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SHORT COMMUNICATION

In Vitro Antioxidant and Antiproliferative Activity of *Portulaca quadrifida* L. Extract on Human Colorectal Cancer Cell Line (HCT-116)

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Abstract

Background: Plants have been used as foods and natural medicines since prehistoric times. Natural products, particularly dietary substances, have become imperative in creating new chemo preventive agents. Natural medicines have been used for treating cancer by inhibiting the promotion process. *Portulaca quadrifida* L. is a small succulent herb used as a vegetable for medicinal purposes like reducing small tumors and inflammation. **Aim:** The study aimed to screen the antiproliferative efficacy of the ethanolic extract of *P. quadrifida* on human colorectal cancer cell lines. The objectives of the study are to evaluate the phytochemical composition of the ethanolic extract besides antioxidant activity and antiproliferative effect. **Methods:** The antioxidant activity was determined by 1,1-diphenyl-2-picryl hydroxyl, nitric oxide, and hydroxyl radical scavenging assay. The antiproliferative effect was determined using human colorectal cancer cell line- 116 by tetrazolium assay. **Results:** The ethanolic extract exhibited maximum inhibition (52.56%) at 300 µg/ml concentration against human colorectal cancer cell line-116, and the inhibitory concentration (IC_{50}) was 269.1µg/ml. The ethanolic extract of *P. quadrifida* was rich in phenolic content. The antiproliferative potential of *P. quadrifida* may be owing to its phenolic components, which were supported by the antioxidant potential of the ethanolic extract of *P. quadrifida*. **Conclusion:** The study concludes that *P. quadrifida* is an edible plant widely used as a vegetable and can be recommended as a dietary supplement for chemoprevention and a medicinal agent for the treatment of colon cancer.

Major Findings: The ethanol extract of the plant *P. quadrifida* quantifies demonstrated potential antioxidant and antiproliferative properties.

Keywords: Antioxidant, Antiproliferative, Human Colorectal Cancer, Phytochemical, *Portulaca quadrifida*

1. Introduction

Colorectal cancer accounts for 10-15% of all cancers and is the second-leading cause of cancer-related death in developed countries[1]. According to the National Cancer Registry of Malaysia, colon cancer is the most widespread cancer among men and the third most common cancer among women. Colon cancer is typically defined as transforming normal colon epithelial cells into oedematous polyps and, eventually, invasive cancer. Since cancer is a disorder of deregulated cell proliferation and cell survival, inhibiting cell proliferation and escalating apoptosis in tumors are effective strategies for preventing tumor growth[2]. Most colon carcinomas and their genetic and familial backgrounds develop due to multiple attacks by various exogenous and endogenous carcinogenic compounds. It is well recognized that colon cancer may be alleviated when risk factors such as lifestyle and environmental factors are modified. Although complete removal of the

