The Anticancer Potency of *Pandanus odoratissimus* Extracts In Human Breast Cancer (MCF-7) Cell Line

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Abstract

Developing new chemical entities, pharmaceuticals, and drug leads frequently involves the exploration of natural sources. *Pandanus odoratissimus* (Pandanaceae), a well-known plant indigenous to Malaysia, has a history of application in Ayurvedic medicine to treat various ailments. This study aims to determine the antioxidant and anticancer properties of *P odoratissimu*s against MCF-7 human breast cancer cell lines. The assessment of antioxidant activity was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity method, which revealed that the core extract exhibited the highest antioxidant value among the various extracts evaluated. The effects of plant components on MCF-7 cell lines were assessed using an MTT assay ((3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide). The samples showed a significant increase in antioxidant activities in *P. odoratissimus* extracts. Our findings revealed that cancer cells treated with the samples for 72 hours experienced a reduction in cell viability. With an IC_{50} value of 88.00 µg/ml, the extracts exhibited poor cytotoxic effects and showed no potential as anticancer agents against MCF-7, indicating a lower effectiveness in killing cancer cells.

Keywords: *Pandanus odoratissimus*, Antioxidant, Anticancer, MCF-7

INTRODUCTION

Cancer is a malignant growth or tumor formed by the abnormal and rapid reproduction of cells (Premila, 2006). In Ayurveda, several predisposing factors have been enunciated as a result of which tumor can develop an imbalance in poor food digestion and absorption, leading to the formation of undigested food particles, deposition in the tissues, and enzyme malfunction that results in tumor development (Gogte, 2000). They estimated 95% of cancers were caused by lifestyle, which takes 20 to 30 years to develop (Bhanot et al., 2011).

Breast tissue is made up of milk glands that produce milk for breastfeeding. Breast cancer usually occurs in the lobules and ducts, part of the glandular tissue that connects the lobules to the nipple. The breast also contains fatty, connective, and lymphatic tissues that provide support and shape to the breast. Screening exams are one way to detect breast cancer, either before symptoms appear or after symptoms develop, such as feeling a lump. They were done before or after symptoms developed when women felt a

lump. According to statistics as reported by Yip (2006), approximately 1 in 20 women in Malaysia developed breast cancer in their lifetime.

Historically, plants have been used in the in the treatment of cancer. More than 60% of anticancer medications are derived from natural resources (Bhanot et al., 2011). According to Cragg and Newman (2005), plants have a long history in cancer treatment, and over 3000 species of plants have been reported to be used in such treatments. Certain antioxidants found in plants have the potential to inhibit breast and lung cancer activities. Natural products are essential as therapeutic remedies in many developing countries.

Studies from the past have highlighted the potent antioxidant properties of *Pandanus odoratissimus*. Antioxidants play a crucial role in preventing cancer by reducing the risk of mutation and neutralizing harmful reactive free radicals in the body, thus preventing lipid and protein oxidation. The damaging process of lipid oxidation in biological membranes has been linked to aging, cancer, and liver damage. Additionally, reactions between oxidized lipid compounds and proteins can lead to various adverse effects, including loss of enzyme activity, damage to DNA, and an acceleration of disease processes.

Numerous treatments, including surgery, radiation, and chemotherapy, have been developed to treat breast cancer. Patients with cancer respond differently to these medicines' adverse effects. Therefore, it's critical to identify natural anticancer sources that, compared to current medical treatments, have less or no adverse impact on patients. Much research has been done on natural sources for anticancer, but this study aims to find another source with anticancer potential. Since antioxidants and anticancer are closely related, previous research shows that *Pandanus odoratissimus* has antioxidant activity. Thus, it might inhibit breast cancer cell growth (MCF-7 cell lines). Hence, this study aimed to determine the antioxidant activity and anticancer potential of fruit and leaf extracts of *Pandanus odoratissimus* on MCF-7 cell lines.

