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SHORT COMMUNICATION

Study of Anti-proliferative Effect and Scratch Wound Migration Assay of Ethanol Extract of *Pyrus communis* L. Leaf on MCF-7 Model

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Abstract

Objectives: The current aim of the study was twofold: first, to identify the primary phytochemical compounds present in the ethanol extract derived from *Pyrus communis* L. leaves and second, to assess the extract's anti-proliferative effect. **Materials and Methods:** The study conducted an *in vitro* anti-proliferative investigation of the *P. communis* leaves extract using the MTT using colorimetric assay against the MCF-7 cell line. These assays collectively provide insights into different aspects of cell behaviour, including proliferation, migration, and invasion, which are important in understanding the overall effect of the extract on the MCF-7 cell line. The protective effect observed in the analysis of the ethanol extract is attributed to the existence of flavonoids and phenols in the extract. **Results:** Total flavonoid and phenolic contents were observed as 56 mg of quercetin/g and 48 mg of gallic acid/g as standard. This extract ascertained cytotoxic against the MCF-7 cell line in a reverse dose-dependent manner. However, the extract is found to be more potent and effective against MCF-7 (human Breast cancer cell lines) with LC_{50} value 265.310978µg/ml. **Conclusions:** The *in vitro* cytotoxic activity of this extract of the plant leaves has been evaluated, revealing a significant anti-proliferative effect and suppression of cell migration against the MCF-7 cell line. This suggests that the extract may possess compounds or properties that inhibit cell proliferation followed by wound migration, which are crucial factors in cancer progression. This approach is motivated by the observed inhibitory effect of cancer cell proliferation and wound migratory effects of the extract against the MCF-7 cell line, as well as the epidemiological evidence suggesting its anti-carcinogenic potential. This avenue of research holds promise for enhancing the effectiveness of cancer therapy and improving patient outcomes.

Keywords: Flavonoids, MCF-7 Cell Line, *Pyrus communis* L., Wound Migration Assay

1. Introduction

The hallmark feature of cancer is uncontrolled cell division, which can result from genetic mutations, environmental factors, or a combination of both. Cancer may damage virtually all parts of the body, and there may spread numerous types of cancer. Early diagnosis and accurate treatment are crucial so the main motto is to improving outcomes and preventing the spread of cancer to all parts of the body[1,2]. Breast cancer and lung cancer were indeed noted as the most frequently reported cancers worldwide in 2020, on account of to this data from the Global Cancer Statistics[3]. Breast cancer accounted approximately 12.5% of all new cancer cases diagnosed globally, making it as most spotted cancer among women. Lung cancer followed closely behind, representing about 12.2% of all new cancer cases universal, and it must be the leading causes of carcinoma-related deaths[4].

Breast cancer's ranking as the second topmost cause of mortality among women underscores its significance as a major public health concern. This emphasizes the critical importance of early detection through regular screenings, such as mammograms,

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as well as ensuring access to high-quality treatment options[5]. On-going research efforts are also crucial for developing our knowledge on breast cancer biology and synthesize more effective treatments also it makes ultimately better outcomes and survival rate for individuals who suffered with this disease condition. By addressing these factors collectively, we can make significant strides in reducing the burden of breast cancer and enhancing the well-being of those affected by it[6-8].

Nowadays most conventional and modern technologies applied against cancers[9]. The current focus of new advanced treatments and modern tools were detecting carcinoma at organs has been contributed to the increased survival rate detection of particular cancer[10,11]. Moreover, many studies have been reported that the issue with treatment is cost uncharacteristically raised. Moreover, the problem persisting until new approaches may come and controls the diseases, because of many failures happens in conventional chemotherapeutic approaches. Therefore, there is a need for generating navel phytocomponents for the prevention and cure to control cancer and death rate[12].

The World Health Organization (WHO) acknowledges that most of the countries may include those in the developing world, continue to utilization of plants based natural products for better therapeutic effect[13]. In fact, approximately 60% of anticancer agents worldwide have their origins in natural source[14]. This underscores the significant role that herbal medicine plays in healthcare, particularly in the treatment of cancer[15].