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causative agents of cancer may not always be possible, chemoprevention of cancer using antimutagens and anticarcinogens present in foods or natural products have been recommended by various studies, and much effort has been dedicated to the search for natural preventive agents that would block or attenuate the colorectal carcinogenesis process[3]. Epidemiological and experimental studies reveal the therapeutic potential of many culinary herbs and demonstrate the importance of compounds derived from plants in decreasing the risk of colon cancer and inhibiting the progress and diversity of tumors in experimental animal models[4]. Spices, and herbs are rich in bioactive compounds that have several health benefits. Regulation of BCL-2, K-ras, and MMP pathways, caspase activation, extrinsic apoptotic pathways, and stress-induced apoptosis of the endoplasmic reticulum are major mediators of the chemo preventive effects of these herbs and spices. Due to their antiproliferative and angiogenesis inhibitory activities against colorectal cancer cells, these herbs and spices may be excellent candidates as chemopreventive or chemotherapeutic agents in managing colorectal cancer[5]. Cancer treatment using plants has a long history. More than 3000 plant species have supposedly been used in cancer treatment. Because of limitations in cell toxicity and adverse reactions, as well as improvements in treatment efficiency, interest in the discovery of new anticancer natural products is increasing[6]. Huge efforts have been made over the past few decades to separate unique natural compounds from bacteria, plants, and other living things to evaluate their anticancer characteristics and learn more about how they work[7]. As a result of these efforts, a panel of anti-cancer medications was discovered. According to estimates, 25% of all recently authorized anti-cancer medications between 1981 and 2019 were thought to be related to natural ingredients. Numerous substances with anticancer properties or distinctive structural benefits in druggable methods have been reported, mentioning Camptothecin, Taxol, Eucalyptin A, Pseudolaric acid B, Parthenolide, Trabectedin, Ulocladol, *etc*[8]. Some nutritional supplements aid in detoxifying the body, and defending it against most cancers, while others work to lessen the side effects of radiotherapy and chemotherapy. Chemotherapy's adverse side effects worsen nutritional problems, so choosing the proper amount of nutrient supplements is essential. By influencing or modifying the biological response, nutritional supplements protect you from the negative consequences of both[9-10]. *P. quadrifida* L. (*Portulacaceae*) is a small, diffused, succulent annual herb found throughout the tropical parts of India. It is used as a vegetable and also for various curative purposes. It reduces small tumors and is used to treat inflammation. It is useful in treating asthma, cough, urinary discharges, inflammations, and ulcers[11]. A plant poultice is applied for abdominal complaints, erysipelas, and hemorrhoids[12]. The plant is enriched with flavonoids, saponins, vitamins, glycosides, and other secondary metabolites. The presence of gallic acid, rutin, and quercetin was reported in the methanolic extract of the plant[13]. The pharmacological activities exhibited by the plant are antibacterial, antifungal, antinociceptive, and hepatoprotective[14-17]. So, the present study intends to evaluate the candidate plant, *P. quadrifida* L., for its antiproliferative property against malignant human colorectal cancer using the HCT-116 cell line.

2. Materials and Methods

2.1 General Materials

HCT-116 cell line was purchased from American type culture collection, Manassas, VA, USA, RPMI-1640 (Roswell Park Memorial Institute) medium, Foetal Bovine Serum (FBS), L-glutamine, 1% Penicillin or streptomycin, MTT (3-[4,5-dimethyl-thiozole-2-yl]-2,5-diphenyltetrazolium bromide) was purchased from Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India, DPPH was purchased from Sigma Aldrich, USA.

2.2 Plant Materials

The plant *P. quadrifida* L. was collected from the road side ponds of Chennai and authenticated (PARC/2011/985) by comparing it with an authentic specimen by Professor P. Jayaraman, Plant Anatomical Research Centre, Tambaram, Chennai.

2.3 Preparation of Extract

The freshly collected whole plant of *P. quadrifida* L. was cut into small pieces, shade-dried for 15 days, and coarsely powdered. The powdered material was charged in an aspirator bottle and extracted with ethanol by cold maceration for 72 hours, 48 hours, and 24 hours. After

decantation and filtration, the solvent was removed by distillation using a rotary flask evaporator. The Ethanolic Extract of *P. quadrifida* (EEPQ) obtained was further dried in a vacuum desiccator, and the yield of the extract was calculated.

2.4 Phytochemical Evaluation

A preliminary phytochemical analysis of EEPQ was carried out to screen for phytoconstituents[18]. The total phenol, tannin, and flavonoid content of EEPQ were estimated using the standard method[19,20]. HPTLC fingerprinting of EEPQ was developed using methanol as the solvent, the stationary phase as aluminum plates precoated with silica gel F_{254}, and the mobile phase as Toluene: Ethyl acetate: Formic acid and Methanol (50:50:2.5:2.5) with the help of CAMAG Automatic TLC Sampler 4 (ATS 4) and developed in a CAMAG twin trough chamber, and the chromatogram was obtained using win CATS planar chromatography manager software.