METHOD

Sample collection

The leaves, fruit, and their seeds and cores were locally sourced from Batu Rakit, Kuala Terengganu, Terengganu, Malaysia. After collection, the plant parts were carefully cut into small pieces and immediately placed in a freezer at -80°C to ensure complete freezing of water molecules before undergoing the freeze-drying process. Subsequently, the samples underwent freeze-drying for seven days to achieve thorough desiccation. Following this, the dried plant materials were finely powdered using a grinder to yield small-sized samples, preparing them for the subsequent extraction process.

(a) Fruit (b) Keys (c) Core (d) Leaf

Figure 1. *Pandanus odoratissimus* fruit (a), part of fruit – keys (b) and core (c) and leaf (d)

Extracts Preparation and Extraction

Plant materials (500 g of dried powder) were soaked in 1 L of 98% methanol using a 1:10 (w/v) ratio to obtain a methanolic crude extract at room temperature. The soaked material was stirred with a sterilized glass rod and left for a minimum of 24 hours. The resulting extracts were filtered using Whatman Filter Paper No. 1, and the filtrates obtained were dried using a rotary evaporator. To ensure the removal of solvent and water, the dried extracts were put under nitrogen steam. The methanolic crude extracts were then stored in a cold room for future use. These extracts were screened for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and those with the highest antioxidant activity were subjected to the solvent partition method. A standard protocol was followed for the fractionation of plant materials, with the crude solution (100 ml) being fractionated using solvents of increasing polarity.

Antioxidant Activity Assay

The Sigh et al. (1995) standard method was used to measure the DPPH radical scavenging activity. 180 µl of DPPH (6 x 105 M) was added to 96 wells containing 20 µl of different concentrations ranging from 0 mg/ml to 10 mg/ml. The stock solution of DPPH was produced in methanol (60 ml methanol buffered with 40 mL acetate buffer), 0.1 M, pH 5.5. For a dark atmosphere conducive to the reaction, the reaction well triplicates were wrapped in aluminium foil and allowed to sit at room temperature for half an hour. Using a microtiter plate reader, a spectrophotometric measurement was performed at 517 nm. The following formula was used to determine the percentage of inhibition:

$$\%inhibition = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} x\ 100$$

The inhibition percentage indicated the antioxidants' ability to extract crude oil to reduce DPPH.

Anticancer Activity Assay

The Institute of Climate Adaptation and Marine Biotechnology (ICAMB) at Universiti Malaysia Terengganu (UMT) provided the MCF-7 human breast cancer cell lines. These cells were cultured in 5 milliliters of Rosewell Park Memorial Institute medium (RPMI) 1640 media, supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The MCF-7 cells were incubated overnight at 37°C in 5% CO2 to facilitate cell attachment (Jin et al., 2010). Upon reaching 95% confluence, the cells were washed twice with 3 ml FBS and subjected to a medium removal step. Subsequently, one milliliter of trypsin EDTA was added to the flask and incubated for ten minutes. Following incubation, the trypsin was removed, and the cells were transferred to a giant preculture flask, where 12 ml of medium was added. The cell mixture was then maintained at 37 °C and 5% CO_2. The MTT assay was performed once the cells reached 80% confluence using [3-(4, 5-dimethyl thiazolyl) 2,5 Diphenyl-tetrazolium bromide].

MTT Assay

More than 80% of confluent cells were chosen. After being trypsinized from the preculture bottle, the cell lines were transferred to a 96-well plate. After making a cell suspension with 9 x 103 cells/mL, a 100 µl aliquot of the suspension was carefully added to each well. The plate was incubated for 24 hours at 37°C with 5% CO_2. The cultured media was discarded after 24 hours, and 100 µl of the crude extract was