Pears belong to the dicotyledonous variety of plant species of the genus of *P. communis,* within the rosaceae family. This family encompasses a diverse range of flowering plants, including many fruit-bearing species such as apples, cherries, and strawberries. Within the genus *P. communis*, there are various cultivars and species of pears, each with its own unique characteristics in terms of flavour, texture, and appearance[16-18]. Pear has full of pretentious phytoconstituents *viz.* many vitamins, most of the minerals, amino acids, fatty acids and secondary metabolites like flavonoids, glycosides, alkaloids, polyphenols and tannins[19-21]. Each and every part of pear tree having highly nutritional value and which reported tremendous medicinal valued properties acting against treatment for tussival effect, diarrheal, wound healing, inflammation, fever, antioxidant, hyperlipidemic, hypoglycaemic, aging, analgesic, spasmolytic, microbial and hepatoprotection[22-25].

This research work was aimed to determine the *in vitro* anti-proliferative activity of an ethanol extract derived from the leaves of a specific plant species against breast cancer cell lines, particularly the MCF-7 cell line[26-28]. This analysis aids to understanding the potential bioactive compounds responsible for the observed anti-proliferative effects against breast cancer cells, thus contributing to the discovery of ideal therapeutic agents or natural remedies for breast cancer treatment. The study constitutes the initial documentation of these compounds within the ethanol extract derived from the leaves of *P. communis*[29-31]. This discovery marks a significant advancement in understanding the chemical composition of this plant species. The migration of cancer cells possessing a pivotal role in the processing of development of cancer into metastasis. Understanding the primary underlying mechanisms of cell migration is crucial for developing strategies to inhibit or prevent metastatic spread[32,33]. By identifying bioactive compounds within the ethanol extract of *P. communis* exhibit anti-proliferative effects against breast cancer cells (such as MCF-7), researchers can potentially uncover novel therapeutic agents capable of impeding cancer cell migration and metastasis, thereby improving outcomes for cancer patients[33-35].

2. Materials and Methods

2.1 Materials

The human breast cancer cell line (MCF-7) was procured from the National Centre for Cell Sciences (NCCS) Pune in India, Dulbecco's Modified Eagle's Medium (DMEM) was bought from Sigma Aldrich, USA, 10% Fetal Bovine Serum (FBS), Sodium bicarbonate sourced from Merck, Germany, L-glutamine, and an antibiotic solution. (penicillin, amphotericin b, streptomycin at a concentration of 100, 2.5, 100 µg/ml.

2.2 Plant Collection and Authentication

Pyrus communis L. leaves were gathered from the hills surrounding Kodaikanal in Tamil Nadu, India, in October (Figure 1). The authentication process was conducted in

the BSI Coimbatore, India. The certificate number is no. BSI/SRC/5/23/2022/Tech/623. After collection, the leaves were thoroughly cleaned, dried in the shadows, crushed to a rough powder, and stored in an airtight plastic container.

2.3 Preparation of Plant Extract

Collected *P. communis* leaf subjected to a drying process where they were placed in the shade to dry. Powder was defatted with petroleum ether for 48 hrs. Then the extract introduced into soxhlet apparatus ethanol used as solvent system[11].

2.4 Total Phenolic Content and Flavonoid Content

Both spectrophotometric methods are commonly employed in plant chemistry research to assess the presence and concentration of phenolic compounds, including phenols (using folin ciocalteu reagent method)[13] and flavonoids (aluminium chloride colorimetric method)[14]. Determining their concentration in plant samples provides valuable information regarding the medicinal and nutritional properties of the plants under study.

2.5 Breast Cancer Cell Line Studies on Ethanol Extracts Leaves of *Pyrus communis*

2.5.1 Cell Treatment Procedure

The required five different concentration of solutions (6.25, 12.5, 25, and 50,100 g/ml) were created using extract. The procedure outlined describes the preparation of cells for an *in vitro* assay to assess the effects of compounds on cell growth or viable. The breakdown of the steps involved are shown in Figure 1[15-17].

Confluent monolayers of cells (presumably MCF-7 cells in this case) were treated with trypsin to detach them from the culture surface. This step allows for the collection of a suspension of individual cells for further processing. Then trypsinized cells were suspended in sterile 10% growth medium. This medium likely contains nutrients and other factors necessary for cell growth and proliferation.

