Abstract:

Cancer is a vicious disease that claims the lives of many every year. In 2012 alone an estimated 8.2 million cases were reported according to the cancer worldwide cancer research organization. Researchers are still understanding its mechanism of action and the exact reason why a cell would ignore instructions dictated by its DNA, escaping apoptosis and start proliferating. Existing methods of treating cancer such as chemotherapy or radiotherapy, display detrimental side effects, in this project we propose a different approach to cancer therapy through plant extracts which have strong anti-oxidative activity. Asplenium polypodioides and Asplenium dalhousiae are used in this study to explore its potential anticancer effect. The derived extracts were dissolved in different polar & nonpolar solvents to screen for optimal dosage (between 5µl-50µl) that would optimise the crude extracts potential to exhibit antitumor activity.

Introduction:

Cancer is known as the second leading life-threatening disease after heart attacks. As current existing medications have fallen short, it is now responsible for one in eight deaths world-wide and is expected to rise within the next few decades. The most dangerous aspect about cancer cells is their ability to evade natural mechanisms that control its proliferation as well as their control over hypermethylation of tumour-suppressor genes on CpG sites, either due to genetic and environmental factors or gene-environment interactions. These cause suppression of the tumour suppressor genes. They also escape programmed cell death, which is otherwise essential for normal body development and maintenance. For this reason, current medicinal approaches focus on targeting cells that have ‘escaped’ programmed cell death, by using chemically derived

drugs (chemotherapy) such as azacitidine and decitabine. Though they may have shown efficacy in reducing metasticity, on the downside they also tend to affect healthy cells. For example, in breast cancer chemotherapy, the treatment permanently affects the immune system by depleting B cells and CD4+ T cells. Another common side effect is Neutropenia which is an abnormally low level of neutrophils. [1],[2],[3] Hence most of the ongoing research is focused on finding alternative treatments that specifically target cancer cells while having minimum cytotoxic effects on healthy cells.

Plant derived treatments have been commonly used as traditional medicine in various countries for many years. They are believed to have the potential to treat a broad spectrum of diseases including cancer amongst others. According to the World Health Organization, it is estimated that 25% of modern medicines are derived either directly or indirectly from medicinal plants, and reaching 60% in the pharmaceutical class of anti-tumoral and antimicrobial medicines. Also in 2007 it was estimated that the net-worth trading of plant-derived drugs was upto US$100 billion in that year alone, and that trade is expected to reach US$5 trillion by 2050 [4]. Medicinal plants are a leading field of research due to their omnipotent ability as an alternative treatment for cancer with its naturally occurring metabolites possessing chemopreventive properties. Although it is practiced in herbal medicine with some success rate, there is no scientific data to support or provide reason for the results, as the metabolites and their biochemical properties have not yet been defined. Research in this area is still scarce but its historical background indicates that it does possess an untapped yet great potential which needs to be further explored. [5]

Isolation of active plant compounds began in the early 19th century, when the analgesic and sleep-inducing agent from opium was isolated and named ‘Morphine’. [6] Following it was the discovery of anticancer medicinal plants in the nineties, of which the most successful against a range of cancers was Taxol, with positive clinical trials and approval by the Food and Drug

Administration (FDA). [7] This success story propelled ethnomedicine into an active field of research by pharmaceutical industries and drug and development sectors, to discover more such medicinal plants. [8]

The plants used in this research were *Asplenium dalhousiae* and *Asplenium polypodioides Blume*. *Asplenium dalhousiae* comes from the Kingdom Plantae, Phylum Tracheophyta, Class Polypodiopsida, Order Polypodiales, Family Aspleniaceae and Genus Asplenium. Its common name is *Spleenwort Fern* characterized by its brownish green to brownish yellow color, and are traditionally used to help pulmonary disorders. It is a common western Himalayan fern usually found in Afghanistan, India, Kashmir, Nepal, Pakistan.