dissolved in each well along with a blank as a reference and varied concentrations (120 µg/ml, 60 µg/ml, 30 µg/ml, 15 µg/ml, 7.5 µg/ml, 3.25 µg/ml, 1.625 µg/ml). For 72 hours, a 96-well plate was incubated. The solution from each well was removed after 72 hours, and each well was then filled with 100 µl of MTT diluted in phosphate-buffered saline (5 mg/ml) and incubated for an additional 4 hours. Add 20 µl of dimethyl sulphoxide (DMSO) and stir gently. The absorbance of the test plate was measured at 571 nm using an enzyme-linked immunosorbent assay (ELISA) reader after it had been shaken. These methods were used to calculate the percentage of cell viability:

$$percentage\ of\ cell\ viability = \frac{Abs\ sample}{Abs\ blank}\ x\ 100$$

The IC_{50}, representing the 50% inhibitory concentration, was determined and subsequently graphed. According to a study by the American National Cancer Institute (NCI), an IC_{50} value falling below 30 µg/mL serves as the threshold for indicating anticancer activity in a crude herb extract (Itharat et al., 2004).

Data Analysis

The experimental results were expressed using the mean ± standard deviation. A graphical representation was employed to facilitate the analysis of the results.

RESULT AND DISCUSSION

Antioxidant Activity

The conventional Sanchez et al. (1999) technique was followed in a quantitative study employing the radical scavenging DPPH test. Plant extracts' antioxidant activity has been compared using quercetin as a reference. Although the antioxidant activity of the core sections of the plants was higher than that of the keys and leaves, these plant extracts still had poor antioxidant activity when compared to the standard, as indicated by the preliminary study of antioxidant activity data in Figure 2. The result also showed that antioxidant activities increased when the concentration of the sample increased and remained constant, beginning at a certain point of concentration. Antioxidant activity in the core and keys was enhanced by further extraction in the solvent partition method where methanolic crude of keys and core been fractionated using hexane, ethyl acetate, and water, which are ascending solvent polarity.

The two methanol extraction extracts, keys, and core, with the highest antioxidant value, were chosen for additional solvent fractionation. The study revealed that the ethyl acetate keys and core had the highest antioxidant content compared to the other extracts. There is a linear association between the number of flavonoids and antioxidant activity, as demonstrated by numerous studies (Ghasemzadeh et al., 2010; Andriani et al., 2019). A high total flavonoid content also raises the level of antioxidant activity. Results in Figure 2 showed that keys have higher antioxidants than the core since the IC_{50} value is lower when obtained from an antioxidant activity assay with a value of 0.80 mg/ml. At the same percentage of inhibition, the core showed antioxidant activity of 1.00 mg/mL. Quercetin exhibited a strong potential for free radical scavenging activity compared to sample extracts with a 0.12 mg/ml value.