2.5 *In Vitro* Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

The reaction mixture was shaken and incubated for 20 minutes at room temperature in the dark with 1 ml of DPPH (1,1-diphenyl-2-picryl hydroxyl) solution (200 mM in ethanol) and various concentrations of the EEPQ[21-24]. The absorbance was recorded at 517 nm, and the percentage inhibition was calculated using the formula,

$$Percentage\ inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

2.5.2 Nitric Oxide Radical Scavenging Assay

The reaction mixture containing 3 ml of sodium nitroprusside (10 mM in phosphate-buffered saline) and various concentrations of EEPQ was incubated at 37° C for 4 h. To the incubated solution, 0.5 ml of Griess reagent [potassium ferricyanide (1%), trichloroacetic acid (10%), and ferric chloride (0.1%) in phosphate buffer (pH 6.6, 0.2 M)] was added, and the absorbance was read at 546 nm[16-19]. The percentage inhibition was calculated using the formula,

$$Percentage\ inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

2.5.3 Hydroxyl Radical Scavenging Assay

The reaction mixture contained 100 µl of 2-deoxy-2-ribose (2.8 mM), 500 µl of EEPQ dissolved in 0.1 mM, phosphate buffer (pH 7.4), 200 µl of ferric chloride and EDTA (1:1 v/v), 100 µl of hydrogen peroxide (1 mM), and 100 µl of ascorbic acid (1 mM). The mixture was incubated for 1 h at 37° F. After the incubation, add 1 ml of thiobarbituric acid (1% w/v) and 1 ml trichloroacetic acid (2.8% w/v). The mixture was then heated at 90° for 20 minutes to develop the color. The mixture was cooled and centrifuged at 3000 rpm. The absorbance of the supernatant was measured at 532 nm against an appropriate blank[25].

2.6 *In Vitro* Antiproliferative Activity

2.6.1 Cell Culture

Human cancer cell lines (HCT-116) were cultured in RPMI-1640 medium with 20% FBS, 2mM L-glutamine, 1% penicillin or streptomycin under a fully humidified atmosphere 5%CO_2 at 37° C.

2.6.2 MTT Assay

The effect of plant extract on the viability of Human colon cancer cell lines (HCT-116) was determined by the MTT (3-[4,5-dimethyl-thiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay. 100 µl of cell suspensions in growth medium were plated in 96-well microtitre plates at 1×10^4 cells/well concentrations and incubated for 48h at 37 °C in a humidified incubator. After 48 h incubation, cells were incubated with various concentrations of EEPQ in 0.1% DMSO for 72 h at 37° C. After removal of the solution and washing with phosphate-buffered saline (pH 7.4), 20 µl of MTT (5 mg/mL) was added to each well of the plate. The plate was incubated for 4 h at 37° C. The solution in each well was aspirated, and 100 µl of buffered DMSO was added to dissolve form a zone. The plates were shaken for 5 minutes. The optical density was measured on a microplate ELISA reader at 540 nm. The cytotoxic potential was obtained by comparing the absorbance between the samples and the control, and the IC_{50} was calculated from dose-response curves. The effect of the EEPQ on cell morphology was determined using an inverted light microscope[26]. The percentage inhibition was calculated as follows:

$$Percentage\ inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} \times 100$$

3. Statistical Analysis

Each assay was performed in triplicate, and the data are given as Mean ± Standard Deviation (SD). The values are plotted as a graph in Microsoft Excel with error bars.

4. Results

4.1 Preparation of Extract

The coarsely powdered plant material was extracted with ethanol through cold maceration, and the Ethanolic Extract (EEPQ) yield was 10.39% w/w.

