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Anticancer Potential of Psoralea Corylifolia L. Ethanol Extract: Cytotoxicity, Apoptosis Mechanisms, and Gene Expression Analysis In Mcf7 Breast Cancer Cells Via qPCR

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Abstract

Aim: The aim of this study was to evaluate the phytochemical profile and anticancer potential of *Psoralea corylifolia* L. extract, specifically focusing on its effects on breast cancer cells (MCF7) and its underlying molecular mechanisms.

Methods: The seeds of *P. corylifolia* were extracted using ethanol and chloroform through Soxhlet extraction. The extracts were analyzed for bioactive compounds through standard phytochemical tests. The anticancer activity of the ethanol extract was assessed using the MTT assay to determine cell viability and IC50 values. Further molecular analysis of apoptosis was performed by quantifying the expression of the Bcl-2 gene using semi-quantitative PCR and evaluating marker of apoptosis.

Results: Phytochemical screening revealed that both ethanol and chloroform extracts contained alkaloids, carbohydrates, and terpenoids, with ethanol extract showing a broader range of bioactive compounds, including flavonoids, phenols, saponins, and proteins. The ethanol extract exhibited significant cytotoxicity against MCF7 cells, with an IC50 value of 10.1 µg/ml. Molecular analysis showed that the extract induced apoptosis through down-regulation of the anti-apoptotic protein Bcl-2 and activation of caspase-3 and PARP, suggesting the involvement of the mitochondrial pathway.

Conclusion: The results demonstrate the anticancer potential of *P. corylifolia* ethanol extract against breast cancer cells, primarily through cell cycle arrest and apoptosis induction. These findings highlight *P. corylifolia* as a promising candidate for further investigation as a safer, plant-based alternative in cancer treatment, particularly for breast cancer, though clinical studies are needed to confirm its therapeutic efficacy in humans.

Keywords: Psoralea Corylifolia, *MCF7 Cells, Cytotoxicity, Bcl-2, Phytochemicals, Natural Compounds.*

Introduction

Cancer is a group of diseases marked by uncontrolled growth and spread of abnormal cells, which can lead to death if metastasis is not controlled. It is caused by a combination of external factors (e.g., tobacco, chemicals, and radiation) and internal factors (e.g., inherited mutations, hormones). Cancer risk is heightened by factors like diet, infections, lack of exercise, and environmental pollutants [1]. In India,

cancer is a major health issue, with over 7 lakh new cases and 3 lakh deaths annually [2]. Carcinogens, substances that damage DNA and promote cancer, include tobacco, asbestos, radiation, and environmental pollutants. These agents generate free radicals that damage cells and disrupt normal function [3]. According to the World Health Organization, cancer is the second leading global cause of death, with 9.6 million deaths in 2018, and 70% of these deaths occur in low- and middle-income countries (WHO, 2020).

Breast cancer, the second most common cancer in women, occurs primarily in women and is most often a ductal carcinoma. Treatment options vary by cancer type and include surgery, radiation, chemotherapy, and hormone therapy. However, chemotherapy may have severe side effects, and new anticancer drugs are still in demand due to the limitations of current treatments [4]. In developing countries, there is a growing interest in medicinal plants as alternative cancer treatments due to their lower side effects compared to synthetic drugs. The National Cancer Institute has investigated over 35,000 plant species, leading to the discovery of key anticancer drugs [5].

Psoralea corylifolia L., a plant used in traditional Chinese medicine, has shown anticancer potential. Its seeds, rich in coumarins and flavonoids, demonstrate cytotoxic and immunomodulatory effects, including against breast (MCF-7), colon (HT-29), and prostate (DU-145, PC-3) cancer cells [6] and [7]. Studies suggest that plant extracts can trigger apoptosis by suppressing anti-apoptosis genes like Bcl-2 and Bcl-xL. For example, extracts from green tea, Ginkgo biloba, and garlic promote apoptosis in cancer cells [8]. The seeds of *P. corylifolia* have demonstrated similar properties, and bioassay-guided isolation is used to identify active compounds for further anticancer research [6]. However, very scare report about the suppression of anti-apoptosis genes like Bcl-2. In this study, the goal is to identify bioactive compounds in *P. corylifolia* seeds that inhibit breast cancer cell proliferation and explore the molecular mechanisms of apoptosis induction through gene expression analysis using RT-PCR.