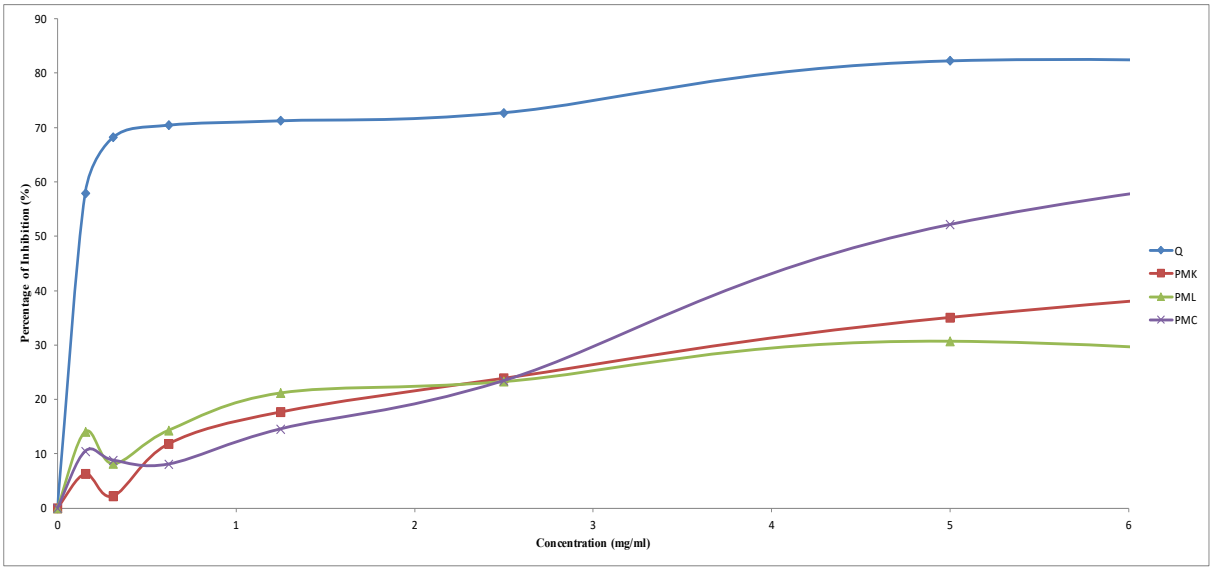

Figure 2. Antioxidant activity of quercetin and P. odoratissimus using methanolic extraction (value is mean of triplicates) [Q= Standard; PMK=Keys; PML= Leaf; PMC= Core]

Antioxidants are gaining popularity, particularly those designed to prevent the breakdown of lipids and other food components and counter the alleged harmful effects of free radicals on the human body (Molyneux, 2004). Antioxidants from natural origins are preferred over synthetic ones in both scenarios (Abdalla and Roozen, 1999). Currently, a prominent method utilizing stable free radicals is known as DPPH. DPPH is a stable free radical because the spare electron delocalizes over the whole molecule rather than fragmenting, as with most other free radicals. When a chemical capable of donating a hydrogen (H) atom is combined with a DPPH solution, the delocalization leads to a violet color, characterized by an absorption band in methanol solution centered at approximately 570 nm. This results in the reduced form, which appears pale yellow.

The data from the DPPH assay is quantitatively analyzed using the 50% inhibition concentration (IC_{50}), representing the substrate concentration at which 50% of the DPPH activity is attenuated. This parameter was introduced initially by Brand-Williams and colleagues (Brand-Williams et al., 1995; Bondet et al., 1997), and since then, several groups of workers have used it to present their findings (Kim et al., 2002; Lebeau et al., 2000, Lu and Foo, 2000; Sanchez-Moreno et al., 1999).

Quercetin is the standard DPPH employed in this study. It is a polyphenolic flavonoid component commonly found in plants and has potent antioxidant potential. Quercetin is a significant bioflavonoid found in human diets and is often in glycoside form. Recent studies have explored quercetin's potential as an anticancer drug and its possible carcinogenicity. However, it is recognized to be one of the most mutagenic flavonoids, as demonstrated in human DNA (Duthie et al., 1997), cell culture (Nakayasu et al., 1986), and the Ames test (Bjeldanes and Chang, 1977).

Nevertheless, mutagenicity does not always imply carcinogenicity. In most research, quercetin has not been shown to have any carcinogenic potential in vitro. Other comparable investigations also suggest that quercetin and its glycosides are not carcinogenic (Ito et al., 1989). Scambia et al. (1994) reported that quercetin demonstrated a marked reduction in the proliferation of human breast cancer cells, specifically MCF-7 and MDA-MB231 cell lines. Furthermore, Du et al. (2010) elucidated the mechanism through which quercetin exerts its inhibitory effects on breast cancer. The antioxidative properties of quercetin are suggested to contribute to its cytoprotective impact against oxidative stress, potentially

correlating its content to its anticancer efficacy. In the current investigation, quercetin exhibited the highest level of antioxidant activity compared to crude extracts, followed by core and seeds.

