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Evaluation of *In vitro* Anticancer Activity (Ovarian Cancer Cells-pa1) and Zebrafish Embryo Toxicity of *Parmelia perlata* Ethanolic Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Phytochemical composition and bioactivity of an ethanolic extract of *Parmelia perlata* were analyzed in this study using established procedures. The extract revealed a rich array of phytochemicals, showcasing its diverse composition, and notably exhibited strong antioxidant activity according to the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) test. Furthermore, the research explored the extract's potential in preventing Bovine Serum Albumin (BSA) denaturation, comparing

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it with the well-known medication Diclofenac. The concentration-dependent inhibition of BSA denaturation by *P. perlata* ethanolic extract was comparable to the effects observed with diclofenac on BSA. The ethanolic extract demonstrated concentration-dependent antibacterial activity against gram-negative and gram-positive bacterial isolates. While gentamicin served as a positive control with larger zones of inhibition, *P. perlata* extract displayed significant antibacterial efficacy against all six tested organisms. Moreover, *in vitro* testing against human ovarian (PA-1) cell lines demonstrated the extract's anticancer potential. A concentration-dependent reduction in cell viability, culminating in the lowest percentage at 1000 µg/ml, was observed. The IC50 value of 31.2 µg/mL showed that the *P. perlata* extract strongly inhibited the PA-1 cell line. This was confirmed by the observed changes in the cellular morphology when exposed to higher concentrations of the extract. Further, the ethanolic extract of *P. perlata* was subjected to various concentrations to evaluate its embryotoxicity on zebrafish embryos. The concentration of extract was determined to have a safety limit of less than 50 µg/L. In summary, *P. perlata* ethanolic extract has emerged as a promising natural resource, exhibiting a spectrum of bioactivities, including potent antioxidant, anti-inflammatory, antibacterial, anticancer properties, and embryonic toxicology study. These findings suggest that its potential utility in pharmaceutical applications is warranted, and further exploration and development are being suggested.

Keywords: Parmelia perlata; antioxidant; anti-inflammatory; antibacterial; anticancer; ovarian cancer cell.

1. INTRODUCTION

Cancer is presently the foremost obstacle to attaining a desirable lifespan in most countries; it is the leading cause of death worldwide [1]. Following uterine and cervical cancers, ovarian cancer ranks as the third most prevalent gynecologic malignancy [2]. The prognosis is the worst, and the death rate is the highest. In contrast to the prevalence of breast cancer, ovarian cancer is considerably rare but three times more lethal. The death rate associated with this disease is projected to increase considerably by 2040. Inadequate screening, the tumor's furtive and silent growth, and the delayed onset of symptoms all contribute to the high mortality rate associated with ovarian cancer [3]. The inadequate diagnostic effectiveness of contemporary cancer screening methods adds to this distress. A comprehensive gynecological examination, including transvaginal ultrasound, blood testing, and Other major early detection methods, including the cancer antigen-125 (CA-125) assay, has failed to substantially reduce the morbidity and mortality associated with this particular cancer [4]. After surgical debulking, traditional medicine prescribes chemotherapy. Chemotherapy [5] is a frequently employed therapeutic approach targeting malignancy. However, the detrimental consequences of chemotherapy medications are highly toxic and abhorrent. There is an ongoing investigation into several novel approaches aimed at the treatment and administration of cancer in human subjects. As cancer treatments, herbal remedies are becoming increasingly popular [6,7] because of their reduced risk and lower cost. Lichens are intricate symbiotic partnerships consisting of fungi, known as "mycobionts," and one or more algae or cyanobacteria, referred to as "photobionts." *P. perlata* is a frequently found lichen that is frequently observed on well-lit surfaces of rocks, belonging to the Parmeliaceae family [8]. In earlier times, lichen was used to treat sexually transmitted diseases like syphilis, reduce inflammation in the gum tissue, and provide relief from disorders such as arthritis, rheumatism, swelling, and heavy menstrual bleeding. The important lichen acids with significant antioxidant properties include salazinic acid, atranorin, and lecanoric acid. Their application in medicine is predicated on the presence of diverse and distinctive biologically active compounds. Lichen metabolites include many biological properties including antiviral, anti-inflammatory, antibacterial, antipyretic, anticancer, antiproliferative, and cytotoxic effects [9]. This study aims to evaluate the *in vitro* anticancer activity of *Parmelia perlata* ethanolic extract on PA-1 ovarian cancer cells and assess its toxicity on zebrafish embryos to investigate its potential as a safer alternative to conventional chemotherapy.

2. MATERIALS AND METHODS

2.1 Chemicals

Ethanol, 1, 1-diphenyl-2- picrylhydrazyl (DPPH), Ascorbic acid, Methanol, Nutrient broth, Muller Hinton agar, Dimethyl sulfoxide (DMSO),

Diclofenac sodium, Potassium Chloride (KCL), Sodium Chloride (NaCl), sodium hydrogen phosphate (Na_2HPO_4), Phosphate Buffer Saline (6.4), potassium dihydrogen phosphate (KH_2PO_4), Bovine Serum Albumin (BSA), fluconazole and MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) were of analytical grade.

