

# Investigation of antitumor activity of different Extracts of Cyanobacterium Westiellopsis prolifica Janet.

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## Abstract

A strain of cyanobacterium *Westiellopsis prolifica* was isolated from soil samples collected from different locations of Pathardi tehsil of Ahmednagar district. Identification using morphological variation and taxonomic methods. Sterile cultures of *Westiellopsis prolifica* were obtained in laboratory. Use a different medium, BG-11 medium, for biomass production. Collect the biomass by filtration through two layers of muslin and dry using a hair dryer. *Westiellopsis prolifica* cultures were isolated by improving soil samples in BG-11 medium (Kaushik 1987). Sterile cultures were obtained by repeated liquid transfer of small particles followed by antibiotic treatment (streptomycin, chloramphenicol and penicillin (10 mg ml<sup>-1</sup>)) (Kaushik, 1987). Medium and clean Unalgal culture. The extraction process requires solvents of different polarities such as (i) hexane (ii) chloroform (iii) methanol and (iv) distilled water. Biomass samples were weighed and added to each weight at a ratio of 1:10 (w/v) for the removal of metabolites. Three consecutive extract filtrations were carried out using Whatman paper and the subsequent filtrates were collected. Treatment of HeLa cells with methanol, chloroform, hexane and aqueous extracts caused dose-dependent cell death as evidenced by the decrease in cell viability assessed by MTT assay. Extracts obtained from *Westiellopsis prolifica* were tested and found to have antitumor activity at different rates. The most potent is the extract of *Westiellopsis prolifica*.

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## Introduction

Cancer is generally defined as the uncontrolled growth of cells with loss of differentiation and often metastasis to other tissues and organs. Cancer is a malignant growth whose treatment includes surgery, radiation and chemotherapy, alone or in combination. Most synthetic antibiotics work by interfering with the growth of cancer cells, but these drugs often affect not only cancer cells but also normal cells that are equivalent to cancer cells. Therefore, cancer exposure to synthetic antibiotics and their side effects are a major issue in cancer treatment today. Cancer is a serious disease that affects millions of people every year. Natural plant products are important in cancer treatment. A study of antibiotics on the market from 1940 to 2002 revealed that only 36% of drugs were synthetic and 64% were of natural origin (Newman et al., 2003). There is increasing interest in the production of many toxic or other toxic substances with potential biomedical and environmental health uses (Moore, 1996; Gerwick et al., 2001; Osborne et al., 2001; Meyer and Gustafson, 2003; Shimizu, 2003). Among compounds with different activities, compounds with anti-inflammatory properties are of particular interest. There are many reports of blue-green algae as antibiotic

producers (Patterson et al., 1994; Wagner et al., 1999; Zorica et al., 2008). Genera such as *Nostoc*, *Scytonema*, *Hapalosiphon*, *Lyngbya* and *Symploca* all contain antibodies. A promising set of cyanobacterial antitumor agents, including cryptophycin, cytophycin, and toxin (Patterson et al. 1991, 1994), has been identified as a microtubule depolymerizing agent and an important cause of apoptosis in human cancer cells. *Hapalosiphon*, *Microcoleus*, *Scytonema*, and *Tolypothrix* have been found to be toxic, but toxins from these genera have not been isolated and characterized (Scott 1991; Skulberg et al. 1992). Oral supplementation of *Spirulina fusiformis* causes regression of vitiligo in individuals with homogeneous leukoplakia (Mathew et al. 1995). *P. tenuifolia* contains many diacylglycerols that inhibit chemically induced tumors in mice (Tokuda et al. 1996). Filamentous marine cyanobacteria have been reported to protect against cancer, neurodegenerative diseases and infectious diseases (Tan 2013). Crude ntawm rau cyanobacterial hom (*Geilerinema* sp., *Arthrospira* sp., *Geitlerinema* sp., *Phormidium* sp., tiab *Leptolyngbya* sp.) muaj concentration-dependent teebmeem ntawm Homo sapiens raum mob cancer tiab Homo sapiens colorectal adenocarcinomas, 2015. glycolysis.

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macrolide, lyngbouilloside (Frayman, K. et al., 2013) was isolated from the marine cyanobacterium *Lyngbya bouillonii* collected in Papua New Guinea. It exhibits moderate cytotoxicity on neuroblastoma cells with an IC<sub>50</sub> value of 17 M (Tan, L.T, et al., 2002). Another 14-membered macrolide, koshikalide (Shishido, T.K, et al., 2015), was isolated from the marine cyanobacterium *Lyngbya* sp. g/mL (Iwasaki, A. et al., 2010). The antimicrobial activity of beneficial cyanobacteria was tested in this study.

### Materials and methods

Culture isolation *Westiellopsis prolifica* culture was isolated by treatment of soil samples in BG-11 medium (Kaushik, 1987). Identification is done by morphological variation and taxonomic methods according to Desikachary (1959). Sterile culture of filamentous *Westiellopsis prolifica* was obtained by repeated liquid transfer of small particles after treatment with antibiotics (streptomycin, chloramphenicol and penicillin (10 mg ml<sup>-1</sup>)) (Kaushik, 1987). Purification of Unalgal culture isolated from the transformation was done aseptically in 100 ml of BG-11 medium (Rippka et al., 1979) in 300 ml glass bottles at pH 7.5 and 24 C. Chemicals & Media SRL Ltd. DMSO, hexane, chloroform, methanol;). The extraction process requires solvents of different polarities such as (i) hexane (ii) chloroform (iii) methanol and (iv) distilled water. Powdered *Westiellopsis prolifica* biomass samples were weighed and added to each weight at a ratio of 1:10 (w/v) to remove metabolites. Perform three successive extractions using what man paper and collect the next filtrate. The aqueous extract samples we stored at 4 C until needed. On the other hand, the organic solvent filtrate was evaporated at room temperature, resulting in the loss of volatile solvent, resulting in a plant extract residue. These residues were weighed and dissolved in 20% DMSO aqueous solution to prepare the crude plant with a final concentration of 2.5 mg/ml. The extracts were further stored at 4 C.

