,0.4,0.6,0.8 and 1mL) were taken in various test tubes. 0.5mL of ethanolic and water extract were taken in a separate test tube. 0.3mL of aluminium chloride (10%) and 0.3mL of sodium nitrate (5%) were added and incubated for 5mins. Finally, 1mL of NaOH (1M) was added. The obtained volume was promptly diluted by adding 10 mL of deionized water. The optical density against blank was read at 510 nm using a spectrophotometer.

Determination of Flavanone and dihydroflavonol content

The quantification of total flavanone and dihydroflavonol content was conducted using the method followed by (18) with minor alterations. 1mL of sample was added to 2mL of DNPH (2,4-dinitrophenylhydrazine) and heated for 50 mins at 50 °C. The mixture was allowed to cool and diluted to 10 mL with 10% KOH. 1 mL of the resultant solution was then diluted to 50mL using methanol. Naringenin was used as a standard. The absorbance against blank was read at 486nm spectrophotometrically.

Determination of Total Phenol Content (TPC)

The total phenolic content was calculated by Folin-Ciocalteu method (19). Different aliquots of gallic acid were taken as (0.2, 0.4, 0.6, 0.8 and 1mL). 0.5mL of ethanolic and water extract were taken separately. Add 0.5 mL of Folin-Ciocalteu reagent, and let it remain for 5mins. After incubation, 0.5mL of sodium carbonate was added and further incubated for 10mins. The optical density against blank was determined at 750 nm using a spectrophotometer.

In vitro antioxidant assays

DPPH Radical Scavenging assay

The scavenging potential of the extracts against free radicals was assessed using the DPPH radical scavenging assay, following the method outlined by (20). Initially, 1mg of DPPH was mixed with 25mL of methanol. 2 mL of this solution was combined with the extract at various concentrations ranging from 20 to 100µg/mL. After extensive vortexing, the mixture was allowed to stand at room temperature for 30 mins while it was kept in the dark. The change in color from violet to yellow indicates the presence of antioxidants. Ascorbic acid was taken as standard. The absorbance of the resulting mixture was then measured using a spectrophotometer at a wavelength of 517 nm. The percentage of radicals scavenged can be determined by the following formula,

$$\% \text{ of DPPH radical scavenging} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} * 100$$

ABTS Radical Scavenging assay

The antioxidant activity of the extracts was assessed through the ABTS radical cation decolorization assay (21). 7mM of ABTS was dissolved in water and 2.45 mM potassium persulfate was mixed together in 1:1 ratio for the formation of ABTS cation radicals. This mixture was stored at room temperature in the dark for 12-16 hours prior to use. After that, methanol was added to the ABTS solution to dilute it and get an absorbance of 0.700 at 734 nm. Following that, the standard and sample were taken in various aliquots (20, 40, 60, 80 and 100 µg/mL) and made it upto 300µL using methanol. The solutions were incubated for 30 mins at room temperature and the absorbance was read at 734 nm using a spectrophotometer. Trolox served as the standard. The ABTS scavenging percentage was calculated using the formula,

$$\% \text{ of ABTS radical scavenging} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} * 100$$

Hydroxyl Radical Scavenging assay

The hydroxyl radical scavenging assay of Pandanus odorifer extracts was determined using the method followed by (22). Different volumes of the standard and sample were taken in the concentration of

(20, 40, 60, 80 and 100 µg/mL). 1mL of iron EDTA was added to all the tubes. Further, 0.5mL of 0.018% of EDTA solution was added. 1mL of DMSO and 0.22% of ascorbic acid were added. The solutions were heated at 80-90^{0}C in water bath for 15mins. Additionally, 1mL of TCA (ice-cold) and 3mL of Nash reagent (12.5g of ammonium sulphate, 0.9mL of glacial acetic acid, 0.6mL of acetyl acetone and made up to 300mL using distilled water) were added to all the tubes. Incubate the tubes at room temperature for 15mins for the development of colour. The yellow colour developed was read at 412 nm using spectrophotometer. Ascorbic acid was used as a standard. The percentage of radical scavenged was calculated by the formula,

$$\% \text{ of hydroxyl radical scavenging} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} * 100$$

Total antioxidant content

The total antioxidant capacity of the extracts was assessed using the phosphomolybdenum method, following the procedure outlined by (23). A reaction mixture containing sulfuric acid (0.6 M), ammonium molybdate (4 mM) and sodium phosphate (28 mM) were mixed with various volumes of extracts (0.2,0.4,0.6,0.8 and 1mL) and incubated for 90mins at 95°C. The absorbance was read at 695 nm using a UV-spectrophotometer. Ascorbic acid serves as a standard.