2.2 Collection of Lichen

The sample was collected from Tirupattur district, Tamil Nadu, India, in sterile polythene bags. The collected lichen material was identified as *P. perlata* using standard manuals (Fig. 1). Following complete rinsing with tap water and drying in the shade, the lichen was ground into a fine powder and stored at 4°C in an airtight bottle.

Fig. 1. Collected *Parmelia perlata*

2.3 Preparation of Ethanolic *P. perlata* Extract

A 10g sample of *P. perlata* lichen powder that had been stored was extracted using the Soxhlet extraction method with 100 mL of aqueous and ethanol-based solvents, which is shown in Fig. 2. The lichen extract was then collected and stored for further analysis (Fig. 3).

Fig. 2. Soxhlet Extraction

Fig. 3. Ethanolic extract

2.4 Phytochemical Qualitative Analysis

Using the standard procedure of [8,10,11], the plant extract was analyzed for the presence of various phytochemicals in the ethanolic extract of *P. perlata*.

2.5 *In vitro* Antioxidant Activity

2.5.1 Antioxidant activity using the DPPH (1, 1-diphenyl-2- picrylhydrazyl) free radical scavenging assay

The radical scavenging activity of *P. perlata* was assessed using the DPPH assay [8-12]. A 1 mL solution of DPPH (0.1 mM) was combined with 3 mL of *P. perlata* extract at various concentrations (10–50µg/ml). Subsequently, the mixture was placed in the dark at room temperature for 30 min. The measurement of absorbance was noted at a wavelength of 517 nm using a UV–visible spectrophotometer (Systron-ics-119). The DPPH radical scavenging activity can be determined based on a previous study by using standard ascorbic acid [13].

2.6 Anti-inflammatory Properties of the *Permalia perlata* Ethanolic Extract

Mendili et al. [14] method was used to determine the inhibition of BSA denaturation. BSA (500 mg) was dissolved in 100 mL of water containing Phosphate Buffer Saline. The pH level is 6.3. Dissolved 8 g of NaCl, 0.2 g of KCl, 1.44 g of NaH_2PO_4, and 0.24g of KH_2PO_4 in 800 mL of purified water. The pH was modified to 6.3 by adding HCl, and the volume was adjusted to 1000 mL with distilled water (H_2O). The test solution (0.5 mL) comprises 0.45 mL of BSA

(0.5%W/V aqueous solution) and 0.05 mL of the test solution with different concentrations.

- The control solution was composed of 0.45 mL of BSA (a 0.5%W/V aqueous solution) and 0.05 mL of H_2O.
- The product control (0.5 mL) was composed of 0.45 mL of H_2O and 0.05 mL of the test solution.
- The standard solution (0.5 mL) comprised 0.45 mL of BSA (0.5% w/v aqueous solution) and 0.05 mL of Diclofenac sodium at different doses.

2.6.1 Procedure

A volume of 0.05 mL of test drugs at concentrations ranging from 100 to 500 µg/mL, as well as a volume of 0.05 mL of diclofenac sodium at concentrations ranging from 100 to 500 µg/mL, were taken separately. These volumes were then combined with 0.45 mL of a solution containing 0.5% w/v BSA. The samples were incubated at 37°C for 20 min, after which the temperature was elevated to maintain the specimens at 57°C for 3 min. After the solutions have cooled, add 2.5 mL of phosphate buffer. The measurement of absorbance was conducted using a UV-visible spectrophotometer at a wavelength of 255 nm. The control demonstrates complete protein denaturation. The results were compared with standard diclofenac sodium. The following calculation is based on a previous study [15,16].

2.7 Antibacterial Activity of the *P. perlata* Ethanolic Extract

The ethanolic extract of *P. perlata* was tested against pathogenic bacterial strains such as *Escherichia coli, Staphylococcus aureus, Enterococcus sp., Bacillus subtilis, Klebsiella pneumoniae,* and *Pseudomonas sp.* Antibacterial activity was assessed using the standard agar well diffusion method [8,11]. Different concentrations of the ethanolic *P. perlata* extract (50, 100, and 150 µg/ml) were prepared using 2% DMSO (dimethyl sulfoxide). The 24 h test cultures were spread onto respective Mueller-Hinton agar media using the spread plate method. Gentamycin was used as the positive control. The plates were placed in an incubator at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone [9, 12-13,17].

2.8 Anticancer Activity of *P. perlata* Ethanolic Extract Against the Ovarian Cancer Cell Line

2.8.1 Cell line and culture

The PA-1 ovarian cancer cell line was acquired from the National Center for Cell Sciences (NCCS), Pune. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C in a humidified environment containing 5% carbon dioxide (CO_2).

