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In-vitro Antioxidant and Anticancer Activity from Senna alata and Senna hirsuta

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Abstract

Cancer is a large group of diseases that all have one thing in common, i.e. cells that grow out of control or, essentially, inability to regulate tissue growth. The goal of this study was to assess the capacity of antioxidants and anticancer with the use of an *Invitro* assay method. The in-vitro assay was analyzed for both the *Senna alata* and *Senna hirsuta* methanolic leaf extract. *In vitro*, DPPH radical scavenging assays examined methanolic extract antioxidant function, inhibition concentration being 383.27 µg / ml and 666.38 µg / ml. The PC-3 (Prostate cell line) cell line analysis was used as the model for MTT assay on *In vitro* cancer. The extract tested showed good dose-dependent free radical scavenging property for methanolic extract in all models with the IC_{50} values, respectively. The aerial component of *Senna alata* and *Senna hirsuta* may be concluded to have possible antioxidant and anticancer activities.

Keywords: DPPH radical scavenging assays, PC-3 cell line, MTT assay, *Senna alata*, *Senna hirsuta*.

1.Introduction

Nature is the paradise of medicinal principles offered to mankind through plants which since time immemorial act as the richest source of phytochemicals. A large number of modern drugs were removed from the floristic resources; many were used in traditional medicine treatments based on their use. Many medicinal plants, worldwide, have been used in everyday life for years to combat diseases. The common use of herbal remedies in preparations for health care, such as those mentioned in ancient texts known as 'ethno-medicines' has been traced as natural medicinal properties products (Debashisha Panda et al., 2012). Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by-products of biological reaction or from exogenous factors (Kikuzaki and Nakatani, 1993). Some of these ROS play an important role in cell metabolism in *In vivo*, including energy production, phagocytosis, and intercellular signaling (Ottolenghi, 1959). Nevertheless, these ROS created by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide range of pathological effects, such as DNA damage, carcinogenesis, and numerous degenerative disorders such as cardiovascular diseases, aging, and neurodegenerative diseases (Gyamfi et al., 2002; Osawa, 1994; Noda et al., 1997).

Cancer is associated with the highest death rates worldwide following cardiovascular disorders (Jemal *et al*., 2007). In 2030 there will be about 17 million deaths from cancer per year and 26 million new cases of tumors worldwide (Thun *et al*., 2010). Cancer is associated with deadly clinical manifestations, characterized by irregular proliferation of cells, replication of faulty DNA, checkpoints and apoptotic pathways (Nojima, 2004). Radiotherapy, surgery, and chemotherapy are common antitumor and anticancer treatments, but they have many side effects and a lower rate of survival in a patient, resulting in the need for new naturally occurring anticancer drugs (Gandhiappan and Rengasamy, 2012). Radiation exposure is normal to health care professionals and patients undergoing radiotherapy or radiation treatment, which can lead to the production of free radicals that cause DNA lesions and eventually cancer-causing genome

instability (Painuli and Kumar, 2016). Free radicals are unstable reactive molecules, which cause extensive intracellular damage, especially to proteins, DNA, and lipids, leading to numerous diseases and disorders such as cancer, diabetes, neurological disorders, pulmonary diseases, and cardiovascular diseases (Lobo *et al*., 2010; Reuter *et al*., 2010).

Prostate cancer is the most common form of male malignancy, particularly in developed countries where most cases are diagnosed in men over 50 years of age (Hariharan and Padmanabha, 2016). Current treatment options available for prostate cancer include surgery, hormone therapy, radiation therapy, and chemotherapy; these therapies have shown success in patients, but generally end up with negative and toxic side effects (Harun-ur-Fiashid*et al*., 2002). Nutritional supplements such as vitamin E, selenium, soy products, lycopene, and green tea catechin have also been used widely in the treatment of prostate cancer (Algotar*et al*., 2013; Kristal et al., 2011; Brausi*et al*., 2008). World Health Organization evidence indicates that approximately 65 percent of the world's population tend to use conventional and herbal medicines to treat disease (Gupta and Tandon, 2004). In India, along with the USA, the use of complementary alternative medicines has increased dramatically over the past two decades (Pandey and Madhuri, 2006).Approximately 60 per cent of anticancer agents are extracted from medicinal plants and other natural resources; however, a number of plants still have an anti-cancer potential but have not yet been fully investigated (Cragg and Newman, 2005). Therefore, the alternative solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines, since very few studies have been recorded on the use of herbal medicine in prostate cancer care (Rao *et al*., 2004).