Asplenium polypodioides Blume comes from the Kingdom Plantae, Phylum Tracheophyta, Class Polypodiopsida, Order Polypodiales, Family Athyriaceae, Genus Diplazium and Species Diplazium polypodioides its synonym is Asplenium polypodioides (Bl.) Mett. Its common name is, *Twin Sorus Fern* and is characterized by its black brown color. [9]

Figure 1: *Asplenium dalhousiae* also known as *spleenwort fern* showing a distinct leaf pattern with colors ranging from brownish-green to brownish-yellow.

Figure 2: *Asplenium polypodioides Blume* also known as the Twin Sorus Fern is a large terrestrial plant with strong black roots.

This study explores the potential anticancer effects these plants may have as they are known on a social aspect to possess medicinal properties, however, there is no scientific data to back this claim. It is tested in 3 different solvents against MG63 an osteosarcoma cell-line with MTT assay assessing its effect.

Materials and method:

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Phosphate Buffer Saline (PBS), Dimethyl Sulfoxide (DMSO) ,Fetal Bovine Serum FBS, 1% penStrep, MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)

Types of cancer cell lines

MG63 cell line from ATCC.

Extract preparation

Stock solutions were made of 7 extracts each dissolved in Acetone, N-Hexane, Methanol and Water; existing in either dry or semi-dry condition. It was weighed and dissolved in Sterile distilled water of at least 10 ml and then dissolved in 0.1% DMSO where a range of varying 8-13mg/ml of stock solution of extracts was reached.

Cell culture and treatments

MG63 cell line obtained from ATCC were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penStrep at 37°C. For seeding; cells were washed two times with phosphate buffer saline (PBS) , PBS was decanted, adherent cells were harvested with 0.75% trypsin-EDTA, then DMEM media was added to make up to the volume of 10ml. Cell suspension was centrifuged at 1200g for 10 min, later the pellet was re-suspended in 10 ml to make a single cell suspension. Cells were seeded in 96-well plates at a density around 5 x 10 3 cells /well in a 200 µl media as a final volume in every well and left for 24 hrs. The anticancer effect of 7 plants extract treatments were tested against the cancer cell line mg63 in 4 different concentrations of 5ug, 10ug, 30ug, 50ug respectively. Each were added in three replicates and tested against three different time intervals of 24hr, 48hr and 72hr, with a separate 96-well plate for every hour. Negative control were added as the last row in each

respective hour plate, where it contained 6 well of cells without treatment and 6 wells of 0.1% DMSO. Treatments were added every 24 hr.

MTT Assay

Medium was removed and cells were washed with PBS, 20 µl MTT solution was added per well to achieve a final concentration of 0.5 mg/ml in 200 µL of medium alone, cells were incubated at 37°C for 3-4 hours. Periodically, cells were checked under an inverted microscope for presence of intracellular purple formazan crystals. Medium was removed at 4 hours mark; when the purple precipitate was clearly visible and cells were washed with PBS, Then 100 µl DMSO solution was added to each well. The plate was wrapped in foil and shaken on an orbital shaker for 15 minutes. Absorbance in each well was measured at 570 nm in 96-well plate.

MTT assay is colorimetric assay to assess cell metabolic activity, it is based on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product [10]. MTT assay was used to provide an indication of the cytotoxic activity of *Asplenium polypodioides Blume* and *Asplenium dalhousiae* extracts in acetone, methanol, water and n-hexane. Selective effects were observed based on concentration and time. MTT assay readings were taken after 24 hours, 48 hours and 72 hours of incubation with treatments added every 24 hours.

