In-Vitro Anticancer Activity Of Hydroalcoholic Extract Of Senna Alata

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Abstract: Cancer is a class of disease characterized by uncontrolled development of cells. Current cancer treatment possibilities are radiotherapy, chemotherapy, hormone therapy and surgery, all causing disagreeable side impacts. Because of their adverse side effects, the development of fresh drugs for cancer treatment is challenging. The researchers are therefore attempting to find noble compounds for the treatment of cancer from natural sources. For the anticancer activity against various cancer cell lines, the hydralcoholic extract of Senna alata leaves was assessed in the current research. The cell lines of breast cancer (MCF-7) and prostate cancer (PC-3) were used to study invitro anticancer with the leaf extracts of Senna alata. Concentration ranges were tested against MCF-7 and PC-3 cells ranging from 50 to 1000 micrograms of Senna alata crude hydralcoholic extracts. 3- [4,5-Di methyl thiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay was conducted with the Senna alata crude hydralcoholic extracts. The cytotoxicity was highly significant in the hydralcoholic extract 456.45 µg in MCF-7 cell line and 628.95 µg in PC-3 cell line.

Keywords: MCF-7 cell line, PC-3 cell line, In-vitro Anticancer Activity, MTT assay, Senna alata

1 INTRODUCTION
Herbal medicine practice dates back to the early days of known human history. In almost all ancient cultures, such as the Indian, Egyptian, Chinese, the Greek and the Roman empires, there is proof that herbs were used in the therapy of illnesses and to revitalize body systems. Plants were the main stem of medicine and credited with mystical and almost supernatural powers of healing. Plants have a vast potential to be used as medicinal products. Recently, the trend in cancer research is moving once again towards finding fresh medications for cancer treatment from natural sources. In present cancer therapeutic methods, wide varieties of compounds are in use. The drug sectors have mainly depended as a source of medicine on natural compounds in the last year. Statistics have shown that over 60% of anticancer medicines lately used are linked to herbal origin (Al-Mamun et al., 2016). Herbal products are worldwide accepted as a source of complementary and alternative medicine in (Ernst and Cassileth, 1998) various diseases especially in cancer (Safarzadeh et al., 2014). They provide us relatively safe, effective, and economical therapeutic options, particularly in case of cancer where treatment is long term and cost is excruciatingly high (Lobo et al., 2008). A vast amount of plant species globally is tested and bio assayed for the drug discovery of multiple illnesses globally (Richardson, 2001). Medicinal plants from systematic screening programs or by serendipity possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures.