4.2 Phytochemical Evaluation

Preliminary phytochemical analysis EEPQ was carried out to identify the phytochemical composition. It showed the presence of flavonoids, carbohydrates, phenols, tannins, glycosides, and proteins. A quantitative phytochemical evaluation was performed to estimate the secondary metabolites such as tannin, phenol, and flavonoid content of EEPQ, and the results are shown in Figure 1(a,b,c). The total tannin content was 1.27 mg tannic acid equivalent/g, and the total phenol and flavonoid contents were 6.17 mg gallic acid equivalent/g and 1.59 mg quercetin equivalent/g, respectively. The EEPQ was fingerprinted using HPTLC. The chromatogram showed about 15 peaks with R_f value ranging from 0.02 to 0.97 Figure 1(d).

4.3 *In Vitro* Antioxidant Activity

4.3.1 DPPH Radical Scavenging Assay

EEPQ was screened for its anti-oxidant potential using the DPPH radical scavenging assay. First, the percentage inhibition was calculated; the results are shown in Figure 2(a). The results showed that EEPQ exhibited minimum scavenging activity at lower concentrations; an increase in concentration showed an increase in radical scavenging activity compared with the standard ascorbic acid.

4.3.2 Nitric Oxide Radical Scavenging Assay

The nitric oxide scavenging activity of EEPQ was evaluated, and the percentage inhibition was recorded in Figure 2. In addition, the nitric oxide radical scavenging activity of EEPQ was tested. The results

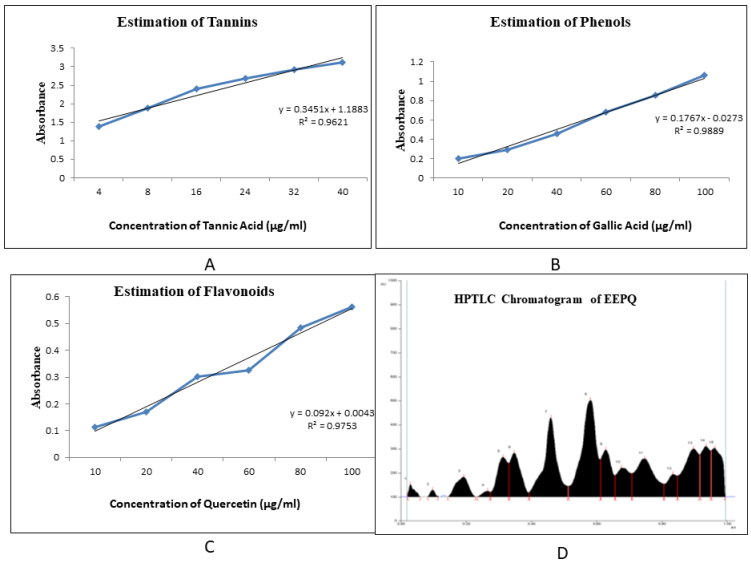


Figure 1. **(a), (b), (c)** Calibration curve of tannic acid, gallic acid, and quercetin respectively. **(d)** HPTLC chromatogram of EEPQ.

The results revealed that the total tannin content was 1.27 mg tannic acid equivalent/g, the total phenol and flavonoid contents were 6.17 mg gallic acid equivalent/g and 1.59 mg quercetin equivalent/g, respectively. The chromatogram of EEPQ showed about 15 peaks with R_f value ranging from 0.02 to 0.9.

showed that it had no radical scavenging activity at the lowest concentration (50 µg/ml).

However, at the highest concentration (1000 µg/ml), it had a moderate inhibitory effect (41.57%), comparable to standard ascorbic acid Figure 2(b).

4.3.3 *Hydroxyl Radical Scavenging Assay*

The hydroxyl scavenging activity of EEPQ was performed, and the % inhibition was recorded in Figure 2(c). In the hydroxyl radical scavenging assay, EEPQ showed a dose-dependent radical scavenging potential and attained the maximum inhibition (88.7%) at 1000 µg/ml, which is comparable with the standard Vitamin E.

4.4 *In Vitro* Antiproliferative Activity

The effect of EEPQ on cell proliferation was investigated using a tetrazolium reduction assay. The extract showed significant dose-dependent antiproliferative activity against the HCT-116 cell line in Figure 2(d).