2.8.2 *In Vitro* assay for anticancer activity by the MTT assay

Cells were plated at a density of 1×10^5 cells per well in 24-well plates and incubated at 37°C in a 5% CO_2 atmosphere. Once the cell achieved its maximum density, different concentrations of the samples (7.8 to 1000 µg/mL) were introduced and incubated for 24 h. Following incubation, the sample was extracted from the well and rinsed with phosphate-buffered saline (pH 7.4) or DMEM lacking serum. Each well was supplemented with 100 µL of a 5 mg/mL solution of 0.5% MTT and incubated for 4 h. Following incubation, 1 mL of DMSO was added to each well. A spectrophotometer was used to measure the absorbance at 570 nm, with DMSO serving as the blank [16,18]. Measurements were conducted, and the concentration needed to achieve 50% inhibition (IC50) was established using graphical methods. The percentage of cell viability was determined using the previous study formula [19].

2.9 Zebrafish Embryonic Toxicity Evaluation of *P. perlata* Ethanolic Extract

Based on the Rajesh Kumar et al. procedure, zebrafish (*Danio rerio*) embryos were maintained and exposed to *P. perlata* ethanolic extract. The progression of *D. rerio* embryos' growth was seen using a stereo microscope. The embryos were exposed to different concentrations of *P. perlata* ethanolic extract (5, 10, 20, 40, and 80 µg/mL) for a duration of 24 to 72 h after fertilization (hpf). The evaluation of embryonic death and hatching rates was conducted at 24 h intervals. The study assessed the mortality of embryos and hatchlings, the rate of hatching, and the identification and documentation of any

abnormalities in the embryos and larvae of both the control and treatment groups. Malformed embryos were observed using a COSLAB- Model HL-10A light microscope, and the number of abnormal embryos was noted every 24 h [20].

2.10 Statistical Analysis

The study conducted triplicates on toxicity test of *P. perlata* ethanolic extract. The error bars shown in the graphical representations are expressed as mean ± SD and percentages using the Origin 2018 software.

3. RESULTS

3.1 Phytochemical Screening of *P. perlata* Extract

The phytochemical screening of the *P. perlata* extract was evaluated using a conventional approach described by Behera et al. and Mursaliyeva et al. [10,11]. Table 1 provides a comprehensive list of phytochemical components found in *P. perlata* extracts. The ethanolic extract contained all phytochemicals such as glycosides, carbohydrates, tannins, saponins, terpenoids, alkaloids, flavonoids, quinone, phenols, and steroids.

Table 1. Phytochemical Screening of *P. perlata* Ethanolic extract

S. No	Test	Ethanol Extract
1.	Carbohydrate	+
2.	Tannins	+
3.	Saponins	+
4.	Alkaloid	+
5.	Flavonoids	+
6.	Glycosides	+
7.	Quinone	+
8.	Phenols	+
9.	Terpenoid	+
10.	Steroid	+

Key: (+) Present and (-) Absent

3.2 Antioxidant Activity of *P. perlata* Ethanolic Extract

Antioxidant activity was assessed using the DPPH assay. It was demonstrated that *P. perlata* ethanolic extract exhibits a higher degree of free radical scavenging activity, indicating a stronger potential for antioxidants, when compared to standard ascorbic acid. By increasing absorbance with concentration (20-100 µg/mL), the DPPH assay indicated a lower power potential. Strong reducing power was shown by the ethanolic extract of *P. perlata* employed in the study; the reported inhibition percentage was 70% shown in Fig. 4.

3.3 Anti-inflammatory Properties of the *Permalia perlata* Ethanolic Extract

Using spectrophotometry at 660 nm, the effects of the common medication Diclofenac and *P. perlata* ethanolic extract ranging from 100 to 500 (µg/mL) on the prevention of BSA denaturation were assessed. The lowest dose of 100 µg of *P. perlata* ethanolic extract effectively inhibited the denaturation of the albumin protein (Fig: 5). As the concentration of *P. perlata* ethanolic extract increased, the proportion of denaturation inhibition grew steadily. In *P. perlata*, 500 µg of albumin-like denaturation inhibition resulted in a maximum denaturation inhibition of 51.72% shown in Table 2. Diclofenac sodium was demonstrated to have a denaturation-inhibiting effect on BSA at dosages of 100–500 µg/mL, whereas *P. perlata* ethanolic extract had the same effect. The lichen extract was examined for a dose-dependent protective effect against the denaturation of BSA caused by heat.