GC-MS Analysis

Using gas chromatography mass spectrometry (GC-MS) analysis, the phytocompounds of Pandanus odorifer flower were examined. Phytocompounds were identified using Agilent gas chromatography (GC7890A) in conjunction with a mass spectrometer (MS5975C). A capillary column (DB5MS) with a length of 30 meters was employed, featuring dimensions of 0.25 mm in diameter and a film thickness of 0.25 microns. The oven temperature was programmed to range from 30°C to 350°C over 4mins at a rate of 10°C per minute. Helium was employed as a carrier gas, and an ionization voltage of 70 eV was utilized for MS detection, with a mass scan range of 45 to 380m/z. The obtained mass spectra were compared to the National Institute of Standards and Technology (NIST) database. The compounds were identified by name, molecular formula and molecular weight (24).

Cytotoxic activity

The MTT assay was used to determine the cytotoxic effect of ethanolic extract of Pandanus odorifer flower in HT-29 colon cancer cells. The cells (1 × 10 4 / well) were plated in 96-well plates with 0.2 mL of media /well. Incubate the cells for 72 hours in a 5% CO_2 incubator. The samples were tested at different concentrations in 0.1% DMSO for 48 hours at 5% CO_2 incubator. The cells were imaged under inverted microscope (40X). After removing the sample, 20 µL of MTT reagent was added. The viable cells were measured at 540nm (25). The percentage of viable cells were measured using the formula,

$$\% \text{ of cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} * 100$$

Statistical Analysis

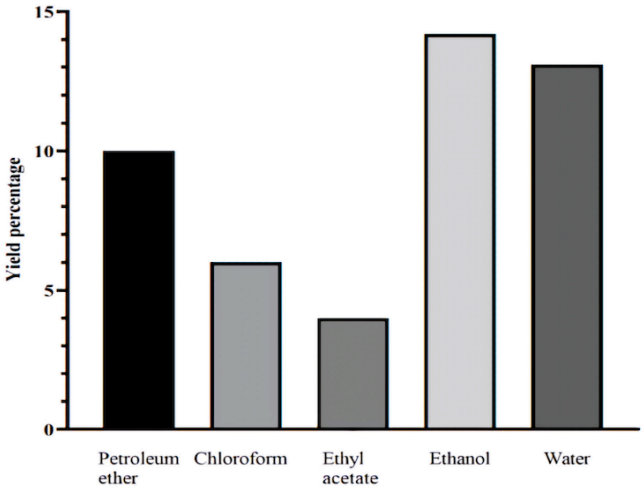
Statistical analysis was performed using GRAPHPAD PRISM 9. The mean and standard deviation (SD) of triplicates were utilized to represent the results of each experiment. One-way ANOVA, followed by Dunnett's test, was conducted to assess variance and identify significant differences between the means, with a significance threshold set at $p < 0.05$. The probability levels were denoted as $p = <0.01$ to 0.05 (*), $p = <0.001$ to 0.01 (**), $p =< 0.0001$ to 0.001 (***).

Results

Yield percentage

Fig. 2 represents the yield percentage of different extracts of *Pandanus odorifer* flower. The extraction yield can vary depending on the type of solvents used and the chemical composition of the sample.