4.4.1 *Cytomorphological Study*

Following 48 h of incubation with various concentrations of EEPQ, the cell morphology was observed. The control group [Figure 3 (f)] represented the uniform accumulation of HCT-116 cells. Compared with the control group, the extract-treated groups showed dose-dependent cytomorphological changes. The minimum concentration, *i.e.*, 0.1 µg/ml of extract, causes cell skeleton rearrangement. Apoptotic features such as cytoplasmic blebbing (orange arrows) were also observed [Figure 3(a)]. In addition to morphological changes, an apparent DNA condensation within the nucleus (red arrow) and cell debris (yellow arrow) were visible at the concentration of 1 µg/ml [Figure 3(b)]. Moderate levels of cells with DNA condensation within their nucleus (red arrow) and cell debris (yellow arrow) were visible in cells treated with 10 µg/ml of the extract [Figure 3(c)]. Higher levels of cells with DNA condensation within their nucleus (red arrow) and cell debris (yellow arrow) were observed in the cells treated with 100 µg/ml of the extract [Figure 3(d)]. Significant reduction in cell number and density, loss of cell adhesion, and apoptotic body formation (white arrows) were observed in the cells treated with 300 µg/ml of extract, which are characteristics of apoptosis, which confirms the cell death [Figure 3(e)].

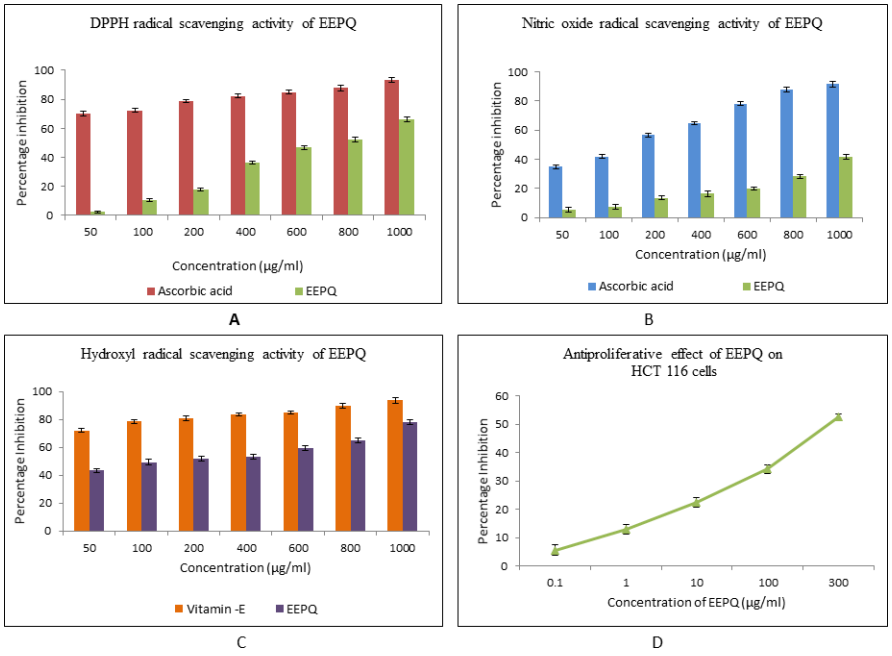


Figure 2. **(a), (b), (c)** DPPH, nitric oxide and hydroxy radical scavenging activity, d- anti-proliferation effect of ethanol extract of *P. quadrifida* on HCT-116 (human colorectal cell line).

The results revealed that EEPQ showed a dose-dependent antioxidant and antiproliferative activity, values are expressed as Mean ± Standard Deviation, n=3.