3.4 Antibacterial Activity of Ethanolic *Parmelia perlata* Extract

The antibacterial properties of an ethanolic extract of *P. perlata* were evaluated against six bacterial strains, consisting of three gram-positive and three gram-negative strains, using the agar well diffusion method. The bacteria included *S. aureus*, *B. subtilis*, *Enterococcus sp., E. coli, K. pneumoniae,* and *Pseudomonas sp.* (Fig. 6 (a), (b), (c), (d), (e), and (f)). Six harmful microorganisms were evaluated using ethanolic extract of *P. perlata* at several concentrations (50, 100, and 150 µg/mL). The increasing concentration gradients indicated a high zone of inhibition (ZOI) in the *P. perlata* ethanolic extract. In all six organisms, the ZOI for the positive control gentamicin was larger than the ZOI for the other three concentrations (50, 100, and 150 µg/mL) of *P. perlata* ethanolic extract. Nonetheless, there was a noticeable ZOI in the ethanolic *P. perlata* extract, which was appropriate to illustrate its antibacterial activity.

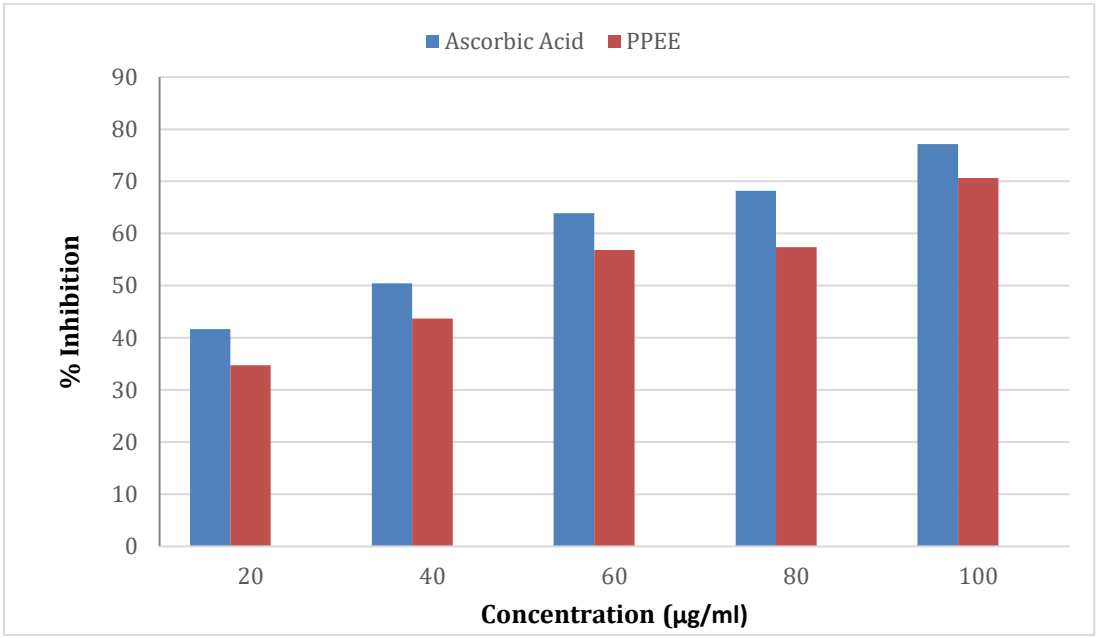


Fig. 4. Antioxidant activity of *Parmelia perlata* ethanolic extract

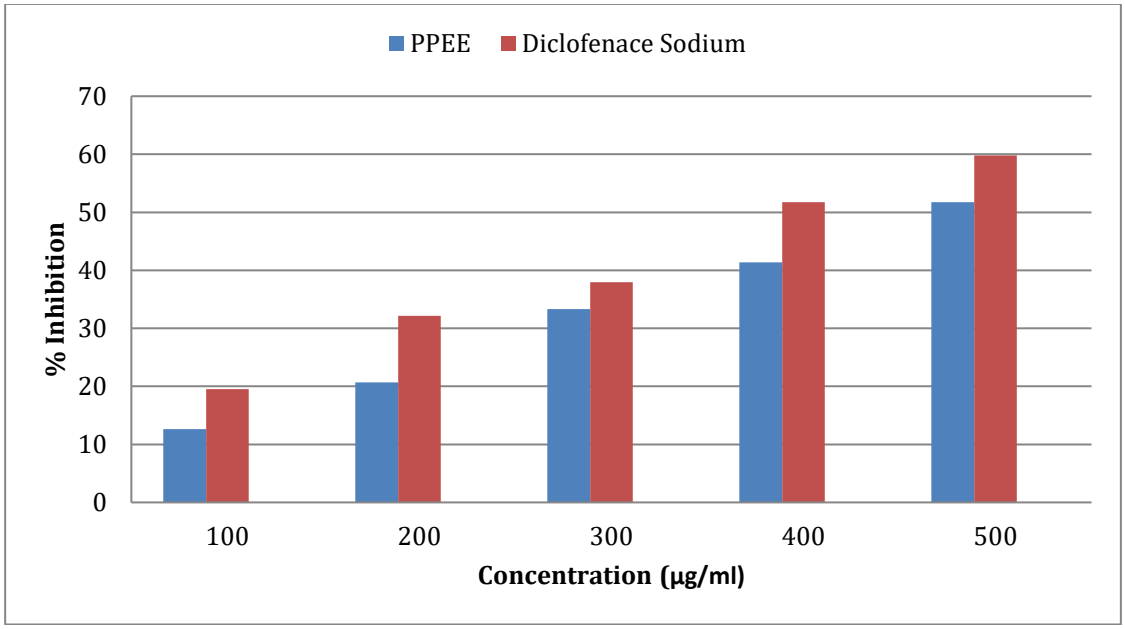
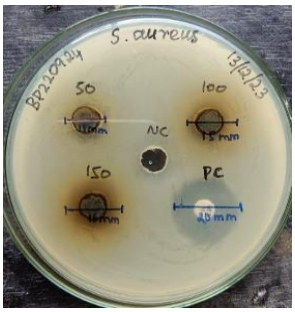