### Cell Culture and Maintenance

Both cells were cultured in 75 cm<sup>2</sup> tissue culture flasks in DMEM growth medium supplemented with 10% FBS and 0.1% antibiotics. They were kept in an incubator at 37 C and 5% CO<sub>2</sub>. Use the appropriate energy mixture to ensure cell stability. Extracts were dried and reconstituted in 0.25% DMSO and distilled water. Cultures were established for 24, 48 and 72 hours (3-4 cells established in culture). The in vitro inhibitory effect of each extract on cancer cells was determined using the

[2,5-diphenyltetrazolium bromide] assay. Use a sterile cell to replicate the HeLa monolayer formation in a 90 cm<sup>2</sup> tissue culture medium. The resulting HeLa cell suspension was centrifuged at 5000 rpm for 5 min and the supernatant was discarded while the cells were suspended in the culture medium. Cell counts were performed using a Neubauer chamber and in each case, 10<sup>5</sup> cells were seeded in 200 l of growth medium (DMEM + 10% FBS + 0.1% antibiotics) in a microtiter plate (96-well plate). In each case, 3 concentrations of extract ranging from 80 g to 150 g were added to the cells in triplicate. Paclitaxel was used as a positive control in the experiment. Place these plates at 37 C for 24 h, then add 20 l of MTT solution to each well and mix well. The plate was incubated again at 37 C for 4 h to allow reduction to occur. Finally, the absorbance of the test and control wells was measured colorimetrically at 540 nm and 620 nm using an ELISA plate reader. Percent cell viability is expressed as:  $(At * 100) / Ac$  [%], where At is the absorbance of the test sample and Ac is the absorbance of the control. The IC<sub>50</sub> is interpolated from the growth curve after 24 h of incubation.

### Results and Discussion

To demonstrate the anti-cancer effect, we tested hexane, chloroform, methanol and water extracts of American ginseng progenitor against HeLa cells. The antitumor activity of *Westiellopsis prolifica* extracts was evaluated by MTT assay. Hexane, chloroform, methanol and aqueous extracts of *Westiellopsis prolifica* showed anti-inflammatory activity. The IC<sub>50</sub> range for all extracts was 140-165 g/ml. The inhibitory effect of the extracts was shown to vary with different solvents. The aqueous extract of *Westiellopsis prolifica* exhibits strong anti-inflammatory properties. The viability of HeLa cells decreased with increasing concentration of methanolic extract, and the lowest viability was observed at the concentration below 80 g/ml. IC<sub>50</sub> of methanol extract was 140 g/ml. Methanol, chloroform and hexane eliminated all the beneficial effects of manual killing. Cell viability was weakly affected after 72 h of exposure. Therefore, the results showed that *S. cerevisiae* biomass extracts used in different solvents exhibited different rates of antitumor activity. The best of these is the methanolic extract of *Westiellopsis prolifica*. It should be acknowledged that the best results are obtained from raw materials, and therefore promising in natural antitumor products. These extracts need to be purified and their bioactive components characterized. In addition, the effects of the extracts on other cancer cells need to be

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tested in vitro and in vivo. Most importantly, the antitumor mechanism of action of the active drug must be determined. Cancer is a disease that causes a lot of fear in people. It can be treated with surgery, chemotherapy and/or radiotherapy. However, there is no cure for this disease, especially in its advanced stages. Therefore, it is important to study new drugs that have low toxicity, high activity and provide better disease control. In this study, the effects of methanol, chloroform, hexane and aqueous extracts of *Westiellopsis prolifica* on HeLa cells were evaluated. Many drugs derived from plants, such as vinca alkaloids, epipodophyllotoxin, and paclitaxel, have been reported to cause cell death. Paclitaxel, used as a concomitant control in this study, reduced cell viability in a dose-dependent manner. Our results suggest that the methanol extract of American ginseng is as cytotoxic as paclitaxel or more potent. Irritant effects seen with low-dose extracts are common and well known in toxic substances. Interestingly, treatment of HeLa cells with low concentrations of *Westiellopsis prolifica* had a cytotoxic effect, while higher concentrations had a stimulatory effect (Tan, 2007; 2001). However, most of the studies on bioactive compounds obtained from cyanobacteria have focused only on marine animals (Moore, 1996; Gerwick et al., 2001; Thacker and Paul, 2004). It has shown activity against MOLT-4 (leukemia cancer cell line) and MCF-7 (cancer cell line) (Tripathi et al., 2010). Curacin A is the most potent molecule isolated from organic extracts of marine cyanobacteria (Gerwick et al., 1994). Lyngbyabellin A and E have been reported to have strong actin polymerization activity from *L. majuscula* (Luesch et al., 2000; Han et al., 2005). Both compounds showed moderate cytotoxicity against many cell lines. Pahayokolide A isolated from freshwater *Lyngbya* is moderately cytotoxic and inhibits various types of human cancer cells (Berry et al., 2004). The mechanism is not proven. The involvement of many unknown mechanisms such as apoptosis and ribozymes may be responsible. Cytophycins are a newly discovered class of natural cytotoxins isolated from cyanobacteria of the family Scytonemataceae.

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