2.5.2 Anticancer Assay by MTT Method

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a universal used method to assess cell viability and proliferation based on the ability of viable cells to convert the yellow MTT dye into a purple formazan product. Here is a breakdown of the protocol described as per reference[16,17]:

- Reconstitution of MTT solution
- Addition of MTT solution to Wells
- Incubation period
- Removal of supernatant

Formula for the percentage of growth inhibition:

% of viability = (Mean OD $_{Sample}$ / Mean OD o $_{control}$) × 100

The LC_{50} value, representing the concentration of a compound at which it is lethal to 50% of the treated cells or organisms, can be calculated using statistical software such as ED_{50} PLUS V1.0.

2.5 Migration Assay of MCF-7 in Breast Cancer Cell Line

By following this reference experimental protocol, researchers can evaluate the ability of the sample to

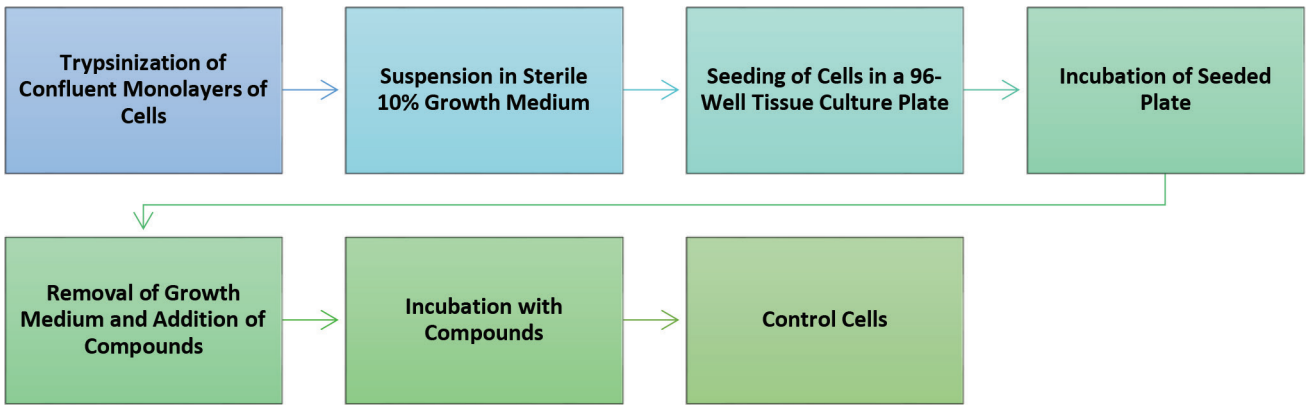

Figure 1. Schematic representation for preparation of MCF-7 cell line.

promote or inhibit wound closure over time, providing valuable insights into its potential therapeutic effects on wound healing processes[14,15].

- Cell seeding and scratch wound formation
- Debris removal and pbs rinse
- Sample incubation
- Image acquisition
- Data analysis

2.7 Statistical Analysis

To ascertain which groups differ significantly from one another, post-hoc tests may be run if the ANOVA test produces a significant result ($p < 0.05$)[17].

3. Results and Discussion

All plant-based research work focus on the phytoconstituents for pharmacological activity. This is importance for identification and quantification of active metabolite. The results obtained for quantifications of flavonoids and phenolic content 56mg quercetin/g, 48mg gallic acid/g. After preliminary presence of secondary metabolites in *P. communis* extracts.

The MCF-7 cell lines was treated in progressive grades include 6.25, 12.5, 25, 50 and 100 g/ml of *P. communis* extracts, and the extracts' cytotoxic activity were tested and identify the changes of cellular morphological under a microscope and viability/inhibition noted using the MTT assay. By using changes in cellular morphology as a marker of cytotoxicity, researchers can observe visible alterations such as cell rounding, shrinking, granulation, or vacuolization in the cytoplasm. These changes suggest cellular stress or damage induced by the extract treatment. The LC_{50} value provides valuable information regarding the potency of the extract in inducing cytotoxic effects on MCF-7 cells. Lower LC_{50} values indicate higher cytotoxicity, while higher LC_{50} values suggest lower cytotoxicity (Figure 2).