Methods

Collection of P. corylifolia

The seeds of *P. corylifolia* were collected from a local market and subjected to an extraction procedure. The seeds were finely powdered using a blender, and 20 g of the powder was loaded into the thimble of a Soxhlet extractor. The extraction was carried out for about 8 hours using heat. The solvents used for extraction were ethanol and chloroform. Both solvent extracts (distinctly condensed vapors) were collected, condensed, and dried using a rotary vacuum evaporator (Equitron, India) at a temperature of 60°C under reduced pressure. After distilling the solvents, the dry crude extract residues were weighed aseptically and re-dissolved in the respective solvents. The extracts were then stored at -20°C in sterile, properly labeled, and airtight containers for further studies.

Preliminary Phytochemical Analysis

Both ethanol and chloroform extracts were assessed for potential bioactive compounds using standard procedures from Solomon *et al*. [9] and Kokate, [10]. A total of 10 distinct phytochemical tests were performed to evaluate the presence of bioactive compounds.

Anticancer Activity of Ethanol Extract against MCF7 Cancer Cells

Cell Culture Maintenance

Human breast cancer (MCF7) cells were obtained from the National Centre for Cell Sciences (NCCS),

Pune, India. Cells were maintained in DMEM supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin, and cultured in a humidified incubator with 5% CO2 at 37°C.

MTT Assay

The cytotoxicity of the ethanol extract on MCF7 cells was determined using the MTT assay [11]. MTT (50 mg) was dissolved in 10 ml PBS, vortexed for 1 min, and filtered through a 0.45 µm filter. The solution was stored in an aluminum-wrapped bottle at 4°C, as MTT is light-sensitive.

Procedure

MCF7 cells were seeded in 96-well plates at a density of 1×10^4 cells/ml and incubated for 24 hours for attachment. The cells were then treated with control or varying concentrations of the extract. After 24 hours of incubation at 37°C in 5% CO_2, the medium was replaced with fresh culture medium, and 5 mg/ml MTT was added. After a 4-hour incubation at 37°C, the purple formazan crystals formed were dissolved in 100 µl DMSO. The absorbance was measured at 540 nm using a multi-well plate reader. Cell viability was calculated as a percentage relative to the control, and the IC50 values were determined for different time points.

$$\text{nhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 10$$

The IC_{50} values were determined from the different dose responsive curve where inhibition of 50% cytotoxicity compared to control cells. All experiments were performed at least three times in triplicate.

RNA Extraction from Cancer Cells

MCF7 cells were treated with plant extract, while control cells were maintained without treatment. After 48 hours of incubation, RNA was extracted from both treated and control cells. Total RNA was isolated using a column-based RNA extraction kit (Aura RNA Extraction Kit, India; ABT-140M) following the manufacturer's instructions. The extracted RNA was eluted in 20 µl of RNase-free water and stored at -80°C for future use. RNA integrity was verified by electrophoresis on a 1% agarose gel.

Procedure for cDNA Conversion

The cDNA synthesis was performed using the Aura cDNA Synthesis Kit (Cat. No: MPC-1). In a fresh, sterile, RNase-free tube kept on ice, the following components were added in the indicated order: 4.0 µl of 5X reaction buffer, 2.0 µl of 10X random primers, 1.0 µl of reverse transcriptase, 0.8 µl of 25X dNTPs, 0.3 µl of RNase inhibitor, and 1.9 µl of nuclease-free water, making the total volume 10 µl. The components were mixed gently, and 10 µl of RNA (ranging from 20 ng to 2 µg) was added to each well, followed by gentle pipetting to mix the sample.