In this study, we studied the antioxidant and anticancer properties of the *Senna alata* and *Senna hirsuta* leaf methanolic extract for *In vitro*. The extracts were prepared using Soxhlet extraction method, with the intention of enriching phenolic extracts (Pietrzak*et al*., 2014). The two plant extracts have been tested for cytotoxicity properties against cell lineage of prostate cancer (PC-3 cell line).

2. Materials and Methods

2.1 Collection of Plant Materials

The fresh samples from yercaurd hills, Senna alata and Senna hirsuta, Tamil Nadu, were collected randomly. The sample materials were washed under running tap water, air dried, and then homogenized to fine powder, and placed in airtight refrigerated bottles.

2.2 Preparation of Extracts

Extract of the raw sample was prepared using Soxhlet extraction method. About 20gm of powdered sample material has been uniformly packed into a thimble and extracted with 250ml of methanolic solvents separately. The extraction process will continue for 24 hours, or until the siphon tube extractor solvent is colourless. Afterwards, the extract was taken in a beaker and placed on a hot plate, heating at 30-40^{o}C until all the solvent was evaporated. Dried extract was placed in the refrigerator at 4^{o}C for potential use.

2.3 DPPH Radical Scavenging Activity

DPPH radical scavenging was performed using Molyneux method (2004). For maintaining specific concentration of liquids,1.0 ml of 100 µM DPPH solution in methanol, equivalent volume of the test sample in specific concentration of methanol was applied and 30 minutes incubated in dark. In terms of absorbance, the improvement in coloration was detected using a 514 nm spectrophotometer. The control tube was filled with 1.0 ml of methanol instead of a test sample. As a reference compound was used the different concentration of ascorbic acid.

Percentage of inhibition was calculated from the equation

[(Absorbance of control - Absorbance of test)/ Absorbance of control] × 100.

IC_{50} value was calculated using Graph pad prism 5.0.

2.4 Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was changed to 1.0x 105 cells / ml using a medium containing 10 per cent FBS and used

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by MTT assays as defined by Francis and Rita (1986) respectively to assess cell viability. The absorbance was measured at a wavelength of 540 nm via a microplate reader. The percentage growth inhibition was determined using the following formula and concentration of the test drug required to inhibit cell growth by 50 percent (CTC50) values is produced for each cell line from the dose-response curves (Wilson, 1983 & Masters, 2000).

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

3. Result and Discussion

The free radicals are constantly generated in the human body because of the use of oxygen by the body's cells. It produces a series of reactive oxygen species (ROS) such as super-oxide anion and hydroxyl radicals, and non-free radical species such as H_2O_2, single oxygen and nitric oxide (NO). Reactive oxygen species and reactive nitrogen species are associated with many pathological conditions such as chronic tissue injury caused by atherosclerosis, ischemia and reperfusion, central nervous system injury, gastritis and cancer (Manoharan *et al*., 2005).

Several biological and pharmacological activities including antioxidant, cytotoxic, anticancer, antimicrobial, antiviral, and anti-inflammatory activities have been documented for phytocompounds such as flavonoids and phenolic acids, commonly found in plants (Middleton *et al*., 2000). Antioxidants have a protective effect by neutralizing free radicals which are harmful by-products of the metabolism of natural cells. The human body has several mechanisms to reduce oxidative stress by generating antioxidants which are either produced naturally in situ or supplied externally by foods and/or supplements.These antioxidants function as free radical scavengers by preventing and repairing ROS damage, thus enhancing the immune defense and reducing the risk of cancer and degenerative diseases (Kumar *et al*., 2011).