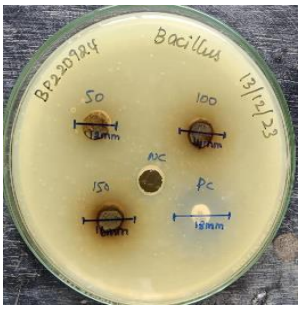
Fig. 5. Anti-inflammatory activity of *Parmelia Perlata* ethanolic Extract

Table 2. Anti-inflammatory activity of *Parmelia Perlata* Ethanolic extract

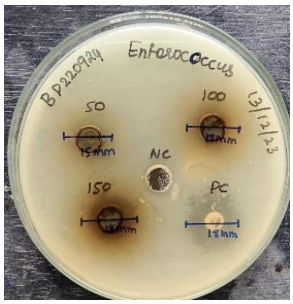
Concentration in µg/mL	Percentage of inhibition (%)	
	Diclofenac Sodium	*Parmelia perlata* ethanolic extract
100	19.54	12.64
200	32.18	20.68
300	37.93	33.33
400	51.72	41.37
500	59.77	51.72



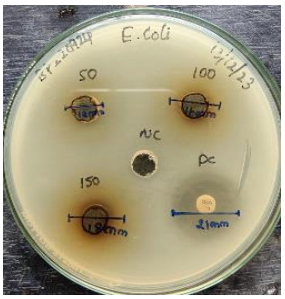
(a) *Staphylococcus aureus*



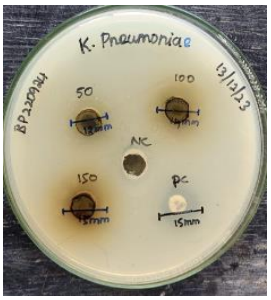
(b) *Bacillus subtilis*



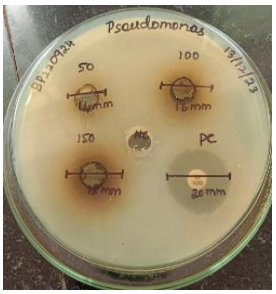
(c) *Enterococcus* sp.



(d) *E. coli*



(e) *K. pneumoniae*



(f) *Pseudomonas* sp.

Fig. 6. Antibacterial activity of ethanolic *Parmelia Perlata* extract

Table 3. Antibacterial activity of ethanolic *Parmelia perlata* extract

S. No.	Organism	DMSO	Gentamycin	Zone of inhibition		
				50 µg/mL	100 µg/mL	150 µg/mL
1.	*Staphylococcus aureus*	-	20mm	11mm	15mm	16mm
2.	*Bacillus subtilis*	-	18mm	12mm	14mm	16mm
3.	*Enterococcus sp.*	-	18mm	15mm	17mm	18mm
4.	*Escherichia coli*	-	21mm	12mm	16mm	19mm
5.	*Klebsiella pneumoniae*	-	15mm	12mm	14mm	15mm
6.	*Pseudomonas sp.*	-	20mm	14mm	16mm	18mm

3.5 *In vitro* Anticancer Activity of Ethanolic Extract of *Parmelia perlata* Against Ovarian Cancer Cell Line

In this study, the anticancer effects of *P. perlata* were evaluated *in vitro* on the viability of the human ovarian (PA-1) cell line. Various concentrations of *P. perlata* ranging from 7.8 to 1000 µg/mL were used for testing. The outcome found that as the concentration of the test substance *P. perlata* increases, the percentage of cell viability of the PA-1 cell line decreases. The lowest percentage of cell viability was found in 1000 µg/ml. It was discovered that the matching IC_{50} value was 31.2 µg/mL (Table 4). Comparing the morphology of Human Ovarian (PA-1) cell lines treated with different doses of the test drug *P. perlata,* the standard control and concentrations are shown in Fig. 7.