Figure 2: Yield percentage of the extracts



Preliminary phytochemical analysis

Table 1: Preliminary phytochemical analysis of different extracts of *Pandanus odorifer* flower

Secondary metabolites	Petroleum ether	Toluene	Chloroform	Ethanol	Water
Resin	+	+	-	+	-
Alkaloids	-	-	-	+	-
Carbohydrates	-	-	-	+	+
Cardenolides	-	-	+	+	-
Amino acids and Proteins	-	-	-	-	+
Flavonoids	-	-	-	+	+
Phenolic compounds	-	-	-	+	+
Tannins	-	-	-	+	+
Carboxylic acids	-	-	-	+	+
Steroids	-	+	+	+	-
Saponins	-	-	-	+	+
+ POSITIVE		- NEGATIVE			

The preliminary phytochemical analysis was conducted on five distinct extracts of Pandanus odorifer flower, as outlined in (Table 1). The ethanolic and water extracts showed the presence of more phytochemicals compared to other solvents. The metabolites like resin, alkaloids, carbohydrates, cardinolides, flavonoids, phenolic compounds, tannins, carboxylic acid, steroids and saponins were found to be present in the ethanolic extract whereas carbohydrates, amino acids, flavonoids, phenolic compounds, tannins, carboxylic acids and saponins were present in water extract.

Determination of phytoconstituents

Table 2: Quantitative determination of phytoconstituents in the ethanol and water extracts of *Pandanus odorifer* flower

Parameters	Ethanolic extract	Water extract
Flavone and flavonol content (mg/g quercetin equivalent)	40±0.110	25±0.090
Flavanone and dihydroflavonol content (mg/g naringenin equivalent)	49±0.268	53±0.120
Total phenol content (mg/g gallic acid equivalent)	108±0.210	87±0.173
Total antioxidant content (mg/g of ascorbic acid equivalent)	1.6± 0.093	1.3± 0.056

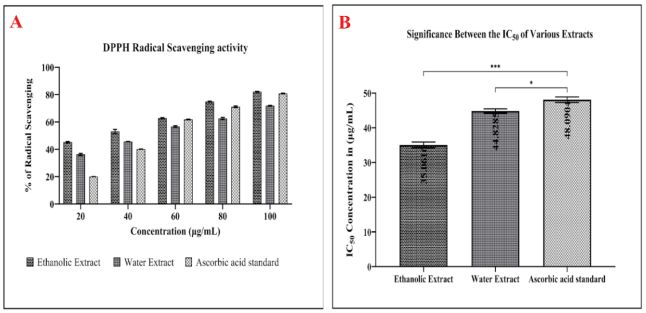
The amount of flavonoids, phenols and antioxidants present in the ethanol and water extracts were determined as shown in (Table 2). In the ethanolic extract, the flavone and flavonol content was determined to be 40±0.110 mg/g quercetin equivalent and flavanone and dihydroflavonol content was found to be 49±0.268 mg/g naringenin equivalent. Moreover, the extract exhibited a significant amount of total phenols, with a value of 108±0.210 mg/g gallic acid equivalent. Further, the extract showed remarkable antioxidant properties, with a total antioxidant capacity of 1.6 ± 0.093 mg/g equivalent of ascorbic acid. Similarly in the water extract, the flavone and flavonol content was determined to be 25±0.090 mg/g quercetin equivalent, the flavanone and dihydroflavonol content was found to be 53±0.120 mg/g naringenin equivalent and the total phenol content was 87±0.173 mg/g gallic acid equivalent. The total antioxidant capacity was determined to be 1.3± 0.056 mg/g equivalent of ascorbic acid respectively.

***In vitro* antioxidant assays**

DPPH Radical Scavenging assay

An evaluation of the antioxidant activity was conducted using the DPPH assay in ethanol and water extracts of *Pandanus odorifer* flower, along with ascorbic acid as a standard (Fig. 3A). The results demonstrated that both ethanol and water extracts exhibited significant scavenging potential. Pandanus odorifer flower extracts showed IC_{50} values of 35.06 µg/mL in ethanol and 44.82 µg/mL in water respectively. The IC_{50} value for ascorbic acid was estimated to be 48.09 µg/mL, as shown in (Fig. 3B).

Figure 3: (A) DPPH radical scavenging assay of flower extracts (B) IC_{50} values of ethanol, water and ascorbic acid indicated with a significance level of p < 0.05.