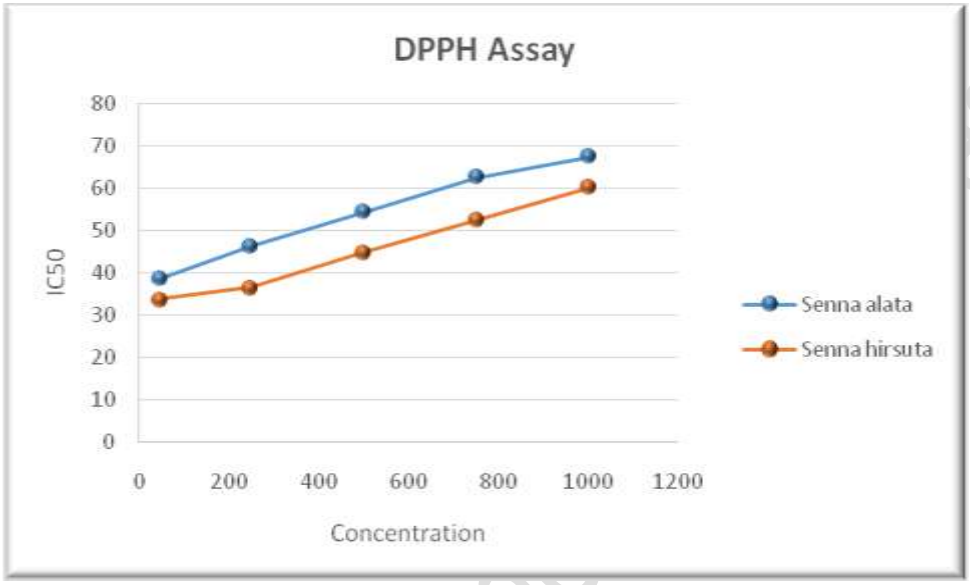
Table 1: DPPH Assay for Synthesized methanolic extract of *Senna alata* and *Senna hirsuta*

S.No	Concentration	*Senna alata*(IC_{50})	*Senna alata*(% IC_{50})	*Senna hirsuta* (IC_{50})	*Senna hirsute* (IC_{50})
1	50	38.78	383.27	34.01	666.38
2	250	46.26		36.73	
3	500	54.42		44.90	
4	750	62.59		52.38	
5	1000	67.35		59.86	

DPPH is a stable free radical based nitrogen that is conventionally used to evaluate the free radical scavenging of antioxidants in plant extract or synthetic compound (Kalaivani and Mathew, 2010; El-Maati*et al*., 2016). DPPH radical's reduction capability is determined by the antioxidant induced decrease in absorbance at 517 nm. The antioxidant efficacy of Senna alata and Senna hirsuta was assessed using the plant's methanolic extract in the present study.The experimental data revealed that the

extracts are likely to have the properties of methanol extract scavenging free radicals showing higher antioxidant capacity. The inhibition concentration of *Senna alata* (383.27 µg / ml) and Senna hirsuta (666.38 µg / ml) methanolic extract. In *Senna alata* is seen as the lowest concentration shows better activity

Figure 1 : DPPH Assay for Synthesized methanolic extract of *Senna alata* and *Senna hirsuta*



*3.2 Invitro*Anticancer Activity (PC3 – Cell Line)

The toxicity of assess, the MTT assay is used to screen the crude extracts. It could also provide an indication of potential cytotoxic properties of the methanolic extract *Senna alata* and *Senna hirsuta.* MTT assay is based on mitochondrial dehydrogenase reduction of MTT by the purple formazan product. It is frequently used as an in vitro model system for measuring the cytotoxic effects against cancer cell lines of various toxic substances and plant extracts (Morshed *et al.*, 2011). In vitro cytotoxicity test was performed using PC-3 lines of prostate cancer cells to screen potentially toxic compounds that affect basic cellular functions and morphology.All *Senna alata* and *Senna hirsuta* methanolic extracts displayed in vitro growth inhibition effects on the cell lines of PC-3 prostate cancer. These selective effects were dependent on concentration as well as time period of incubation. Every extract was analyzed with respect to concentration (50 µg / mL, 250 µg / mL, 500 µg / mL, 750 µg / mL, and 1000 µg / mL). The various concentrations of 1000 µg / mL of methanolic extract were the most effective in producing inhibition of percentage growth. For a single 72-hour time point, the methanolic extract showed less effect across the spectrum of concentrations examined in PC-3 prostate cancer cell lines.The results showed that methanol extract inhibited the PC-3 cell lines substantially and was the most effective IC50-value extract for *Senna alata* (427.77 µg / mL) and *Senna hirsuta* (623.50 µg / mL). The findings also verified the differential effect of PC-3 cell lines caused by the methanolic extract. Hence, the inhibition of cell

growth by *Senna alata* extracts may be due to the solvent's power in exceeding the effect of several bioactive constituents, the presence of phenolic compounds such as gallic acid and other antioxidants present in L. Indica (Rahman and others, 2013; Dalu*et al*., 2014).