Cells undergoing cytotoxicity often undergo changes in shape, becoming rounded or shrinking in size. This alteration is typically associated with cell detachment from the culture substrate and is a hallmark of cell death processes such as apoptosis or necrosis. Granulation refers to the appearance of granules within the cytoplasm of cells. These granules may represent aggregates of cellular components or metabolic by products and can indicate cellular stress or damage. Cytoplasmic vacuolization refers to the formation of

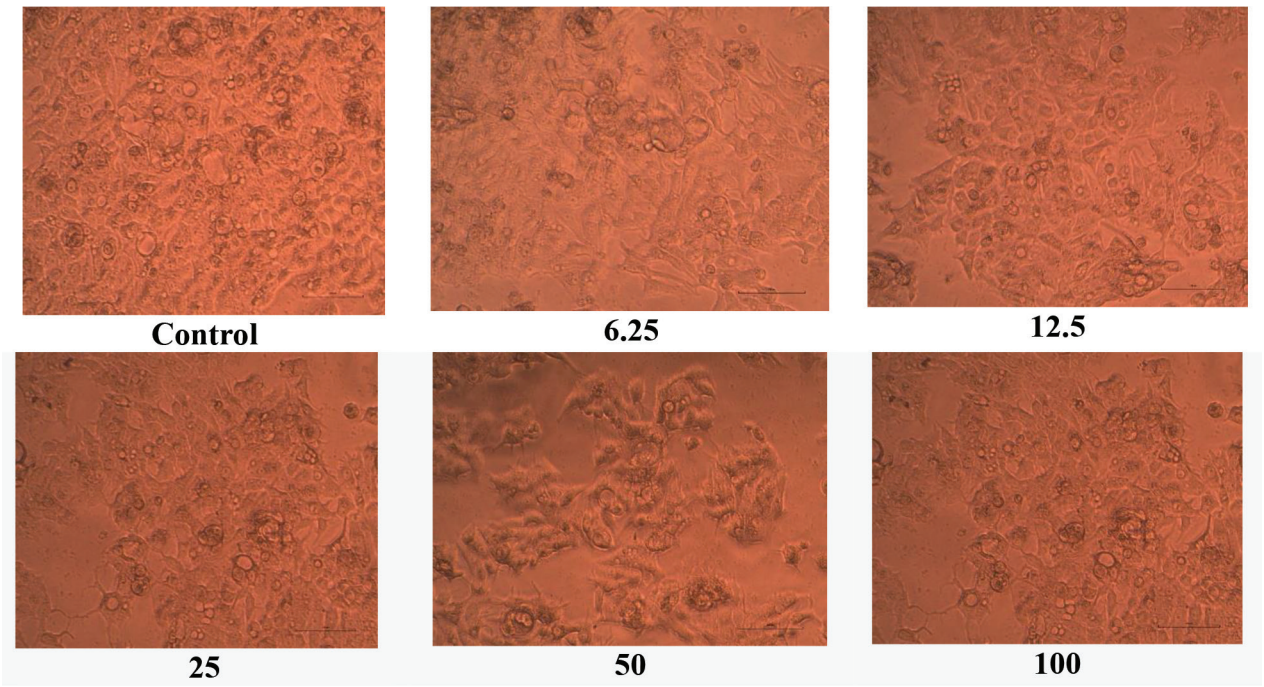

Figure 2. Cellular morphology changes of incubated MCF-7 cells in various concentrations like control, acetaminophen 1.5, 3.1, 6.25, 12.5, 25.

vacuoles, or fluid-filled sacs, within the cytoplasm of cells. Vacuoles can arise due to various cellular processes, including autophagy, lysosomal storage disorders, or perturbations in cellular metabolism. Vacuolization is often observed in cells undergoing stress or damage and is considered a marker of cytotoxicity. By monitoring these cellular alterations, researchers can assess the impact of compounds or treatments on cell health and viability. These morphological changes provide valuable insights into the mechanisms underlying cytotoxicity and can help guide further investigations into the potential therapeutic effects of test compounds, such as the ethanol extract from *P. communis* leaf in the case you mentioned. We discovered that cytotoxic alterations in incubated MCF-7 cells were directly proportional to the concentration of *P. communis* extract.