The positive effects of oxidative stress can be counteracted or prevented by natural antioxidants present in herbs and spices. Spices and herbs contain free radical scavengers such as polyphenols, flavonoids, and phenolic compounds. In a methanol extract, the core crude exhibited high antioxidant activity with an IC50 value of 4.60 mg/ml, although still lower than the standard quercetin with a 0.20 mg/ml value. A lower IC50 value indicates higher antioxidant activity. The methanolic extracts' free radical scavenging activity was confirmed in this study, with the leaf crude showing the lowest antioxidant activity (See Figure 2).

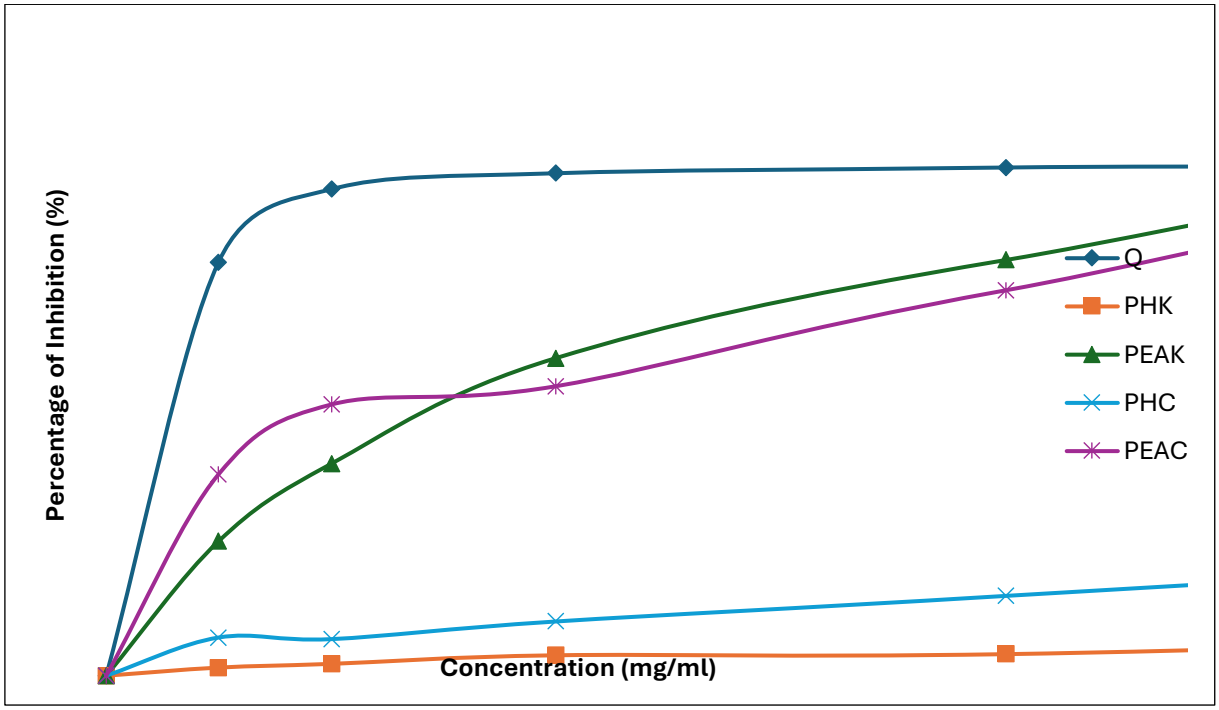

Figure 3. Antioxidant activity of quercetin P. odoratissimus from solvent partition method (value is mean of triplicates) [Q= Standard; PHK= Key (hexane); PEAK= Key (ethyl acetate); PHC= Core (hexane); PEAC= Core (ethyl acetate)]

In the fractionation method, the IC_{50} value for quercetin measured was 0.12 mg/ml. The results indicate the antioxidant activity of the ethyl acetate extract of core and keys with IC_{50} of 0.80 µg/ml and 1.00 µg/ml. The antioxidant activity is presented in Figure 3 above. Compared to the standard, the standard has lower IC_{50}, indicating the most active antioxidant activity. As a result, the core extract may have antioxidant properties. Compared to the standard and core, the antioxidant activity of the Keys extract was deficient. Thus, antioxidant compounds are abundant in the kingdom of plants. One such member's capacity for antioxidants has been made clear by this investigation.