Cytotoxic activity criteria for the crude extract are IC50 < 20 µg / mL, according to the National Cancer Institute (NCI) (Boik, 2001). The IC50 values indicated that the isolated flavonoid fraction's anticancer activity was higher than that of ethanol extract against MCF7 cell lines, and the isolated flavonoid fraction's IC50 falls within the NCI criteria, thus the flavonoid fraction is considered a promising potential for anticancer.

Table 2: Cytotoxicity assay of PC-3 cell line for methanolic extract of *Senna alata* and *Senna hirsuta*

S.No	Concentration	*Senna alata*			*Senna hirsuta*		
		CTC_{50}	Cell Viability	% CTC_{50}	CTC_{50}	Cell Viability	% CTC_{50}
1	50	35.39	64.61	427.77	16.26	83.74	623.50
2	250	41.36	58.64		25.72	74.28	
3	500	58.02	41.98		49.18	50.82	
4	750	61.52	38.48		60.49	39.51	
5	1000	68.52	31.48		66.46	33.54	

Figure 2: Cytotoxicity assay of PC-3 cell line for methanolic extract of *Senna alata* and *Senna hirsuta*

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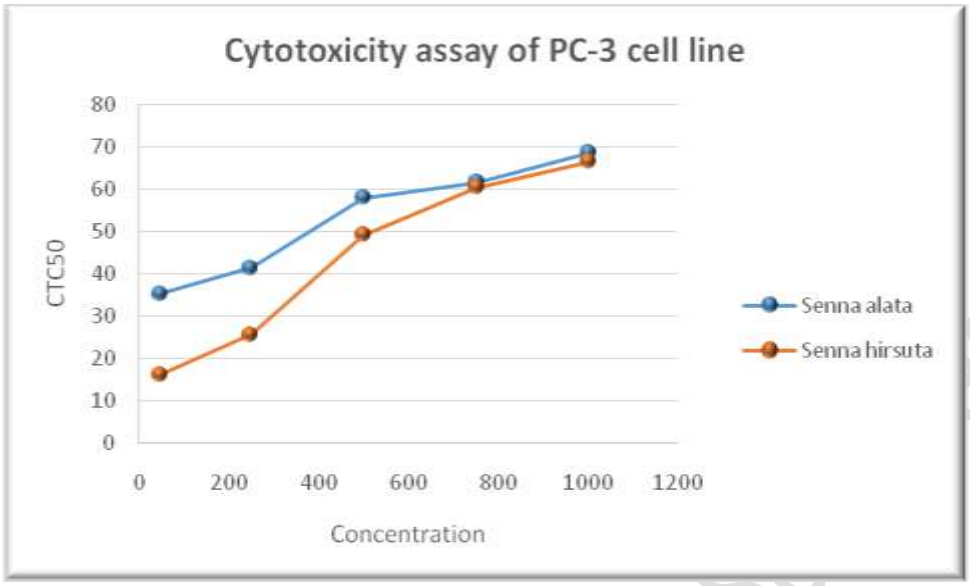

4. Conclusion

The present study, which notes that *senna alata* methanolic leaf extract showed significant antioxidant and anticancer activity *in vitro* against PC-3 cells. Such important and significant preliminary findings can be taken as the basis on which further studies should be carried out to delineate the detailed profile of *Senna alata* and *Senna hirsuta* antioxidant and anticancer activity.The components present on *Senna alata* and *Senna hirsuta* could act as a drug in the future, further *in-vivo* studies should be conducted. Considerable work has been done on the cancer treatment medicinal plants, and some plant products have been marketed as anticancer drugs. By destabilizing body equilibrium and conditioning the body tissues, these plants can promote host resistance to infection.

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