The cDNA synthesis was carried out using the following program: the tubes were incubated at 25°C for 10 minutes, then at 55°C for 60 minutes. Afterward, the reaction was terminated by incubating at 85°C for 5 minutes, and the tubes were stored at 4°C until further use. To verify the cDNA, electrophoresis was performed on a 2% (w/v) agarose gel in 1% TBE buffer (containing 2 mM EDTA and 40 mM Tris-

acetate). The gel was run at 50V for 30 minutes, and the cDNA bands were observed under UV illumination.

Semi-Quantitative PCR for Bcl2 Anti-Apoptotic Gene Expression

The semi-quantitative PCR for the Bcl2 anti-apoptotic gene was performed based on the method described by Prihantono *et al*. [12]. A reaction mixture was prepared by adding 2 µl of cDNA, 1 µl of each 10 µM primer, 10 µl of master mix (containing 2.5 U of Taq polymerase, 2.5 mM dNTPs, and $MgCl_2$), and molecular-grade water to a final volume of 20 µl. After an initial denaturation at 94°C for 5 minutes, the PCR was carried out for 33 cycles with the following conditions: denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 59 seconds. A final extension was performed at 72°C for 5 minutes. The PCR products were then separated by electrophoresis on a 2% agarose gel. The following primers were used for amplification of the BCL2 gene: 5'F-CCCTGTGGATGACTGAGTAC'3 and 5'R- GCATGTTGACTTCACTTGTG'3.

Result and discussion

The use of plant extracts for the treatment of cancer has been studied for over a century. While plant extracts have been used in traditional medicine for centuries, research into their anti-cancer properties has only gained significant attention in recent years. Many different plant extracts have been investigated for their anticancer effects, including those from green tea, turmeric, ginger, garlic, ginseng, and others. Studies have shown that these extracts may have a variety of effects, such as inhibiting the growth of cancer cells, inducing cell death, and blocking the formation of blood vessels that supply tumors with nutrients [13].

The exact mechanisms of action of these extracts are not yet fully understood, but they may involve the activation of certain pathways in the body that help fight cancer. For example, some plant extracts may contain compounds that block the production of pro-inflammatory molecules, which can promote the growth of cancer cells. The use of plant extracts for cancer treatment is still in its early stages, and further research is needed to better understand their potential benefits. In the present study, our goal was to determine the phytochemical profile of *P. corylifolia* and evaluate its anticancer activities. In particular, more clinical trials are needed to assess the safety and effectiveness of these extracts in humans [14].

Phytochemicals are natural compounds found in plants that have beneficial effects on human health. They are present in fruits, vegetables, nuts, and grains, and are known for their antioxidant, anti-inflammatory, and anticancer properties. Some examples of phytochemicals include flavonoids, carotenoids, phenolic compounds, terpenes, and phytosterols [15]. Phytochemicals can be consumed by eating a healthy diet that includes a variety of plant-based foods or through dietary supplements [16] and [17]. Additionally, some phytochemicals can be applied topically to the skin for their beneficial effects [18].

In the present study, the phytochemical screening of *Psoralea corylifolia* extracts revealed distinct differences between the ethanol and chloroform solvent extracts. Both ethanol and chloroform extracts contained alkaloids, carbohydrates, and terpenoids, indicating the presence of these compounds in both solvents. However, the ethanol extract was found to contain a broader range of phytochemicals compared to the chloroform extract. Specifically, the ethanol extract contained flavonoids, phenols, saponins, and proteins, which were absent in the chloroform extract. In contrast, the chloroform extract