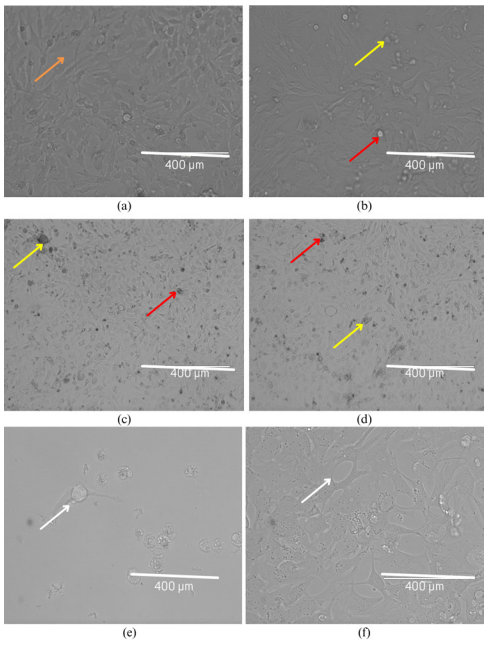


Figure 3. Cytomorphological alterations of HCT-116 cells treated EEPQ.

(a), (b), (c), (d), (e) HCT-116 cells treated with 0.1, 1, 10, 100, 300 µg/ml respectively for 48 hr, **(f)** Control (without treatment), magnification: 40X, Uniform accumulation of cells are present in the control Group (f), the treatment groups show cell skeleton rearrangement and cytoplasmic blebbing as an apoptotic feature (orange arrow), DNA condensation within the nucleus (red arrow) and cell debris (yellow arrow). The cells treated with 300 µg/ml EEPQ show a significant reduction in cell number and cell density, loss of cell adhesion, and apoptotic body formation (white arrows).

5. Discussion

Humans have used plants as food since time immemorial, and different types of plants, depending on location and season, have been part of their diet since prehistoric times. The major breakthroughs were the discovery and development of the vinca alkaloids; vinblastine and vincristine and the isolation of the cytotoxic podophyllotoxins. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents[27]. Since plants have been proven to be an important natural source of anti-cancer therapy for several years, in the present study, an attempt was made to determine the antiproliferative effect of an ethanol extract of *P. quadrifida* against the human colorectal cancer cell line HCT-116. According to some research, increased dietary consumption of natural antioxidants is positively correlated with a lower death rate from coronary heart disease and cancer and a higher life expectancy. *P. quadrifida* is an edible plant that can serve as a natural antioxidant in food[28]. The phytochemical screening revealed the presence of secondary metabolites such as tannins, phenols, and tannic acids. The quantitative estimation was performed to determine the constituent that attributed to the activity. EEPQ was found to be rich in phenols, tannins, and flavonoids. Many polyphenolic substances, including phenolic acids, flavonoids, anthocyanidins, and tannins, which have outstanding antioxidant and anticancer properties, are abundant in plant materials[29-31]. Flavonoids may interfere with angiogenesis, inflammation, and metastasis as well as cancer cell growth, differentiation, and apoptosis[32-33]. So, the pharmacological activity of EEPQ may be due to these secondary metabolites present in the extract. For the purposes of species authentication, quality assessment, and maintaining the consistency and stability of herbal pharmaceuticals and their related products, chromatographic fingerprint analysis of herbal drugs constitutes an all-encompassing qualitative method[34]. In the present study, HPTLC fingerprinting of the EEPQ was developed, and it will help as a tool in the identification of the plant as well as the extract. Previous research has shown that the colonic mucosa of cancer patients and people with predisposing inflammatory conditions like ulcerative colitis and Crohn's disease produces significantly more reactive oxygen species than normal tissue[35]. So, the extract was subjected to screen the antioxidant potential using various methods. Antioxidants with DPPH radical scavenging activity may donate hydrogen to free radicals, primarily the lipid peroxides or hydroperoxide radicals that are the primary propagators of the chain auto-oxidation of lipids, resulting in the inhibition of the disseminating phase of lipid peroxidation[36]. Repetitive exposures to the nitric oxide radical are coupled with various carcinomas and inflammatory conditions, including diabetes, multiple sclerosis, arthritis, and ulcerative colitis. The toxicity of NO increases significantly when it reacts with the superoxide radical, forming the highly reactive peroxynitrite anion[37]. Hydroxyl radicals can destabilize disulfide bonds in proteins, particularly fibrinogen, causing them to unfurl, merge, and refold into an irregular spatial arrangement. The outcome of this reaction is evident in many diseases, such as