Once inside the cells, mitochondrial enzymes such as succinate dehydrogenase reduce MTT to formazan, resulting in the formation of purple-coloured crystals. Since only metabolically active cells contain sufficient levels of mitochondrial enzymes to reduce MTT, the MTT assay specifically measures cell viability and metabolic activity. Cells that are non-viable or metabolically inactive will not produce formazan crystals, and therefore will not contribute to the absorbance signal measured in the assay. Thus, the MTT assay is commonly used as a surrogate marker for cell viability and proliferation in cell culture experiments. It provides a quick and relatively simple method for assessing these effects of compounds or treatments on cell health and viability, making it a valuable tool in biomedical research. Viable cells were converted MTT into purple colour formazan crystals which directly assessment upon the intensity of colour obtained was proportionate to the quantity of viable cells. The vitality of the cells was demonstrated by the development of purple formazan crystals, the intensity of which was assessed in terms of Optical Density (OD). The OD value was recorded for control as well as samples of increasing directly to the concentration of *P. communis* extract.

We discovered that the viability of the cells dropped into the concentration of garlic extract increased, i.e., the viability of cells was 94.91% at the lowest concentration of *P. communis* extract 6.25 and 82.76% at the highest concentration of *P. communis* extract 100 **µg/ml** (Table 1). In our investigation, we discovered that as the quantity of *P. communis* extract increased, the average OD value decreased, indicating a decrease viable cells percentage (Figure 3).

Table 1. Cytotoxic effect of ethanol extract of *P. communis* leaves

Concentration of Sample (µg/ml)	Value of OD	value of OD	value of OD	Average OD	Viable cells Percentage
Control	0.7599	0.7617	0.7584	0.76	100
6.25	0.7232	0.7154	0.7254	0.7213	94.91
12.5	0.7046	0.6958	0.7025	0.7009	92.23
25	0.6866	0.6654	0.6845	0.6788	89.32
50	0.6598	0.6571	0.6525	0.6546	86.37
100	0.6238	0.6242	0.6391	0.6290	82.76

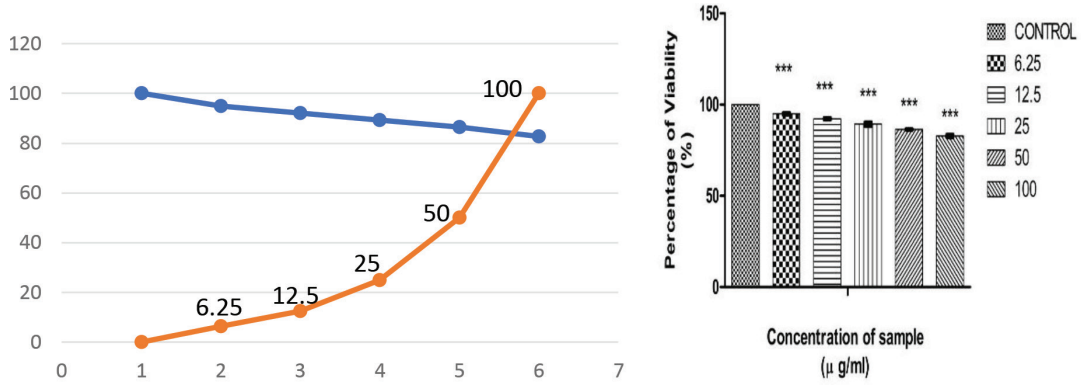

Figure 3. Graphical depiction of the anticancer effect of *P. communis* extract by MTT assay.

The LC_{50} value of 265.310978 µg/ml indicates in the concentration of the *P. communis* leaf extract at which 50% of the population cells are killed under the experimental conditions showed in Table 2. This value provides important information about the cytotoxicity of the extract and its potential as a therapeutic agent. A lower LC_{50} value indicates higher cytotoxicity and this extract is more effective at killing cells at lower concentrations. Conversely, a higher LC_{50} value suggests lower cytotoxicity, meaning that higher concentrations of the extract are needed to achieve the same level of cell death. From the result of *in vitro* scratch wound healing assay, the scratch area of the extract is higher when compared to the control after 72 hrs of incubation (Figure 4). This result findings demonstrates that the extract suppresses the migration of MCF-7 cells. Typically, Table 2 would present data related to cytotoxicity assays, including cell viability percentages or other relevant measurements at different concentrations of the extract.