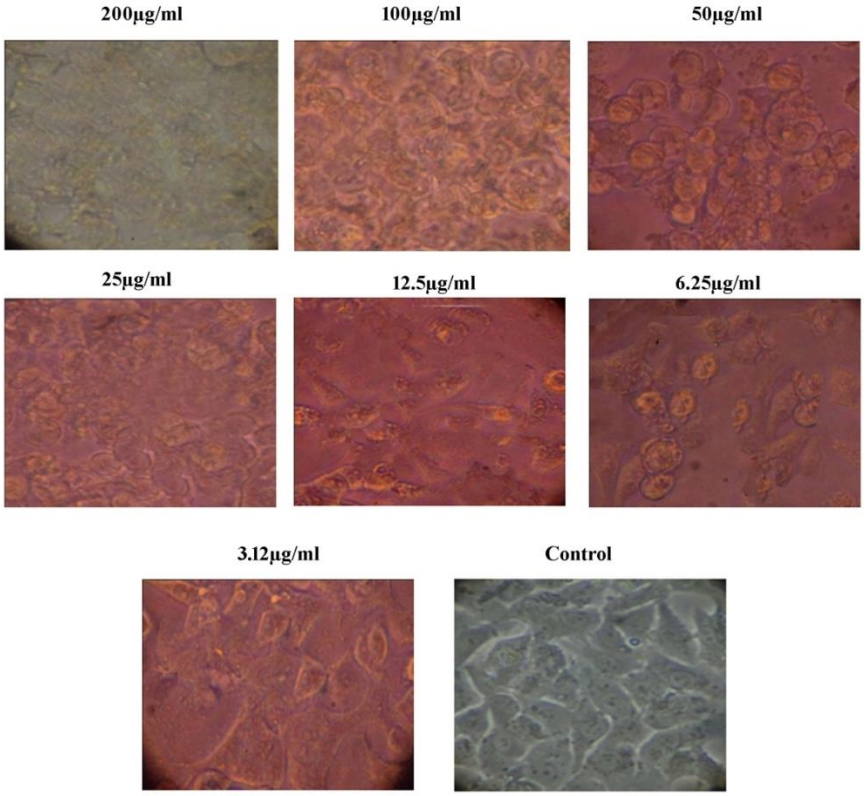
exhibited the presence of tannins and quinines, compounds that were not detected in the ethanol extract. The effectiveness of ethanol aligns with previous studies, such as those by Nabi and Shrivastava. [19] and Pandey *et al*., [20] which highlight ethanol's ability to disrupt cell membranes and release phytochemicals.

Sterols were absent in both solvent extracts, suggesting that this compound is not present in significant quantities in *P. corylifolia*. Overall, the results demonstrate that ethanol is more effective in extracting a wider variety of phytochemicals, a finding that is consistent with the study by Ares *et al*. [21]

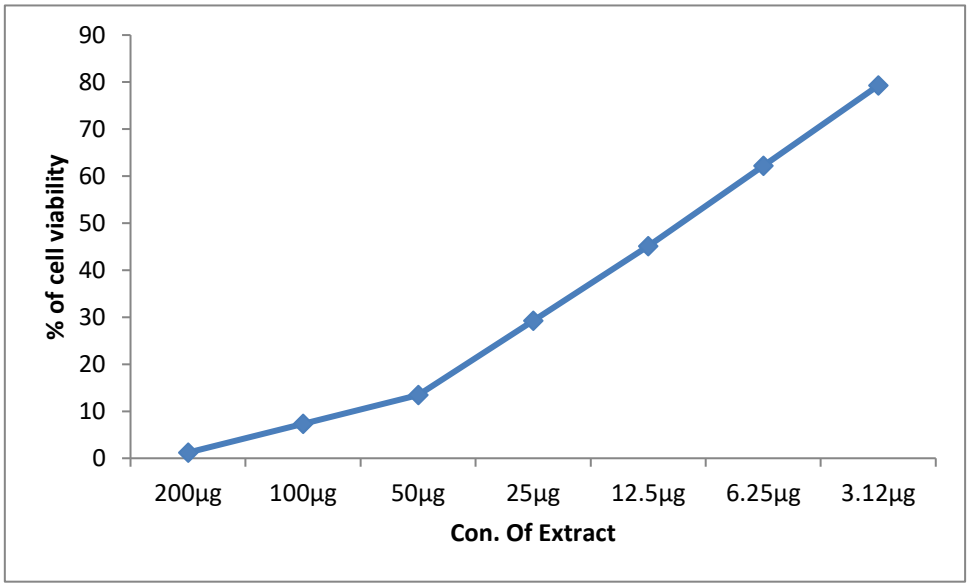
Cancer chemotherapy and chemoprevention may be improved by agents that inhibit cancer cell proliferation and induce apoptosis. Despite the development of various anticancer agents, successful cancer treatment is often hindered by associated adverse side effects and acquired drug resistance [22]. As a result, there is growing interest in the development of novel, safe, and effective treatments for cancer using plant-based compounds.