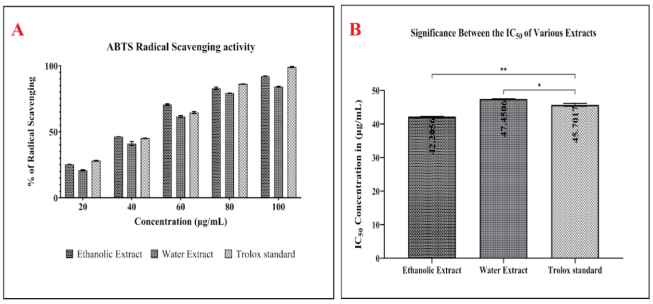


Note: p = <0.01 to 0.05 (*) and p = < 0.0001 to 0.001(***).The graphs were given as mean ± SD in triplicate

ABTS Radical Scavenging assay

(Fig. 4A) illustrates the ABTS assay for the ethanol, water extracts. The IC_{50} values for both extracts and the standard were shown in (Fig. 4B). The IC_{50} value for the ethanolic extract was determined to be 42.20 µg/mL, while for the water extract it was 47.45 µg/mL. Similarly, the IC_{50} value for trolox was found to be 45.70 µg/mL.

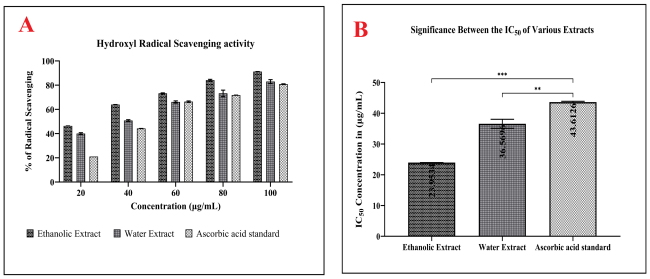
Figure 4: (A) ABTS radical scavenging assay of flower extracts (B) IC_{50} values of ethanol, water and trolox indicated with a significance level of p < 0.05.



Note: p = <0.01 to 0.05 (*) and p = <0.001 to 0.01 (**).The graphs were given as mean ± SD in triplicate.

Hydroxyl Radical Scavenging assay

Figure 5: (A) Hydroxyl radical scavenging assay of flower extracts (B) IC_{50} values of ethanol, water and ascorbic acid indicated with a significance level of p < 0.05.



Note: p = <0.001 to 0.01 (**) and p = < 0.0001 to 0.001(***).The graphs were given as mean ± SD in triplicate.

The hydroxyl radical scavenging assay was carried out in the ethanol and water extracts of Pandanus odorifer flower which was compared with the standard ascorbic acid (Fig. 5A). The IC_{50} values of the ethanolic extract was found to be 23.95 µg/mL and for water extract 36.56 µg/mL. The ascorbic acid showed the IC_{50} value of 43.61 µg/mL which was shown in (Fig. 5B).

GC-MS Profiling

The compounds that are bioactive present in the ethanolic extract of Pandanus odorifer flower were determined using GC MS analysis. The identified bioactive compounds and their chromatogram were shown in (Table 3 & Fig. 6) respectively. 22 bioactive compounds were selected from the total compounds identified. These compounds exhibited significant biological activity and were determined using their retention times, mass spectra, and comparison with spectral databases.

Figure 6: Chromatogram of GC MS analysis of ethanolic extract of *Pandanus odorifer* flower