atherosclerosis, cancer, and neurological disorders[38]. Since there is a strong association between free radicals and the etiology of cancer and various reports divulge that antioxidants impact cancer prevention and treatment, an attempt was made to interrelate the efficacy of the selected plant extract through antioxidant studies. The results obtained from all the antioxidant assays showed that EEPQ exhibited a unique pattern of radical scavenging activity in each assay. When the three radical scavenging assays were compared, EEPQ had the highest scavenging activity against the hydroxyl radical, followed by the DPPH radical and the nitric oxide radical. Plant phenolics such as flavonoids, phenolic acid, and tannins have varied biological activities, including anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic activities. These activities might be associated with their antioxidant activity[39].

The antioxidant potential of EEPQ may be due to the presence of phenols, tannins, and flavonoids. Antioxidants eliminate detrimental chemicals in the body and protect against heart disease, arthritis, cancer, and many other chronic diseases. As a result, consuming antioxidants on a daily basis protects cells from damage. Uncontrolled cellular proliferation is a hallmark feature of cancer cells. As a result, the efficacy of anticancer drugs can be calculated by their ability to reduce cell proliferation, decrease cell viability, or arrest cells in interphase (G0/G1, S, or G2/M)[40]. The MTT (3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay is based on converting MTT into formazan crystals by living cells, which determines mitochondrial activity. Since, for most cell populations, the total mitochondrial activity is related to the number of viable cells, this assay is widely used to measure the *in vitro* cytotoxic effects of drugs on cell lines. The effect of an ethanol extract of *P. quadrifida* on cell proliferation and its cytotoxic effects were investigated using a tetrazolium reduction assay. The results showed that the extract showed significant dose-dependent antiproliferative activity against the HCT-116 cell line. From the results, it was found that the extract exhibited maximum inhibition (52.56%) at a concentration of 300 µg/ml, and the Inhibitory Concentration (IC_{50}) was found to be 269.1 µg/ml (Figure 2d). Cytomorphological modification could represent the activation of programmed cell death[41]. Since intracellular suicide programs encompass morphological changes like cell shrinkage, oxidative stress, coiling, and the biochemical response leading to apoptosis. Thus, the cytomorphological changes caused by the extract on HCT-116 cell lines at different concentrations were studied. The impact of the extract on the cells is also in line with the antiproliferative efficacy observed in various concentrations of extract-treated groups and confirms the antiproliferative potential of EEPQ.

6. Conclusion

The daily consumption of certain food has also been known to have anticancer effects. Therefore, it emphasizes the significance of environmental factors such as diet in chemoprevention. Also, it is important that over 60% of presently used anti-cancer agents are derived in one way or another from natural plants. *P. quadrifida was* found to have significant antioxidant potential, thereby protecting the cell from the damage caused by free radicals, a major cause of various diseases. The phenolic compounds such as flavonoids, phenolic acid, and tannins found in the EEPQ might be responsible for the antioxidant activity. Also, the anti-proliferative potential of the EEPQ against the HCT-116 (Human Colorectal Cancer) cell line was proven. In conclusion, the present observations provide preliminary data on the anticancer activity of the plant. It calls for further studies on the active components for chemotherapeutic properties and their possible development as promising anticancer drugs from this plant. Also, this study suggests that *P. quadrifida,* an edible plant widely used as a vegetable, can be recommended as a dietary supplement for chemoprevention.

7. Author Contribution

Latha Somasundaram, Maphibanri Maring, Shanthi S, Shabna Roupal Morais were involved in the design of the study and compilation. Merlin Roshma Sherin, Prathap S, Priyadarshini S, Priyanka H, Pushkarapriya S were involved in the execution of the research work. All authors have read and agreed to the published version of the manuscript.

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