One important source of new chemotherapeutic agents comes from chemicals derived from herbal sources. Several phytochemicals have been shown to possess anticancer effects. The use of complementary and alternative medicine, such as botanical extracts, is becoming increasingly popular among cancer patients [23]. In our study, we demonstrated the potent cytotoxic effects of *Psoralea corylifolia* L. extract using breast cancer cell lines. The highest cytotoxicity was observed at a concentration of 200 µg/ml, with 98.79% cell viability. It was noted that the percentage of growth inhibition decreased as the concentration increased, with an IC50 value of 10.1 µg/ml. In 2014, Rajan *et al* [24] determined the anticancer activity of *P. corylifolia* against a breast cancer cell line. More recently, Telang *et al*. [25] also assessed the growth inhibitory effects of *P. corylifolia* extract in a model of triple-negative breast cancer.

Cytotoxic Effect Of *Psoralea Corylifolia* Extract On Mcf-7 Breast Cancer Cells



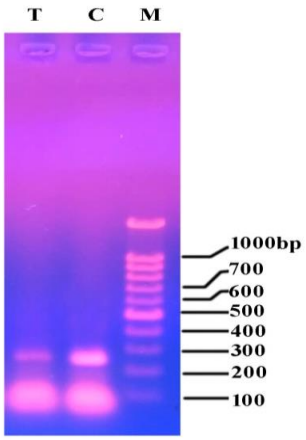
Effect of *Psoralea corylifolia* Extract on Cell Viability of MCF-7 Breast Cancer Cells



To further investigate the molecular basis by which *Psoralea corylifolia* extract inhibits tumor cell growth, we analyzed the expression of the Bcl-2 gene, which is involved in cell cycle regulation [27]. *P. corylifolia* extract significantly reduced the expression levels of Bcl-2 proteins. These findings suggest that *P. corylifolia* extract inhibits the growth of breast cancer cells by inducing cell cycle arrest. In addition to cell cycle arrest, the induction of apoptosis is considered a key mechanism through which various naturally occurring phytochemicals inhibit tumor growth [28].

In breast cancer cells treated with *P. corylifolia* extract, the expression of the apoptosis-inhibiting protein Bcl-2 was down-regulated, while the apoptosis effectors protein caspase-3 was strongly activated, accompanied by the activation of PARP protein. These changes triggered the apoptotic process of programmed cell death. Taken together, these results suggest the involvement of the mitochondrial pathway in *P. corylifolia* extract-induced apoptosis in MCF-7 cells. Several studies have shown that psoralen derivatives act as inhibitors of the NF-κB pathway and other signaling pathways. According to the review of the literature, this is the first study to determine the expression of Bcl-2 while using the ethanol extract of *P. corylifolia* seed extract.

Semi-Quantitative PCR For Bcl2 Anti-Apoptotic Gene Expression



T- Test (Treated with plant), C- Control (not treated with plant)
M-100 DNA marker

In conclusion, the present study highlights the promising anticancer potential of *Psoralea corylifolia* L. extract, particularly in inhibiting breast cancer cell growth. Phytochemical screening showed that the ethanol extract contains bioactive compounds, such as flavonoids, phenols, saponins, and proteins, which likely contribute to its anticancer effects. The extract inhibits cell proliferation through mechanisms like cell cycle arrest and apoptosis, supported by the down-regulation of Bcl-2, suggesting the involvement of the mitochondrial pathway. These findings align with previous studies, but clinical research is needed to assess its safety and effectiveness in humans. Given the growing demand for safer, plant-based alternatives to chemotherapy, *P. corylifolia* shows potential as a viable option for cancer treatment, particularly in breast cancer, and warrants further in vivo and clinical investigation.

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