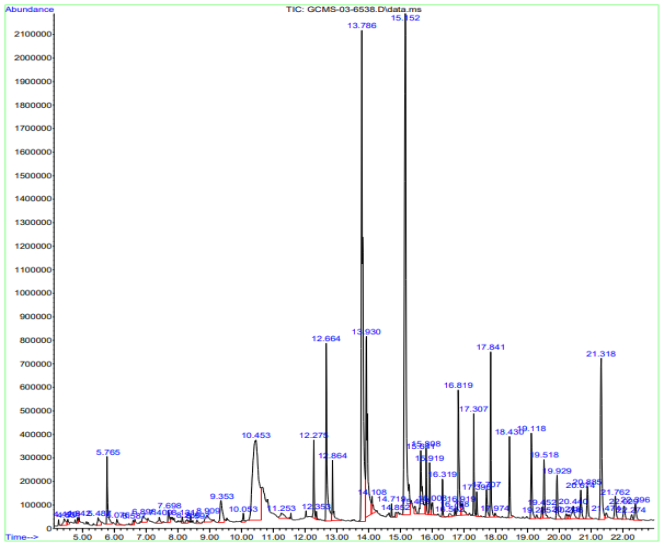


Table 3: GC MS profile of ethanolic extract of *Pandanus odorifer* flower

S.No	Peak Number	Retention time	Molecular weight (g/mol)	Molecular formula	Area %	Compound name	Similarity Index
1	4	5.487 min	124.13	$C_7H_8O_2$	0.40	Phenol, 2-methoxy-	95
2	5	5.765 min	122.16	$C_8H_{10}O$	1.39	Phenylethyl Alcohol	97
3	16	10.053 min	268.5	$C_{19}H_{40}$	0.25	Nonadecane	93
4	19	12.275 min	234.34	$C_{14}H_{22}N_2O$	1.22	Lidocaine	80
5	20	12.353 min	290.5	$C_{20}H_{34}O$	0.12	Geranylgeraniol	95
6	21	12.664 min	256.42	$C_{16}H_{32}O_2$	4.25	n-Hexadecanoic acid	99
7	22	12.864 min	284.5	$C_{18}H_{36}O_2$	1.16	Hexadecanoic acid, ethyl ester	97
8	23	13.786 min	280.4	$C_{18}H_{32}O_2$	16.58	9,12-Octadecadienoic acid (Z,Z)	99
9	24	13.930 min	308.5	$C_{20}H_{36}O_2$	6.36	Linoleic acid ethyl ester	99
10	26	14.719 min	296.57	$C_{21}H_{44}$	0.17	Eicosane, 10-methyl-	81
11	31	15.808 min	282.5	$C_{20}H_{42}$	0.94	Eicosane	97
12	32	15.919 min	330.50	$C_{19}H_{38}O_4$	0.90	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	90
13	36	16.708 min	364.5	$C_{20}H_{35}F_3O_2$	0.13	Oleyl alcohol, trifluoroacetate	91
14	40	17.396 min	410.7	$C_{30}H_{50}$	0.39	Squalene	87
15	42	17.841 min	240.5	$C_{17}H_{36}$	2.75	Heptadecane	95
16	45	19.118 min	380.7	$C_{27}H_{56}$	1.98	Heptacosane	90
17	47	19.452 min	430.7	$C_{29}H_{50}O_2$	0.27	Vitamin E	99
18	48	19.518 min	386.7	$C_{27}H_{46}O$	1.45	Cholesterol	99
19	52	20.440 min	400.7	$C_{28}H_{48}O$	0.67	Campesterol	91
20	53	20.674 min	412.7	$C_{29}H_{48}O$	0.88	Stigmasterol	98
21	55	21.318 min	414.7	$C_{29}H_{50}O$	5.20	gamma.-Sitosterol	99
22	60	22.396 min	410.7	$C_{29}H_{46}O$	0.62	Stigmasta-3,5-dien-7-one	93

Cytotoxic activity

The cytotoxic effect of the ethanolic extract of Pandanus odorifer flower on HT-29 colon cancer cells were assessed using the MTT assay as shown in (Fig.7& 8). The viability of cells treated with different concentrations of extract was contrasted with the untreated control cells. The viability of the cell decreased with increasing extract concentration. The IC_{50} value was found to be 28.08 µg/mL. A significant dose-response relationship was found, with higher concentrations of the extract inhibiting cell viability more effectively.