Many natural compounds such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids have been found from plant sources that have significant antioxidant activity and play an important part in the therapy of cancer (Rajandeepe et al., 2011). Several studies have shown that antioxidant compounds demonstrate anti-inflammatory, antitumor, antimutagenic, and anticarcinogenic activity (Kabir et al., 2013). Natural antioxidant compounds can directly prevent the proliferation of cells and boost the immune system (MacDonald et al., 2002). The cancer and infectious disease study regions have a leading role on the use of medicinal plants as a drug discovery source. The FDA endorsed extremely validated anticancer and anti-infectious drugs for 60-75 percent of natural drugs such as medicinal crops (Newman et al., 2003). These drugs have been regarded from the discovery of natural drugs in chemoprevention for chemotherapeutics in cancer studies (Kucuk, 2002; Balunas and Kinghorn, 2005). Cancer addresses the burning health problem and is currently one of the most life-threatening illnesses in both advanced and developing nations (Tague et al., 2014). Cancer places a severe strain on public health and is still scientifically difficult in its treatment and healing procedures (Rajesh et al., 2011). Development of cancer is connected with oncogene modification, tumor suppressor genes, and genes for DNA repair (Mukherjee et al., 2007). Both external factors such as tobacco, chemicals, radiation, and infectious organisms and internal factors such as inherited mutations, hormones immune conditions are considered to be responsible or the risk factors for causation of cancer (Ganapathy et al., 2015). Chemotherapy, radiotherapy, hormone therapy, and surgery are the standard approaches to cancer treatments. But there are serious side effects in each of these standard therapy modules (Krishnamoorthy and Ashwini, 2011). The enhanced incidence of death and the adverse effects of anticancer drugs are the primary factors that inspired scientists to search for fresh and more efficient drugs with minor side impacts (Haghighi et al., 2017). Breast cancer is the second most common cancer in females and leads to death (Parkin et al., 2001). About one-third of females with breast cancer created metastases and eventually died from the disease. MCF-7 is an estrogen-positive line of human cancer cells obtained from metastatic breast cancer patients (Levenson and Jordan,
The objective of medicinal plant testing is to search for an outstanding anticancer agent that can be averted to human malignancies. Prostate cancer is the most common forms of malignancy in men, particularly in developed countries where the majority of cases are diagnosed in men aged above 50 years (Hariranan and Padmanabha, 2016). Present treatment choices available for prostate cancer include surgical therapy, hormone therapy, radiation therapy, and chemotherapy; these medications have shown patient enhancement but usually end up with negative and poisonous side impacts (Harun-ur-Flashid et al., 2002). Nutritional supplements such as vitamin E, selenium, soy products, lycopene, and green tea catechin have also been widely used in prostate cancer therapy (Algotar et al., 2013; Kristel et al., 2011; Brausi et al., 2008). Senna alata (L.) Roxb, a soft wood shrub (Hutchinson, 1963) known as winged Senna (Palanichamy & Nagarajan, 1990), ringworm shrub or craw-craw plant (Irvine, 1961), king of the forest, or candle cassia (Seaforth, 1962), is an important medicinal plant in many developing countries. Extracts from the leaves alone or mixed with lime juice, local gin, or oil, are applied for the treatment of skin diseases and many eruptive skin infections (Dalziel, 1937; Fuzellier et al., 1982; Palanichamy et al., 1991; Olorundare et al., 1992). In Ghana, the method of application is to rub the affected skin until it bleeds and then rub the mixture of Senna alata and Piper guineensis plants into the sores.

2 MATERIAL AND METHODS

2.1 COLLECTION AND EXTRACTION OF PLANT MATERIAL

Senna alata's leaves were gathered and the specimen was placed in the research center of Alpha Omega Hi-Tech Bio. With running tap water, the new plant materials were cleaned and dried shade. The leaves and barks have been crushed to grind roughly powdered. Through the use of Soxhlet apparatus, these coarse powders (25 g) were subjected to consecutive extraction in 250ml hydroalcoholic. The extract gathered was stored and then used for further inquiries. For these extracts, DMSO (Dimethyl sulfoxide) acts as dissolved solvents.

2.2 CYTOTOXICITY ASSAY

2.2.1 MTT-Assay-Chemicals

The chemicals were obtained from Hi-Media Laboratories Ltd., Mumbai 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle Medium (DMEM) and Trypsin from Sigma Aldrich Co, St Louis, USA, E.Merck Ltd., Mumbai, India, Dimethyl Sulfoxide (DMSO) and Propanol

2.2.2 CELL LINES AND CULTURE MEDIUM

Cell cultures were procured from from the National Center for Cell Sciences (NCCS), Pune, India. MCF-7 (Human, Breast Cancer) and PC-3 (Human Prostate Cancer Cell Line). Stock cells were grown in the altered medium of Dulbecco's Eagle (DMEM), Medium was supplemented by 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU / ml), streptomycin (100 µg / ml) and amphotericin B (5 µg / ml) in a humidified environment of 5% CO2 at a concentration of 37°C. TPVG solution dissociated the cells (0.2% trypsin, 0.02% EDTA, 0.05% PBS glucose). The inventory crops were cultivated in flasks of 25 cm2 and all tests were conducted in 96 microtitre plates (Tarsions India Pvt. Ltd., Kolkata, India).