A. (B) (C)

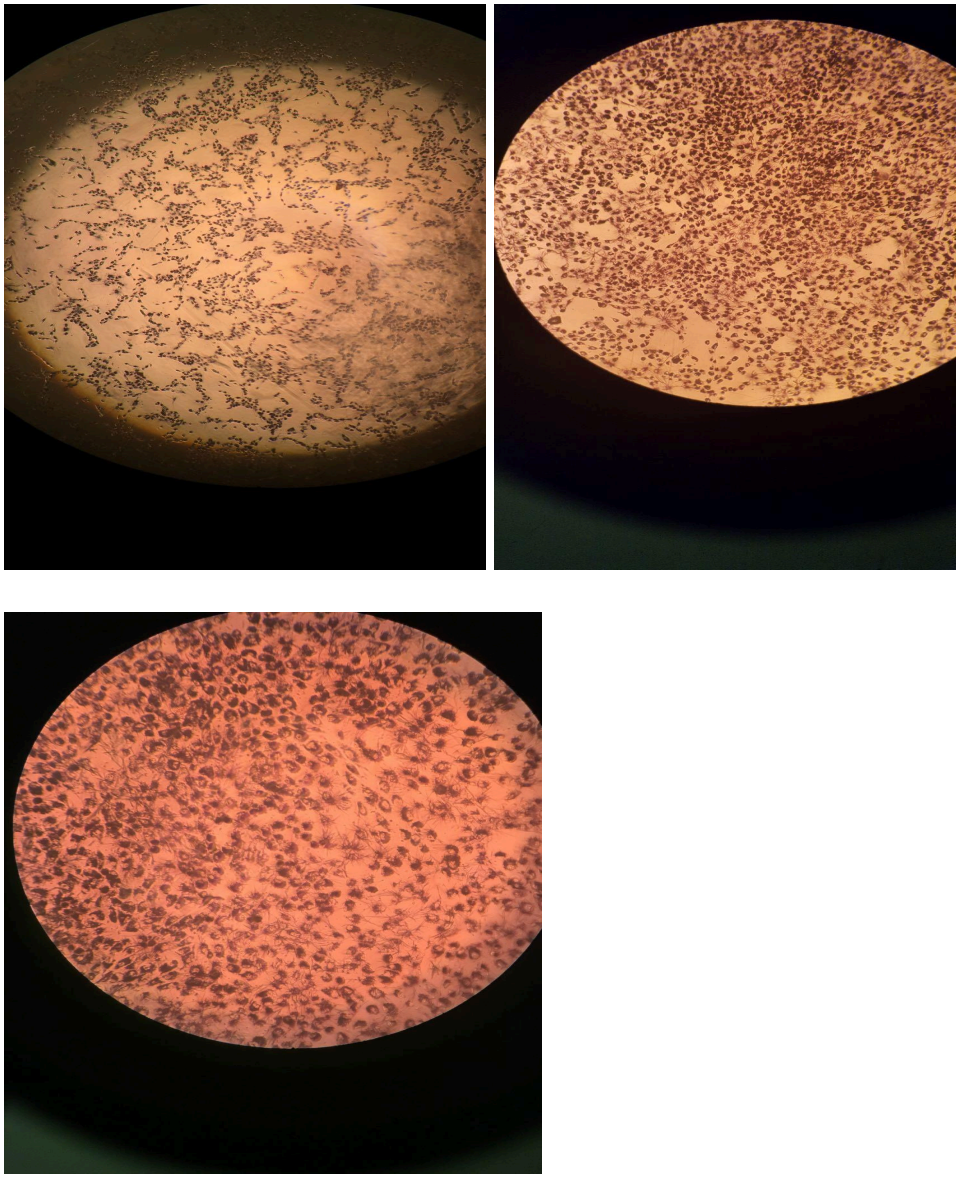

Figure3: MTT Assay performed on MG63 cells treated with *Asplenium polypodioides Blume* and *Asplenium dalhousiae* extracts in acetone, methanol, water and n-hexane show purple color due to formazan crystals in the cells. (A) Treated cells at 24 hours and incubated with MTT for 4 hours. (B) Treated cells at 48 hours and incubated with MTT for 4 hours. (C) Treated cells at 72 hours and incubated with MTT for 4 hours.