3.6 Embryonic Cytotoxicity Study of *Parmelia perlata* Extract Using Zebrafish

The impact of *P. perlata* extract on zebrafish embryos was investigated by examining its effects on various developmental parameters such as hatching rates, mortality rates, deformities, and morphological abnormalities. The treated embryos were observed at 24, 48, and 72 h post-fertilization (hpf).

Zebrafish embryos were treated with *P. perlata* ethanolic extract at doses ranging from 50 to 250

µg/mL after 4 h of fertilization. Developmental abnormalities were observed in the treated embryos. The hatching rate of the embryos at a concentration of 50–150 µg/mL was roughly 90%, indicating no harm to their growth. However, at a concentration of 200 µg/mL, hatching was delayed to 85%. At a concentration of 250 µg/mL, the embryos were slightly affected, resulting in an 80% decrease in growth. An increasing concentration of *P. perlata* ethanolic extract gradually affects the hatching rate of the embryos (Fig. 9).

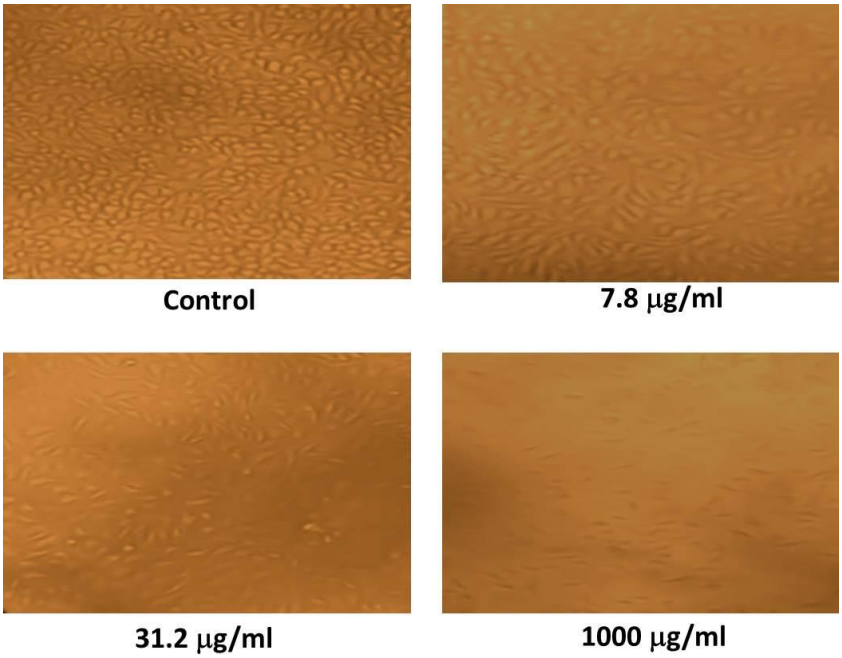


Fig. 7. Morphological comparison of PA-1 cells treated with *Parmelia perlata* at different concentrations and control