2.2.3 PREPARATION OF TEST SOLUTIONS

For cytotoxicity studies, each weighted test drug was dissolved separately in distilled DMSO and the volume was made up of DMEM supplemented with 2 percent inactivated FBS to obtain a 1 mg / ml concentration stock solution and filtration sterilized. Serially two-fold dilutions for cytotoxic research were prepared from this.

2.2.4 DETERMINATION OF CELL VIABILITY BY MTT ASSAYS

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells / ml using a 10% FBS medium and used by MTT assays as described by Francis and Ritas (1986) respectively to determine cell viability. The absorbance was measured using a 540 nm wavelength microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of the test medication required to inhibit cell development by 50% (CTC50) values is produced for each cell row from the dose-response curves.

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\% \text{ Growth inhibition} = \frac{\text{Mean OD of control group} - \text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100
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3 RESULT AND DISCUSSION

There has been no extensive study of cytotoxicity experiments with ordinary cell culture systems of plant extracts or folk medicinal plant extracts, and this is essential for assessing safety or extracting herbs. The aim of this research was therefore to assess the prospective cytotoxic activity of senna alata hydroalcoholic extract against cell line MCF-7 and PC-3. For in-vitro anticancer activity, the leaves of Senna alata hydroalcoholic extracts have been assessed. The standardization of Senna alata hydroalcoholic extracts was performed in accordance with the normal rules and the extract anticancer activity was performed against various cell lines showing the anti-cancer activity against breast cancer (MCF-7) and cell lines of Prostate cancer (PC-3). In addition, Senna leaf was active against Breast cancer (MCF-7) cell line whereas Prostate cancer (PC-3) cell line shows less activity when compared to breast cancer cell line. The MTT assay is a colorimetric assay that is delicate, quantitative and accurate, measuring cell viability. The assay is based on the ability of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) yellow water-soluble material into a water-insoluble dark blue / purple formazan product. The quantity of formazan generated in a range of cell lines is directly proportional to the cell number (Riss et al., 2016; Gerlier and Thomasset, 1986). Cancer chemotherapy's main objective is to target cancer cells specifically without showing toxicity to ordinary cells. This is the restriction on the use of several chemotherapeutic agents; therefore, when discovering leads for cancer treatment, selective toxicity must be considered (Wong et al., 2012).
Cytotoxicity of Senna alata leaf extract was evaluated by MTT (3,4,5-dimethyl thiazole-2-yl)-5-5-diphenyltetrazolium bromide) in the development of MCF-7 cells (Human breast cancer cells) and PC-3 (Human prostate cancer cells). Cytotoxic activity was investigated using MTT assay for cell line breast cancer (MCF-7), whereas prostate cancer (PC-3) is treated at different levels (50, 250, 500, 750 and 1000 μg / ml-1). Currently used for cancer treatment are agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction (De Flora and Ferguson, 2005). In cancer treatment, the use of various chemopreventive agents or agents with various cancer cell objectives is regarded more efficient (Howells and Manson, 2005). Breast cancer is the most common malignancy among women. MCF-7 cells have become a prominent model system for breast cancer research because they relate to the cells’ susceptibility to apoptosis. Although many tumors originally react to chemotherapy, breast cancer cells may eventually survive and acquire therapy resistance (Campbell et al., 2001).

4 CONCLUSION
This study disclosed that the studied senna alata hydroalcoholic extract showed important cytotoxic activity, demonstrating greater cytotoxicity with MCF-7 cell line and PC-3 cell line. This credits the ethnopharmacological approach to selecting specific plant species for bio-discovery of the natural product. Senna alata is one of the powerful medicinal plants used in India’s traditional medicinal systems. All plant components have the powerful medicinal property and have been used to heal many illnesses. The current research also added one more potent activity of the leaf of the plant. Further study is needed in the pharmaceutical industries to design the drugs for cancer illnesses. However, further study is underway to verify this and/or recognize novel natural products, which are in reality the interesting cytotoxic compounds.

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6. REFERENCES