Table 2. Result of migration assay of PCE against MCF-7 cell line

Time interval (Hrs)	Scratcharea (px)
C 0^{th}	2438508
S 0^{th}	2499806
C 24^{th}	2316534
S 24^{th}	2394332
C 48^{th}	2131556
S 48^{th}	2353930
C 72^{th}	917000
S 72^{th}	2331090

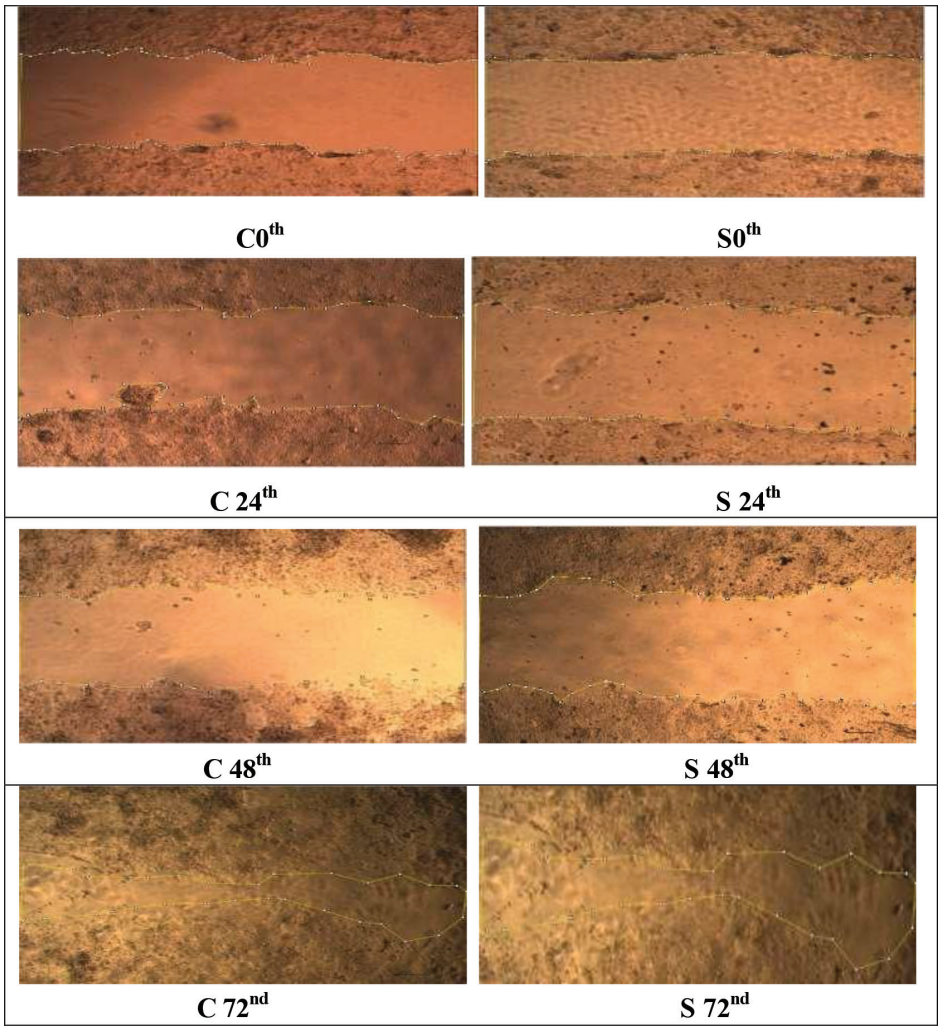

Figure 4. MCF-7 cell migration effect of control and PCE.

4. Conclusion

Investigating the phyto-composition of such extracts can provide insights into the chemical constituents responsible for their biological activities. These bioactive compounds may offer opportunities for the development of new therapeutic agents or natural remedies for various ailments. Wound migration and anti-proliferative activity against MCF-7 cell line in pear cultivars were determined findings demonstrates that the extract suppress the wound migration of MCF-7 cells. The LC_{50} value of 265.31 µg/ml indicates the concentration at which the extract is lethal to 50% of the population of cancer cells under the experimental conditions. However, its LC_{50} concentration against the cell line shows, the leaves may be a better choice against human breast cancer cells. Through this process, active phytoconstituents isolated from *P. communis* leaf could potentially lead to the growth of novel drugs for best treatment of human breast cancer. This research holds promise for discovering new therapeutic options that are effective, safe, and well-tolerated by patients, contributing to advancements in cancer therapy.

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