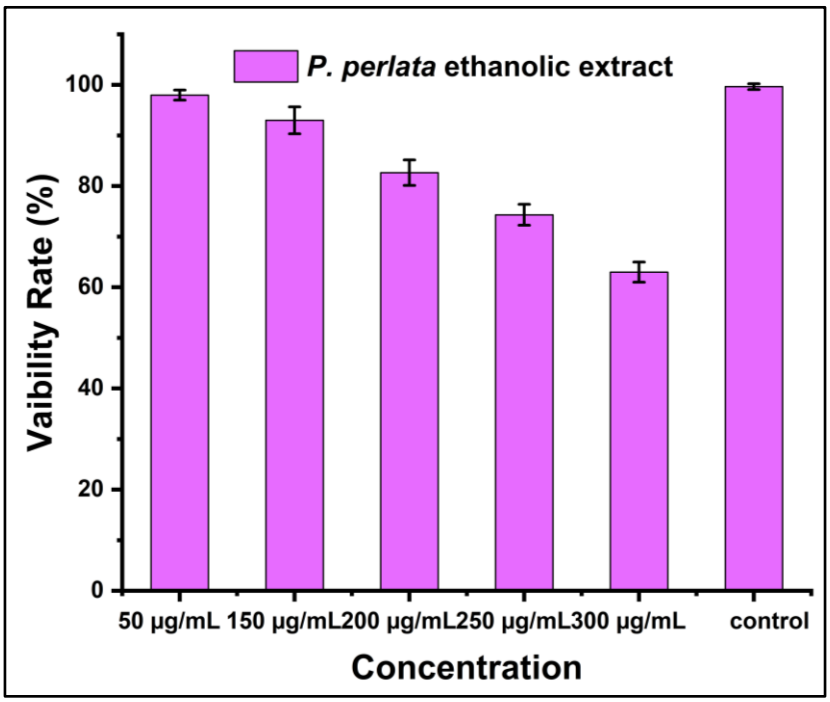


Fig. 8. Viability Rate of Zebrafish treated with *Parmelia perlata* extract

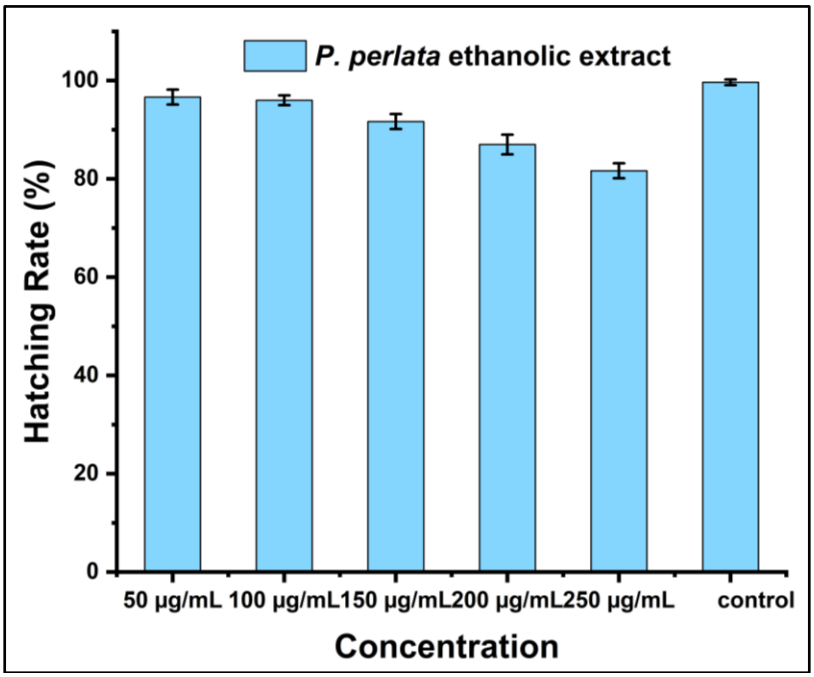


Fig. 9. Hatching Rate of Zebrafish treated with *Parmelia perlata* extract

Table 4. Anticancer effect of *P. perlata* on PA-1 cell line

S. No.	Concentration (µg/ml)	OD value	Cell viability (%)
1	1000	0.089	14.75
2	500	0.132	21.89
3	250	0.174	28.85
4	125	0.217	35.98
5	62.5	0.261	43.28
6	**31.2**	**0.304**	**50.41**
7	15.6	0.349	57.87
8	7.8	0.392	65.00
9	Cell control	0.603	100

The results exhibited a uniform viability rate for every concentration range (50–150 µg/mL) examined (Fig. 8), suggesting the absence of toxicity in comparison to the control group. The findings indicated that lower amounts of *P. perlata* ethanolic extract did not show considerable toxicity; however, higher concentrations had a severe negative impact on the survival of zebrafish embryos. The study found that the zebrafish embryos showed reduced toxicity when exposed to the ethanolic extract of *P. perlata.*

The findings of the present study indicated that exposured to *P. perlata* extract at a concentration of 150 µg/mL had a significant impact on the hatching rates and viability rates, as shown by Figs. 8 and 9. The embryos successfully hatched at a high rate. In addition, there were no significant differences in morphology and heart rate between the group exposed to *P. perlata* extract and the normal control group (Figs. 8 and 9).

4. DISCUSSION

The Indian Siddha system was a renowned traditional form of treatment that had been employed since ancient times to heal a wide range of illnesses. Ayurvedic Indian medicine was used in several lichen extracts as remedies for various ailments [21]. The phytochemical test of *P. perlata* extract was evaluated for qualitative analysis. Similarly, Momoh and Adikwu [22] study showed, that the lichen *P. perlata* ethanolic extract contained saponins, flavonoids, tannins, glycosides, steroidal aglycone, and carbohydrates. Kello et al. [9] assessed the

antioxidant properties of acetone extracts from several lichen species, comparing them to ascorbic acid, which showed scavenging activities. According to Kello et al., DPPH radical scavenging activities of lichen methanolic extracts increased proportionately with concentration. Absorbance values of these extracts and standard antioxidants were assessed at 24.75 µg/mL [9,12].

Further, Tartouga et al., the extract demonstrated a 38.39% inhibition of BSA denaturation at a concentration of 1000 µg/mL [23]. Tatipamula et al. reported that extracts from lichen had significant anti-inflammatory properties *in vitro*. At a concentration of 1 mg/mL, the extracts acetone and ethyl acetate showed inhibition levels that were comparable to indomethacin, with acetone inhibiting inflammation by 89.85% and ethyl acetate by 82.36% [24]. When compared to the present study, the concentration of extract at 31.2 µg/mL showed 50.41%.