Figure 7: Inverted microscopic images (40X) of cytotoxic activity on HT-29 cells

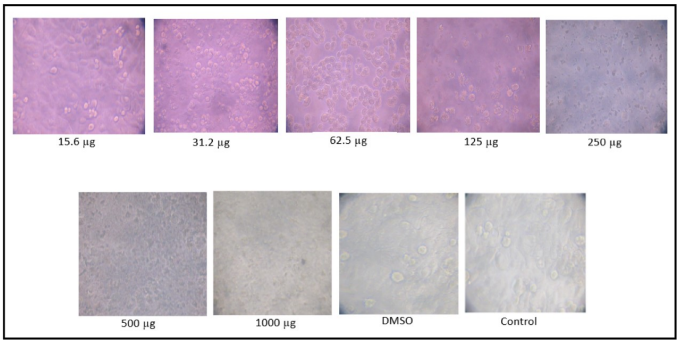
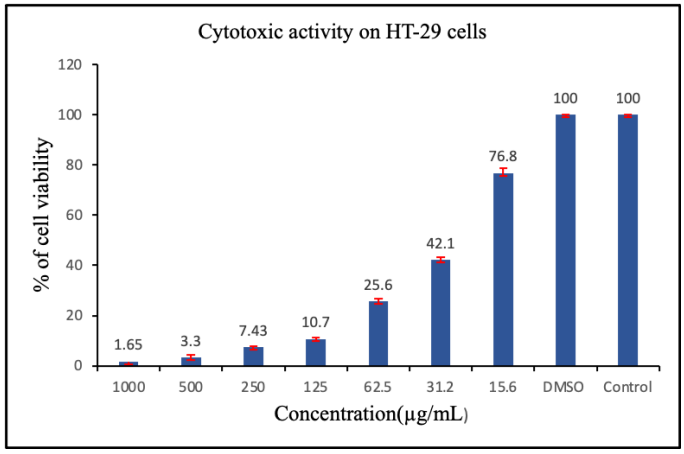


Figure 8: Cytotoxic activity in the ethanolic extract of *Pandanus odorifer* flower



Discussion

According to our knowledge, this study was the first to employ the antioxidant potential of *Pandanus odorifer* flower. This study investigated that the phytochemical compounds, antioxidant potential and the bioactive compounds of *Pandanus odorifer* flower. The preliminary phytochemical analysis of various extracts revealed the presence of resin, alkaloids, carbohydrates, cardinolides, flavonoids, phenolic compounds, tannins, carboxylic acid, steroids and saponins. These phytochemicals are naturally occurring organic substances present in plants with specific characteristics and potential health advantages (26). The ethanol and water extracts exhibited the maximum phytochemical compounds and were employed for further assays. Subsequently, the chloroform extract was found to have the least phytochemical compounds. Flavonoid compounds, derived from plants, are naturally occurring substances distributed throughout various parts of the plant. Most categories of flavonoids possess antioxidant properties. Many studies suggested that the flavones and catechins are particularly potent among flavonoids in safeguarding the body against reactive oxygen species (27). Flavonoids can activate signaling pathways that induce apoptosis and significantly inhibits cancer cell survival and multiplication (28). Antioxidants prohibit the activity of free radicals through various mechanisms, with phenolic compounds, especially phenolic acids, demonstrating significant free radical scavenging capabilities. These compounds fight against oxidative stress and its effects by inhibiting enzymes that produce reactive oxygen species (29,30). In this study we also estimated the flavonoid and phenolic contents. Investigating the flavonoid levels in *Pandanus odorifer* flower can offer valuable insights into its possible medicinal uses and applications in traditional medicine. Our findings demonstrated a substantial presence of flavonoids in the *Pandanus odorifer* flower extracts, as indicated by spectrophotometric analysis, suggesting potential pharmacological importance for this plant species. These results are consistent with prior studies that have highlighted flavonoid presence in various parts of Pandanus species, including leaves and roots.

Similarly, the assessment of phenolic compounds in natural products holds significant importance due to their varied biological activities and potential health benefits (31). Determining the phenol content in *Pandanus odorifer* flower emphasizes their potential as a natural source of antioxidants with diverse pharmacological effects. Consequently, the presence of flavonoids and phenolic compounds in *Pandanus odorifer* flower are likely to possess considerable antioxidant capabilities (32).