Anticancer Analysis

Despite recent advancements in synthetic chemistry for drug discovery and production, the potential for discovering new and unique compounds from bioactive plants or their extracts for treating and preventing diseases is still vast (Kviencinski et al., 2008). Plant-derived medications such as vinblastine, vincristine, taxol, and camptothecin have been frequently used in the field of antitumor drugs, where they have been proven to improve the chemotherapy for certain cancers (Yousefzadi et al., 2010). Therefore,

the current work conducted MTT assays to evaluate *P. odoratissimus* extracts in the search for a novel anticancer agent.

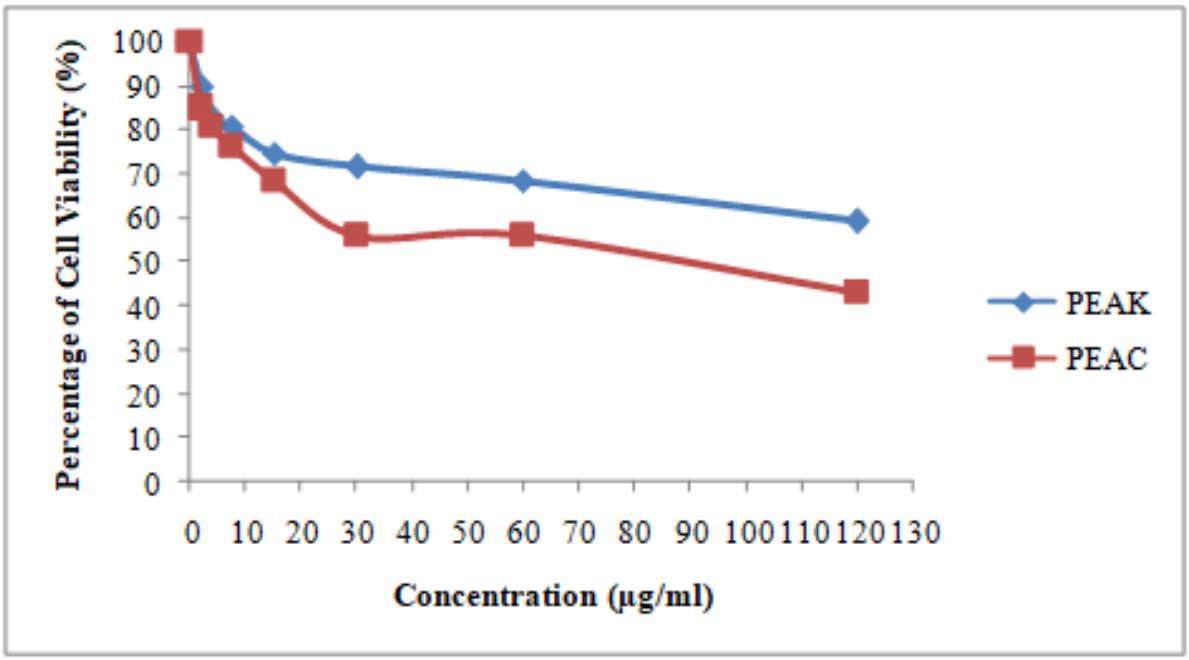

Figure 4. Cytotoxicity assay using MTT analysis for 72 hours (value is mean of triplicates) [PEAK= Key (ethyl acetate); PEAC= Core (ethyl acetate)]