The results indicate that the acetone extract of lichen had superior antibacterial activity compared to the methanolic extract, particularly against Gram-positive bacteria. Nevertheless, it had little efficacy against Gram-negative bacteria such as *E. coli, S. enterica, S. typhimurium,* and *K. pneumoniae* when assessed using MIC and MBC techniques as per Kocovic et al. study in [8]. Similarly, Kello et al., evaluated by extracting seven lichen extracts. These extracts were then tested against both Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) at a concentration of 1 mg/mL. The treatment showed no efficacy against *E. coli,* but showed efficacy against *S. aureus*, with lichens exhibiting the most pronounced inhibitory impact [9]. The methanol extracts of five lichen species exhibited antibacterial properties against 11 bacteria and 1 fungus. *C. calcarea, N. chlorophylla,* and *B. capillaris* showed significant effectiveness against *B. cereus, B. subtilis, C. perfringens,* and *E. coli*, resulting in ZOI ranging from 9 to 15 mm. In a study conducted by Us et al., it was shown that *C. calcarea* exhibited the most activity, resulting in a 15 mm inhibition zone against both *E. coli* and *B. cereus* [12]. The study assessed the antibacterial efficacy of solvent extracts derived from 12 lichen species against pathogenic bacteria (*P. aeruginosa, E. coli, S. aureus,* and *K. sp.*) and the fungus *Aspergillus sp*., employing the disc diffusion method. The acetone extract of lichen species exhibited the most potent antibacterial action, namely against *E. coli, P. aeruginosa,* and *S. aureus*. The fungal strain examined by Khan et al., showed the greatest inhibitory zones in *P. sulcata*, *Evernia mesomorpha,* and *Sticta lambata* [17]. In the present study, the antibacterial activity of *P. perlata* ethanolic extract was mostly reflected in a higher ZOI in *Enterococcus sp., E. coli,* and *Pseudomonas sp*., which is shown in Table 3. The remaining cultures showed less ZOI in measurement. Among the bacteria, *P. perlata* extract showed the best effect against *E. coli* in 150 µg/mL concentration.

According to Nikhitha et al. *Adhatoda vasica* extract was used to treat PA1 teratocarcinoma cells and showed significant anticancer effects. Observations were conducted for a duration of 48 h to evaluate the suppression of metastatic characteristics. The mRNA obtained from both treated and untreated cells was transformed into complementary DNA (cDNA) and then amplified to assess the gene expressions of p53, p21, and GAPDH. The findings demonstrated the efficacy of *A. vasica* extract in combating ovarian cancer [25]. Similarly, Fankam et al. examined the potential role of apoptosis in the cell death of ovarian cancer cells induced by guttiferone (GBL) isolated from *Allanblackia gabonensis.* The study of the cell cycle revealed a significant prevention with 8.5% of cells in the sub-G0 phase at a low dosage and 30.7% at the IC50 dosage, in contrast to 5.4% in cells that were not treated [26]. In the present study, *P. perlata* ethanolic extract was used for the *in vitro* anticancer activity, which shows significant activity against the human ovarian (PA-1) cell line.

Shenbagam et al. treated zebrafish with *P. tinctorum* extract for 7 days. The behavior study revealed no observable physiological alterations or notable behavioral consequences, such as immobility, over the whole duration of the exposure [27,28]. Shenbagam et al. investigated the harmful effects of *P. tinctorum* on zebrafish embryos by exposing them to doses ranging from 50 to 250 µL for a duration of 72 h after fertilization. There were no notable variations in growth, survival, and heart rate across the different concentrations. The embryos exhibited good health and did not have any morphological defects. The results indicated that the safety was maintained at concentrations of up to 200 µg/mL [21].

Finally, the ethanolic extract of *P. perlata* was very good at killing bacteria, reducing inflammation, and fighting cancer, especially

human ovarian (PA-1) cancer cells. Additionally, zebrafish embryos confirmed its safety profile up to certain concentrations, suggesting its potential as a safer alternative to conventional chemotherapy. Further in-depth studies are necessary to validate these findings and explore clinical applications.

5. CONCLUSION

The ethanolic extract of *P. perlata* was used to identify phytochemicals and several biochemical activities, including antioxidant, anti-inflammatory, and antibacterial properties by *in vitro* methods. The MTT test was employed to determine how various *P. perlata* concentrations affected the survival of PA-1 cell lines. This *in vitro* model was extensively utilized to assess the cytotoxicity of plant extracts against various types of cancer cells. *P. perlata* extract identified its toxic level by observing the morphological characteristics such as mortality, malformation rate, and heartbeat of the zebrafish model with compared to the control zebrafish model. The extract induced toxicity in embryonic development in a dose-dependent manner. This toxicity adversely impacted the development of the embryos, resulting in deformities, delayed development, and potentially even mortality. Numerous herbs found in the test medication contain phytochemicals that function as antioxidants, antibacterial and anticancer agents. Antioxidants could be effective in preventing and treating cancer and other ailments because of their ability to protect cells from the harmful effects of highly reactive oxygen molecules, commonly referred to as "free radicals."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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