Generally, aromatic species are rich in antioxidants. The increased level of free radicals generated through biological oxidation leads to disturbances in both the structure and operations of intracellular proteins. This disruption extends to membrane impairment caused by the peroxidation of polyunsaturated fatty acids in lipids, alterations in nucleic acid bases, and changes in chromosomes, including breaks in DNA that occur in single and double strands, as well as cross-links between DNA and proteins. Additionally, it results in cell demise and oxidative stress to cellular structures and constituents, including the depolymerization of polysaccharides and the deterioration of carbohydrates (33). Antioxidants are a system that shields the organism from the negative effects of free radicals by reducing or repairing damage. It is widely recognized that the mechanisms triggered by the rise in reactive oxygen species due to oxidative stress are closely associated with the pathogenesis of numerous diseases (34). From the (Fig. 3), (Fig. 4) and (Fig. 5), it was clear that the ethanolic extract of the *Pandanus odorifer* flower exhibited greater antioxidant activity compared with water extract and ascorbic acid.

The results of gas chromatography mass spectrometry showed 60 peaks in the chromatogram, from which 22 compounds were chosen based on their similarity index with NIST library. The compounds such as Phenol, 2-methoxy-, Phenylethyl Alcohol, Nonadecane, Lidocaine, Geranylgeraniol, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester,

9,12-Octadecadienoic acid (Z,Z), Linoleic acid ethyl ester, Eicosane, 10-methyl-, Eicosane, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Oleyl alcohol, trifluoroacetate, Squalene, Heptadecane, Heptacosane, Vitamin E, Cholesterol, Campesterol, Stigmasterol, gamma.-Sitosterol and Stigmasta-3,5-dien-7-one were found to be present in the ethanolic extract of the *Pandanus odorifer* flower. The majority of the compounds detected in the GC-MS analysis are primary metabolites, including fatty acids, esters, alcohols, hydrocarbons, sterols and lipids. Although these compounds are mostly implicated in fundamental metabolic processes, they also possess significant potential for providing important insights in cancer research. It also helps in understanding the volatile profile, nutritional composition, and bioactive characteristics of the extract. The study conducted by (35) concerning the essential oil of *Pandanus odorifer* flowers revealed the predominant presence of compounds such as 2-Phenylethyl methyl ether, terpinen-4-ol, and α-terpineol. According to the literature (36-38), compounds like Phenol, 2-methoxy-, Geranylgeraniol, 9,12-Octadecadienoic acid (Z,Z), Vitamin E and Cholesterol are known to scavenge free radicals and prevent oxidative damage in biological systems.

The MTT assay depends on measuring mitochondrial activity by forming formazan crystals from MTT in living cells. This assay is frequently used to investigate the *in vitro* cytotoxic properties of the drug on cell lines, for the majority of cell populations, total mitochondrial activity is associated with the proportion of live cells (39). The study by (40) on HT-29 cells using *Pandanus canaranus* found substantial cytotoxic properties in the crude plant extracts, indicating that the Pandanus species has a greater ability to inhibit cancer cells.

Conclusion

In conclusion, the study conducted on *Pandanus odorifer* flower has provided valuable understanding into its phytochemical composition, antioxidant potential, cytotoxic activity and chemical constituents as determined by GC-MS analysis. The phytochemical screening revealed the presence of various secondary metabolites indicating its potential therapeutic benefits. Moreover, the antioxidant activity assessed through different assays demonstrated significant free radical scavenging potential, highlighting its potential use as a natural antioxidant agent. Furthermore, GC-MS analysis identified several compounds, providing a deeper understanding of the chemical composition of *Pandanus odorifer* flower. The ethanolic extract of *Pandanus odorifer* flower showed a potential cytotoxic activity on HT-29 colon cancer cells. Overall, these findings emphasize the importance of *Pandanus odorifer* flower as a possible supply of bioactive substances with antioxidant properties, justifying further exploration for its pharmaceutical applications. In future, we investigate deeper into its pharmacological mechanisms and conduct its anti-cancer potential to validate its therapeutic potential and determine its suitability for various healthcare applications.

Conflict of Interest: The authors declare no conflict of interest

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