Figure 4 showed that the parts of *P. odoratissimus* (keys and core) were found to express MCF-7 cancer cells inhibitory activity when tested at the concentration range of 1.625-120.00 µg/ml. At the concentration of 88.00 µg/ml, though, the core extract of ethyl acetate exhibited 50% inhibitory activity (IC_{50}) towards MCF-7, meanwhile, at this concentration, key extract of ethyl acetate exhibited a low percentage of inhibition with 36%. However, the standard has not been tested in MTT assay but by comparison with previous research, quercetin has IC_{50} of 4.9 µM (Miodini et al., 1999) and 15 µM (So et al., 1997). Quercetin (248 micoM) was found to down regulate the expression of mutant the protein of p53 which are a common genetic abnormality in human cancer (Du et al., 2011) until nearly undetectable levels in MCF-7 cell lines (Avila et al., 1994). In contemporary medicine, plants utilized in folk and traditional medicine have come to be recognized as potential sources for new medicinal drugs. Because *P. odoratissimus* is used as a medicinal agent in other parts of the world and in traditional medicine by Native Americans, it was selected for this investigation. As a result, an in vitro assessment of the cytotoxicity was conducted. Numerous bioactive substances, including tannins, alkaloids, steroids, saponins, terpenoids, and flavonoids, have been shown in earlier research to display a range of biological activities. These substances, which can be found in many foods, have a lot of potential as medication candidates because of their low toxicity, safety, and widespread public acceptance.

This study examined the cytotoxic effect of *P. odoratissimus* extracts on cancer cells using the malignant MCF-7 cell lines as a test subject. After 72 hours of extract incubation, the current study showed the cytotoxicity indices as a measure of the percentage of cell viability determined by MTT assay in MCF-7 cells in a dose-dependent manner. The test system for this investigation, which was driven by the need for the most effective treatment to address the rising prevalence of breast cancer globally, was the breast cancer phone line MCF-7. As demonstrated in Figure 4, the extract stopped the cancer cell from proliferating at 88 µg/ml. The limit of activity for crude extracts after a 72-hour exposure period is defined by the American National Cancer Institute (NCI) recommendations at an IC_{50} of less than 30 µg/mL. Because of this, the IC_{50} value of 88

μg/ml is regarded as a non-cytotoxic activity. Nonetheless, the core of the ethyl acetate extract is thought to be the least harmful when its IC_{50} is greater than 30 μg/ml (Ghasemzadeh et al., 2010). According to the study's findings, the ethyl acetate extract *of P. odoratissimus* exhibited ineffective cytotoxic effects on MCF-7 cells. The NCI, USA's IC_{50} value for classifying a pure substance as an anticancer agent was higher than the organization stipulated. MCF-7 cells, which do not meet NCI requirements, were found to have an IC_{50} value higher than this strict threshold, indicating a low anticancer potential against the MCF-7 cell line. Nevertheless, additional experiments employing distinct cell types could be required to investigate whether the extract has anticancer properties against other cancer cells. *P. odoratissimus* is rich in phenolics and flavonoids, which are known to have potency as anticancer agents against various types of cancer cells, including ovarian cancer cells (SKOV-3, OVCAR-3, and A2780), prostate cancer cells LNCaP and PC3 cells, and many others (Andriani et al., 2019; Kopustinskiene et al., 2020; de Luna et al., 2023). According to the investigation, *P. odoratissimus* extracts may have anticancer action against cancer cells other than MCF-7.

CONCLUSION

In conclusion, the study's results suggest that *P. odoratissimus* may potentially promote health if it is acknowledged that increased consumption of naturally occurring antioxidants that contain phenolic are linked to long-term health advantages. The antioxidant and multitherapeutic qualities of *P. odoratissimus* core and keys may be the cause of their health-promoting qualities. *P. odoratissimus* may, therefore, help create medicinal raw materials. According to this study, *P. odoratissimus* keys have lower cytotoxicity test results than core but greater antioxidant values than core. According to the current observation, *P. odoratissimus* extracts have less intense cytotoxic action against MCF-7 cells.

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